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Characterization of a population of *Pratylenchus hippeastri* from bromeliads and description of its related new species, *P. floridensis* n. sp. and *P. parafloridensis* n. sp. from grasses in Florida

Francesca DE LUCA[1], Alberto TROCCOLI[1]*, Larry W. DUNCAN[2], Sergei A. SUBBOTIN[3,4], Lieven WAEYENBERGE[5], Maurice MOENS[5] and Renato N. INSERRA[6]

[1] *CNR, Istituto per la Protezione delle Piante, Via G. Amendola 122/D, Bari 70126, Italy*

[2] *University of Florida, IFAS, Citrus Research and Education Center, Department of Entomology Lake Alfred, FL 33850-2299, USA*

[3] *Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832,USA*

[4] *Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow, 117071, Russia*

[5] *Agricultural Research Center, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Gent, Belgium*

[6] *Florida Department of Agriculture and Consumer Services, DPI, Nematology Section, P.O. Box 147100 Gainesville, FL 32614-7100, USA*

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* Corresponding author, e-mail: a.troccoli@ba.ipp.cnr.it

Summary – Morphological and molecular analyses confirmed the presence of *P. hippeastri* in regulatory samples collected in commercial bromeliad operations from genera *Guzmania*, *Neoregelia* and *Vriesea* in central and south Florida, USA. These *P. hippeastri* from bromeliads contained males which were not detected in the type population from amaryllis. The rDNA sequences of these males matched those of *P. hippeastri* female type material. *Pratylenchus hippeastri* and related root-lesion nematodes from several hosts in Florida were characterized at the morphological and molecular level, whereas other samples from Russia and South Africa at the molecular level only. Phylogenetic and sequence analysis using the ITS rRNA gene of these root-lesion nematodes revealed the presence of eight putative new species (spH1-H8) closely related to *P. hippeastri*. However, detailed morphological and molecular analyses are still required to confirm their unique species status. Here we describe two Florida representatives of the amphimictic root-lesion nematodes from Bahia grass (N1) and maidencane (N2), previously characterized by Inserra *et al.* (1996) and Duncan *et al*. (1999), as two new species phylogentically related to *P. hippeastri* and named *Pratylenchus floridensis* n. sp. and *P. parafloridensis* n. sp., respectively. The small round or oval, rarely rectangular and occasionally oblong and enlarged spermatheca and the bluntly pointed or subacute tail with smooth and occasionally indented terminus separate *P. floridensis* n. sp. from *P. parafloridensis* n. sp., which has a quadrangular spermatheca and a sub-hemispherical or bluntly pointed tail with generally smooth and rarely indented terminus. However, these characters may overlap in some specimens making the morphological separation problematic without the use of molecular analysis. The close phylogenetic relationships shared by the species characterized in this study indicate that they are representatives of a *P. hippeastri* species complex.

Keywords – Bahia grass, bottlebrush, *Callistemon rigidus,* D2-D3, 28S rDNA, *Fraxinus caroliniana*, ITS, maidencane, morphology, morphometric, *Panicum hemitomon*, *Paspalum notatum*, phylogeny, pop ash, root-lesion nematodes, Russia, species complex, St. Augustine grass, South Africa, *Stenotaphrum secundatum*, systematics.

In the early 1990s, two amphimictic populations of root-lesion nematodes were found on bahia grass (*Paspalum notatum* Flueggé) (N1) and maiden cane (*Panicum hemitomon* Schultes) (N2) in Florida, USA. The populations have regulatory significance because they are morphologically similar to *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941 which is a regulated nematode in Florida. Morpho-biological studies of these two populations provided evidence that they share similarities (undivided smooth face, with two lip annuli and prominent spermatheca filled with sperm) with *P. loosi* Loof, 1960, a species closely related to *P. coffeae*. In spite of the fact that these populations have a more anterior vulva (75-79%) than *P. loosi* (79-85%), they were tentatively identified as Florida populations of *P. loosi* (Inserra *et al*., 1996). Subsequent molecular analyses of these populations showed that they were phylogenetically unrelated to both *P. coffeae* and *P. loosi* (Duncan *et al*., 1999) and consisted of two morphologically similar species of root-lesion nematodes, designated as *Pratylenchus* N1 and N2 (N1 = *P. loosi* Zolfo Springs and N2 = Lithia in Table 1). Their morphological characteristics were given by Inserra *et al*. (1996) and Duncan *et al*. (1999).

A root-lesion nematode, *P. hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007, was recently described from amaryllis in Florida, USA (Inserra *et al*., 2007). This species, lacking males, was characterized by having a flat and smooth face with two lip annuli, a rectangular empty spermatheca, a conoid tail with a bluntly pointed terminus and anterior vulva position (75-78%). *Pratylenchus hippeastri* clustered in a clade with the amphimictic *Pratylenchus* N1 and N2 populations from pasture grasses in Florida when its D2-D3 expansion domains of the 28S rDNA sequences were compared with those of 30 *Pratylenchus* populations (Inserra *et al*., 2007). The close relationships between *P. hippeastri* and *Pratylenchus* N1 and N2 were confirmed by Subbotin *et al.* (2008), who considered *Pratylenchus* N1 and N2 conspecific with *P. hippeastri* in spite of the difference in their reproductive behavior and morphological differences.

Recently, root-lesion nematodes with few males and numerous females morphologically similar to *P. hippeastri* were detected in regulatory samples collected in commercial bromeliad operations in central and south Florida. Bromeliads are ornamental epiphytes that are grown and traded for their attractive foliage and flowers. These epiphytes produce roots that anchor the plant to branches and twigs of trees, but also take up nutrients when

bromeliads are in contact with or grown in soil and other media. In spite of the presence of males, which are unknown from the type population of *P. hippeastri* from amaryllis, these root-lesion nematodes from bromeliads share major diagnostic morphological features with *P. hippeastri*. Additionally, non-amphimictic and amphimictic lesion nematodes with two or occasionally three, lip annuli and morphologically similar to *P. hippeastri* and N1 and N2 occur in Florida on turf grasses, ornamentals, and native trees. However, the taxonomic status of these species as well that of N1 and N2 and those infecting bromeliads is uncertain and requires clarification.

The main objectives of this study were to: (i) characterize morphologically and molecularly populations of *P. hippeastri* from bromeliads and confirm their species identity; (ii) provide updated morphological and molecular data on the root-lesion nematodes N1 and N2 and describe them as two new species; (iii) reconstruct phylogenetic relationships between *P. hippeastri* and N1 and N2 along with other closely related species from Florida, Russia and South Africa (Table 1) using the ITS and D2-D3 expansion segments of 28S rRNA gene sequences.

Materials and methods

ROOT LESION NEMATODE POPULATIONS FROM BROMELIADS, GRASSES AND OTHER PLANTS

Three bromeliad production operations located near Apopka and Miami, Florida were surveyed in 2007-2008. Sixty composite root and soil samples were collected from containerized bromeliads (Tables 2) in all of the production operations. Root lesion nematodes were hand picked from infested bromeliad samples and transferred to carrot disks at 23 °C (Huettel, 1985). Cultured nematodes were used for morphological and molecular analyses and sex ratio determination.

Pratylenchus hippeastri from amaryllis and related non-amphimictic root-lesion nematodes from bromeliads and other hosts in Florida (spH5, spH7 and spH8) tentatively identified morphologically as representative of *P. hippeastri*, *P. zeae* Graham, 1951 and *P. jordanensis* Hashim, 1983 (= *P. zeae*) (Table 1) were selected for this study. Two additional Florida amphimictic root-lesion nematodes (spH1 and spH6) similar to N1 and N2 identified

as *Pratylenchus* sp. (Table 1) were also included for molecular analysis only, along with two other samples identified as *P. subranjani* Mizukubo, Toida, Keereewan & Yoshida , 1990 and *P. zeae* (spH3, H2) from Russia and South Africa, respectively, and a Florida population with divided face indentified as *P. scribneri* Steiner in Sherbakoff & Stanley, 1943 (spH4) (Hernández *et al.,* 2000) (Table 1). Nematodes were extracted from soil by the sieving and decanting method, and from bromeliad, bottlebrush, pop ash, St. Augustine grass and mixed species of turf grass roots by incubation in jars.

MORPHOMETRIC AND MORPHOLOGICAL ANALYSIS

Adult root-lesion nematodes from bromeliads were used for this study. Live specimens were immobilized by gently heating and then mounted in water agar on a slide (Esser, 1986) for measurements and photographs. Additional measurements and drawings were made from specimens killed and fixed in hot aqueous 2% formaldehyde + 1% propionic acid, dehydrated in ethanol vapor and mounted in dehydrated glycerin (Hooper, 1970). Measurements of specimens were made with an ocular micrometer and drawings with a *camera lucida*. Photographs were taken with two Leica (Wild MPS 46/52 and Leica DFC 320) cameras mounted on Nikon (Optiphot) and Leica DM 2500 compound microscopes.

The morphological information on the root-lesion nematodes from Bahia grass (N1) and maidencane (N2) provided by Inserra *et al.* (1996) and Duncan *et al.* (1999) was augmented by further microscopic examination of additional preserved specimens kept in the nematode collection (CNR-IPP, Bari) by the second author of this paper. Morphometrics of mature females of root-lesion nematode species studied by Duncan *et al*. (1999) and *P. hippeastri* from bromeliad and the original description were subjected to principal component analysis using (Minitab 13; Minitab Inc., State College, PA). Populations were characterized based on the lip morphology (smooth or divided) and the weakly-allometric characters/ratios V, a, and stylet length (Duncan *et al*., 1999).

Specimens for scanning electron microscope (SEM) observations were cold fixed in glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.2), post fixed 1hour in 2% osmium tetroxide, dehydrated in a graded series of ethanol, critical point dried with CO_2 and sputter coated with gold palladium (Eisenback, 1985). Nematodes were observed with a Hitachi S530 microscope at 15 to 20 kV accelerating voltage.

MOLECULAR ANALYSIS

DNA was extracted from individuals of both female and male root-lesion nematode specimens. Specimens were handpicked and singly placed on a glass-slide in 3 μl of the lysis buffer (10mM Tris-HCl, pH8.8, 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 samples were incubated at 65 °C for 1 h and then at 95 °C for 15 min to deactivate the proteinase K. DNA-extraction from individual root-lesion nematode specimens, PCR, cloning and sequencing have been done in three laboratories: Istituto per la Protezione delle Piante, Italy; ILVO, Belgium; PPDC, CDFA, USA. The protocols were described in detail by De Luca *et al.* (2004), Waeyenberge *et al.* (~~2000~~2009) and Subbotin *et al.* (2008), respectively. The following sets of primers were used for amplification of two gene fragments in the present study: (*i*) D2-D3 expansion segments of 28S rRNA using forward D2A (5'-ACAAGTACCGTGGGGAAAGTTG-3') and reverse D3B (5'-TCGGAAGGAACCAGCTACTA-3') and (*ii*) ITS1-5.8-ITS2-rRNA using forward 18S-Int (5'-CGTAACAAGGTAGCTGTAGG-3') and reverse 26S-Int (5'-TCCTCCGCTAAATGATAT-3'), ~~or~~ forward TW81 (5´-GTTTCCGTAGGTGAACCTGC-3´) and reverse AB28 (5´-ATATGCTTAAGTTCAGCGGGT-3´) or forward PRATTW81 (5´-GTAGGTGAACCTGCTGCTG-3´) and reverse AB28 (5´-TATGCTTAAGTTCAGCGGGT-3´). ~~.~~ PCR products were purified using the protocol listed by the manufacturers of Nucleospin Extract II (Macherey-Nagel, Duren) or QIAquick (Qiagen, USA) gel extraction kits and used for cloning or direct sequencing in both directions with the primers given above or M13 forward and M13 reverse primers. TOPO-TA cloning kit (Invitrogen) or pGEM-T Vector System II kit (Promega) were used for cloning of PCR products. Newly obtained sequences were deposited in the GenBank under accession numbers given in Table 1.

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The newly obtained sequences for both ribosomal regions of *P. hippeastri* from bromeliad and of root-lesion nematodes from other hosts including those from amaryllis, Bahia grass (N1), maidencane (N2) (Table 1) and *P. jaehni* Inserra, Duncan, Troccoli, Dunn, dos Santos, Kaplan & Vovlas, 2001 (Duncan *et al*., 1999; Waeyenberge, unpublished) were aligned using ClustalW (Thompson *et al*., 1997) with default parameters. *Pratylenchus jaehni* was used as an outgroup taxon (Subbotin *et al*., 2008). Phylogenetic analysis of the

sequence data sets were performed with maximum parsimony (MP) using PAUP* 4b10 (Swofford, 2002) and Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). For MP we used heuristic search setting with ten replicates of random taxon addition, tree bisection-reconnection branch swapping to seek the most parsimonious trees. Gaps were treated as missing data. To obtain an estimation of the support for each node, a bootstrap analysis (BS) with 1000 replicates was carried out. BI analysis under GTR + I + G model for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. The log-likelihood values of the sample points stabilized after approximately 10^3 generations. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades.

SPECIES DELIMITING IN STUDIED *PRATYLENCHUS*

Species delimiting of the studied populations was done by applying an integrated or polyphasic approach, which was based on consideration of results of morphological and morphometrical studies, phylogenetic and sequencing analysis, and analysis of host-plants and geographic distribution of studied samples. This approach integrates any significant information on the organisms, and results in a consensus and transition type of classification (Subbotin & Moens, 2006). Two new species named here as *P. floridensis* n. sp. and *P. parafloridensis* n. sp. and several unidentified, putative new species defined here as *Pratylenchus* spH1–H8 were delimited in this study using this approach. More detailed morphological and molecular analysis is still required to confirm the unique species status of *Pratylenchus* spH1–H8.

Results

ROOT-LESION NEMATODE POPULATIONS COLLECTED FROM BROMELIADS AND OTHER PLANTS

Bromeliads belonging to the genera *Guzmania*, *Neoregelia* and *Vriesea* were found infected by root-lesion nematodes similar to *P. hippeastri* (Table 2). Population levels were usually < 10 specimens/g fresh roots. In some cases bromeliad roots were found infected concomitantly with a few specimens of *P. brachyurus* (Godfrey, 1929) Filipjev &

Schuurmans Stekhoven, 1941, endoparasitic *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961, and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949. Carrot cultures inoculated with female root-lesion nematodes from bromeliads produced a large number of nematodes at different life stages and only a few males (usually 3 males per 100 females). The population levels of other Florida root-lesion nematodes varied from 30, 160, and 100 specimens/ g fresh roots for spH1, H5 and H6, respectively. The number of males in the amphimictic spH5 and H6 was about 40/ 100 females.

MOLECULAR CHARACTERIZATION OF *P. HIPPEASTRI* SPECIES COMPLEX

The amplification of the ITS containing region produced a single fragment of approximately 970-1000 bp for the studied samples. The sequence alignment for *P. hippeastri* and related species with consensus sequence for each putative species is given in figure 1. The ITS alignment included 44 sequences and was 1050 bp in length. Sequence diversity within all studied root-lesion nematodes including *P. jaehni* reached 19% (174 nucleotides); for *P. hippeastri* from bromeliad and from amaryllis varied from 0 to 0.6% (0 to 6 nucleotides), whereas sequence diversity within the other root-lesion nematodes related to *P. hippeastri* reached 6.2% (57 nucleotides). Phylogenetic relationships within *Pratylenchus* species as inferred from Bayesian inference are given in figure 2. Four main moderate or highly supported clades (PP = 90-100) were distinguished within the tree. Clade 1 grouped *P. hippeastri* populations along with root-lesion nematodes spH1-H5. Populations of *P. hippeastri* from amaryllis and bromeliads clustered together forming one highly supported (PP = 100) subclade within clade 1. The ITS sequences for *Pratylenchus* spH2 and spH3 did not form distinct subclades and relationships between them were not resolved. The root-lesion nematode N2 (= *Pratylenchus parafloridensis* n. sp. in Table 1 and Figs 1-3) formed a moderately supported (PP = 90) clade 2 together with *Pratylenchus* spH6. Root-lesion nematodes spH7 and H8 clustered together and were not well separated. The two sequences of N1 (= *Pratylenchus floridensis* n. sp. in Table 1 and Figs 1-3) formed highly supported clade 4 at the basal position of the tree.

Sequence alignments of the D2-D3 of 28S rDNA included 32 sequences of 713 bp in length. Sequence diversity reached 7.4% (47 nucleotides) for all root-lesion nematodes

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studied; 2.1% (14 nucleotides) for the species closely related to *P. hippeastri* and varied from 0 to 0.3% (0 to 2 nucleotides) within *P. hippeastri* populations. Phylogenetic relationships within *Pratylenchus* species based on D2-D3 of 28S rDNA sequences is given in figure 3. Five main weak to highly supported clades (PP = 72-98) were distinguished within the tree, which corresponded to the clades on the ITS-rRNA tree. Populations of *P. hippeastri* from amaryllis and bromeliads formed a moderately supported (PP = 93) clade 1 together with *Pratylenchu*s spH1, H2 (Russia) and H5 (South Africa). Root-lesion nematodes spH7 and H8 clustered in a moderately supported clade 3. The amphimictic N1 formed a highly supported clade 4 at the basal position of the tree. The relationships between *Pratylenchus* species were not well resolved. MP and BI analyses generated congruent trees with similar branch supports for the ITS and D2-D3 gene alignments, respectively.

The close phylogenetic relationships shared by the species (spH1-H8) characterized in this study indicate that they are representatives of a *P. hippeastri* species complex. These results confirmed the identity of males and females of bromeliad populations as *P. hippeastri* and provided evidence that the rDNA sequences of *P. hippeastri* males from bromeliads matched those of *P. hippeastri* female type material. Furthermore the phylogenetic findings also provided support for the description of N1 and N2 as two new species named *P. floridensis* n. sp. and *P. parafloridensis* n. sp., respectively.

MORPHOLOGICAL CHARACTERIZATION

Comparative measurements of females and males of *P. hippeastri* from bromeliads and those reported from amaryllis in the original description are reported in Table 3.

Female

Morphometric values of the females from bromeliads did not differ from those of *P. hippeastri* from amaryllis, except in the length of tail of fixed specimens, which was slightly shorter than in females from amaryllis. Small differences were observed also in the mean values of some characters of bromeliad live specimens, such as body, tail and post uterine sac length, which were shorter than those of specimens from amaryllis. Bromeliad females also showed smaller maximum and vulval body diameter and a shorter vulva-anus distance. However, their range values overlapped. Their lip pattern was similar to that of *P. hippeastri*

and consisted of a flat and undivided face with two lip annuli (in some specimens with an incomplete third annulus) and with the second annulus larger and thicker than the first annulus (Fig. 7A, B) as those described for *P. hippeastri*. These females showed also a small and empty spermatheca indicating that fertilization by the males in the cultures did not occur. The small and empty spermatheca was observed also in females collected directly from bromeliad roots from the nurseries. These morphometric and morphological characters indicate that the lesion nematode females from bromeliads are *P. hippeastri*.

The principal components analysis for the morphometrics listed in Table 3 and those reported by Duncan *et al.* (1999) positioned bromeliad *P. hippeastri* in a group (VI) with N1 and N2 and separate from the other *Pratylenchus* species studied by Duncan *et al*. (1999) (Fig. 4).

Male

The few males present in carrot cultures and bromeliad roots exhibited a slight sexual dimorphism. Males had smaller head width (6.0-6.7 *vs* 7-7.8 μm), stylet knobs (2.3-3.0 across and 1.7-2.0 high *vs* 3.0-4.7 and 2.0-2.7 μm) and metacorpus (7.3-8.7 across and 10.0-11.3 high *vs* 10.0-11.3 and 13.3-17.3 μm) compared to those of the females. This sexual dimorphism has been reported for many amphimictic root lesion nematodes. The male lip pattern, in spite of a slightly more collapsed appearance of the cuticle, did not differ from that of the female and showed a flat and undivided face with two lip annuli of different size with the second annulus larger and thicker than the first annulus (Figs 5B, 6C, 7C, D). The oral disc was slightly raised and the amphid apertures were broader than in female. The stylet was more slender than that of female; the knobs ellipsoidal to triangular in profile, with rounded margins. The pharynx had a small, muscular metacorpus, rather long isthmus and a slender gland lobe, overlapping intestine about 3 times the body width. Spicules were curved, weakly cephalated, with two prominent expansions at the base of their proximal third. Gubernaculum simple, slightly curved. Tail conical, enveloped by a crenate, moderately protruding bursa, extending to the tail tip. Lateral field with four smooth incisures, occupying slightly less than 1/3 of body diameter.

MORPHOLOGICAL CHARACTERIZATION AND DESCRIPTION OF ROOT-LESION NEMATODES N1 AND N2 AS TWO NEW *PRATYLENCHUS* SPECIES

In this study we collected enough molecular and morphological data to describe N1 and N2 as two new root-lesion nematodes, *Pratylenchus floridensis* n. sp. and *P. parafloridensis* n. sp., respectively. The major objective of these descriptions was to clarify the identity of these two species that were reported in the literature and GenBank with acronyms and incorrect scientific names. The morphological description of the other putative species in the *P. hippeastri* species complex from Florida and other countries was not attempted.

***Pratylenchus floridensis*[*] n. sp.**

[Figs 8-11]

MEASUREMENTS

Measurements of this species, originally identified as *P. loosi* from Bahia grass and later as N1 root-lesion nematode, were reported in Table I and Results and Discussion in Inserra *et al.*(1996) and Table 2 and Results in Duncan *et al.* (1999), respectively.

Additional selected measurements (present study) are reported in Table 4.

DESCRIPTION

Female

Body of dead females almost straight. Labial region with two annuli, 2 μm high, 7 μm wide on average (present study), offset from the body by a slight constriction; second lip annulus wider and higher than first annulus. SEM *en face* view characterized by un undivided pattern, with all labial sectors fused together and partially with an oval oral disc (Fig. 11A, B); amphidial openings rather wide, obliquely oriented, at the sides of the oral disc. Stylet with ellipsoidal knobs or rounded with slightly flattened anterior surface, 4 μm across, 2 μm high (mean values). Dorsal pharyngeal gland opening 2-2.5 μm posterior to stylet base (o% range = 12.9-16.7). Pharyngeal metacorpus oval, 11-13 μm high, 8.5-11 μm

[*] Specific epithet derived from Florida, the only geographical area where this species has been detected.

in diam. Isthmus slender, encircled by the nerve ring in its upper part, just posterior to metacorpus. Pharyngeal gland lobe overlapping intestine ventrally, 35 ± 7.8 (27-52) μm in length. Lateral fields with four lines, not areolated. Anterior genital tract 144-240 μm long, 32-47 % of body length. Spermatheca small, rounded or oval, rarely rectangular in shape, occasionally oblong and large, filled with sperm. Post uterine sac approximately 1.5 body diam. long, undifferentiated. Tail bluntly pointed (*sensu* Frederick & Tarjan, 1989) or subacute, with smooth terminus. In few specimens, a slight indentation was observed at the tail tip (Fig. 8L).

Male

Body generally straight when heat-relaxed, similar to female except for sexual dimorphism and slightly smaller body size. Lip region slightly offset, 2 μm high and 5.7 μm wide, with two annuli (second annulus higher than first one). Stylet more slender and shorter than in female, with minute, slightly cupped knobs, 2.7 μm across, 1.5 μm high. The lip pattern in SEM *en face* view shows a plane, undivided face, two lip annuli (second annulus larger and thicker than the first annulus) and an oral disc more rounded than in females (Fig 11C). Pharynx with oval metacorpus (10.5 × 7.3 μm in longitudinal and cross diam., respectively) and gland lobe overlapping intestine for 37 μm. Hemizonid just anterior the secretory-excretory pore; hemizonion eight annuli posterior to it. Lateral field with four smooth lines. Testis outstretched, 238 μm long. Tail conoid, with a narrowed jaline tip, 4 μm long. Spicules arcuate, weakly cephalated; gubernaculum simple, slightly arcuate.

TYPE HOST AND LOCALITY

The type population is from Bahia grass (*Paspalum notatum* Flueggé) roots collected from a sod farm in Zolfo Springs, Hardee County, Florida USA (latitude 27º41’95”N; longitude 81º64’25”W). The soil type is sandy, the annual precipitation 1500 mm and the climate is subtropical.

DIAGNOSIS AND RELATIONSHIPS

Pratylenchus floridensis n. sp. female is characterized by the following morphological characters: slender body, undivided, plain and smooth face with all labial sectors fused

together and partially with an oval oral disc, head with two lip annuli and with the second annulus larger and thicker than the first annulus, ellipsoidal stylet knobs or rounded with slightly flattened anterior surface, rounded, oval or rarely rectangular spermatheca filled with sperm, tail bluntly pointed with smooth terminus (in rare specimens slightly indented). The matrix code (*sensu* Castillo & Vovlas, 2007) for this species is: A1, B2, C2, D2, E2, F4, G3, H1, I2, J1, K1.

Few morphological and morphometrical characters separate *P. floridensis* n. sp. from *P. parafloridensis* n. sp., hereinafter described. The present study revealed that *P. floridensis* n. sp. differs from *P. parafloridensis* n. sp. in having a shorter female body (average 450 *vs* 532 μm) an oval *vs* round oral disc, a small, round to oval and sometimes rectangular spermatheca *vs* quadrangular or large rectangular in *P. parafloridensis* n. sp. and a bluntly to finely pointed (rarely indented) tail tip *vs* sub-hemispherical or bluntly pointed tail with smooth or, less frequently, indented tail terminus. However, these characters may overlap in some specimens making the morphological separation of these two species unreliable without the corroboration of the molecular analysis.

The amphimictic reproductive habits, presence of males and a large spermatheca filled with sperm separate morphologically *P. floridensis* n. sp. from other male-less lesion nematode species with undivided and smooth face, two lip annuli and having a non-functional spermatheca such as *P. acuticaudatus* Braasch & Decker, 1989, *P. angulatus* Siddiqi, 1994, *P. brachyurus*, *P. estoniensis* Ryss, 1982, *P. hippeastri* and *P. tenuis* Thorne & Malek, 1968. This new species differs from the amphimictic root lesion nematodes with the same head features for the following characters: from *P. alleni* Ferris, 1961 it differs for the tail shape (bluntly pointed *vs* rounded) and a more anterior vulva position (77 *vs* 80% mean value); from *P. araucensis* Múnera, Bert & Decraemer, 2009 for the long *vs* short pharyngeal overlap, lateral field smooth *vs* areolated in outer lateral ridges and shape of tail terminus (smooth *vs* variable); from *P. artemisiae* Zheng & Chen, 1994 for the longer stylet length (14-15.5 *vs* 11.5-14.5 μm) and more anterior vulva position (75-79 *vs* 76-81); from *P. brzeskii* Karssen, Waeyenberge & Moens, 2000 for the shorter stylet (14-15.5 *vs* 18-19 μm); from *P. coffeae* for the tail shape (bluntly pointed *vs* rounded, truncate or indented) and a more anterior vulva position (77 *vs* 81% mean value); from *P. flakkensis* Seinhorst, 1968 for the tail (bluntly pointed with smooth terminus *vs* conical with faintly annulated terminus) and stylet knob

shape (ellipsoid to rounded *vs* anteriorly pointed), from *P. gutierrezi* Golden, Lopez & Vilchez,1992 for the undivided face *vs* divided and from *P. kumamotoensis* Mizukubo, Sugimura & Uesugi, 2007 for the pharyngeal gland lobe (ventral *vs* frequently dorsal), shorter PUS (23-32 *vs* 37-45) and lateral field (smooth *vs* areolated in vulval region). *Pratylenchus floridensis* n. sp. differs also from *P. jaehni* Inserra *et al.*, 2001, *P. loosi*, *P. neobrachyurus* Siddiqi, 1994, *P. panamenis* Siddiqi, Lopez & Vilchez, 1991 *P. roseus* Zarina & Maqbool, 1998 and *P. silvaticus* Brzeski, 1998 for the more anterior vulva position (75-79 *vs* 77-80, 79-85, 80-84, 77-83, 81-83 and 80-83%, respectively). In addition, *P. floridensis* n. sp. has a longer tail than *P. jaehni* (24-41 *vs* 21-31 μm), a longer body than *P. neobrachyurus* (421-744 *vs* 310-410 μm), a different tail shape than *P. panamensis* (bluntly pointed with mostly smooth terminus *vs* subclavate with annulated terminus), different vulval margins, number of lateral lines and tail terminus than *P. roseus* (no vulval flaps, four lateral lines and smooth tail terminus *vs* presence of vulval flaps, six lateral lines and coarsely annulated tail terminus) and different tail shape than *P. silvaticus* (slightly clavate with irregularly striated tail terminus).

We would like to point out that lip patterns of *P. acuticaudatus*, *P. alleni*, *P. artemisiae*, *P. angulatus*, *P. brzeskii*, *P. estoniensis*, *P. flakkensis*, *P. gibbicaudatus*, *P. kumamotoensis*, *P. neobrachyurus*, *P. panamensis*, *P. roseus*, *P. silvaticus* and *P. tenuis* are not known (Castillo & Vovlas, 2007).

Pratylenchus parafloridensis[†] **n. sp.**

[Figs 8-11]

MEASUREMENTS

See Table 4 and Table I, Results and Discussion *in* Inserra *et al.* (1996) and Table 2 and Results *in* Duncan *et al.* (1999).

DESCRIPTION

Female

[†] Specific epithet consisting of *para* = close + *floridensis* and indicating the close similarity of this species with *P. floridensis* n. sp.

Body of dead females almost straight or in open C. Labial region with two annuli, 2.3 μm high, 7.5 μm wide on average, offset from the body by a slight constriction; second lip annulus distinctly wider and higher than first annulus. SEM *en face* view characterized by un undivided pattern, with all labial sectors fused together and with a rounded oral disc (Fig. 11D-F); amphidial openings obliquely oriented, at the sides of the oral disc. Stylet with rounded or ellipsoidal knobs, 4 μm across, 2.1 μm high (mean values). Dorsal oesophageal gland opening 2-2.5 μm posterior to stylet base (o% range = 12.5-17.2). Pharyngeal metacorpus oval, 10-15 μm high, 7.5-13 μm in diam. Isthmus slender, encircled by the nerve ring in its superior half. Pharyngeal gland lobe rather long, overlapping intestine ventrally, 43 ± 9.3 (30-56) μm in length. Lateral fields with four lines, not areolated. Anterior genital tract 171-211 μm long, 31-39 % of body length. Spermatheca filled with sperm, quadrangular or large rectangular in shape, sometimes with a constriction in its equatorial diam., giving the appearance of a bilobed structure (Fig. 8P). Post uterine sac approximately 1.5 body diam. long, often with rudimentary cellular elements at the end. Tail conoid, subhemispherical or bluntly pointed with smooth or slightly indented (about 30% of specimens observed) terminus.

Male

Body generally straight when heat-relaxed, similar to female except for sexual dimorphism and body size, which is slightly smaller. Lip region 2.0 ± 0.1 (2-2.1) μm high and 6.4 ± 0.3 (6-6.7) μm wide. Stylet more slender and shorter than in female, with rounded knobs, 2-2.7 μm across, 2 μm high. Pharyngeal metacorpus rounded to oval, 10.5 × 7.7 μm (longitudinal and cross diam., respectively). Pharyngeal gland lobe overlapping intestine for 43 ± 7.4 (37-51) μm. Hemizonid just anterior to excretory pore. Lateral field with four, smooth lines. Tail conical, rather short. Testis outstretched, 211 ± 34.6 (174-254) μm long. Spicules arcuate, slender and weakly cephalated; gubernaculum simple, slightly arcuate.

TYPE HOST AND LOCALITY

The type population is from maidencane (*Panicum hemitomon* Schultes) roots collected from a pasture land in Lithia, Hillsborough, Florida, USA (latitude 27º79’63”N; longitude

82º21'13"W). The soil type is sandy, the annual precipitation 1500 mm and the climate is subtropical.

DIAGNOSIS AND RELATIONSHIPS

Pratylenchus parafloridensis n. sp. female is characterized by the following morphological characters: slender body, undivided, plain and smooth face with all labial sectors fused together and with a round oral disc, head with two lip annuli and with the second annulus larger and thicker than the first annulus, generally rounded stylet knobs, quadrangular or large rectangular, sometimes bilobed spermatheca filled with sperm, tail subhemispherical or bluntly pointed with smooth or, less frequently, slightly indented terminus. The matrix code for this species is: A1, B2, C2, D2, E2, F5, G3, H1, I3, J1, K1.

The relationship of *P. parafloridensis* with other members of the genus *Pratylenchus* is similar to that described above for *P. floridensis* n. sp.

Discussion

PRATYLENCHUS HIPPEASTRI FROM BROMELIADS

This study provides evidence that *P. hippeastri* is a tropical root lesion nematode reported so far only in Florida where it parasitizes tropical ornamentals such as amaryllis and bromeliads. The application of phylogenetic and sequence analysis of the ITS-rRNA gene confirmed co-specificity of the root lesion nematode population found parasitizing bromeliads with *P. hippeastri* a previously known parasite of amaryllis only. Our observations indicate that populations of this species from bromeliads can produce males in both carrot discs and bromeliad roots. So far no males have been found in other populations of this nematode. The function of the males in the bromeliad populations is unclear since they are present in very small number and are consistently in association with un-mated females showing an empty and small spermatheca. There are reports of occurrence of males in non-amphimictic root lesion nematodes such as *P. zeae* (Loof, 1991). The identity of these males may be questioned since contaminating male specimens belonging to different species may be

Opmerking [lw6]: I suggest to switch because sequencing comes before phylogenetic analysis?

Met opmaak: Engels (Verenigde Staten)

Met opmaak: Engels (Verenigde Staten)

associated with non-amphimictic species. In spite of the occurrence of a few males, our observations do not provide any evidence that *P. hippeastri* is an amphimictic species.

USEFULNESS OF THE ITS-RDNA SEQUENCES FOR SPECIES DIFFERENTIATION IN *PRATYLENCHUS*

The ITS containing region allowed better discrimination among the closely related species studied, because it evolved faster than the D2-D3 expansion segments of 28S rDNA and accumulated more substitution changes. The present analysis of the ITS-rDNA dataset clearly separated *P. hippeastri* from other amphimictic and non-amphimictic root-lesion nematodes confirming that they are probably new *Pratylenchus* species belonging to the *P. hippeastri* species complex. The fact that they share morphological affinity, show minimal sequence differences in the rRNA gene and sometimes their positions are not well resolved in the phylogenetic trees suggests that these species are derived by recent speciation events with insufficient time to attain complete morphological differentiation. The phylogenetic analysis of the ITS-rDNA does not confirm the conclusion of co-specificity of *Pratylenchus* N1 and N2 populations with *P. hippeastri* previously made by Subbotin *et al.* (2008) based on analysis of the D2-D3 of 28S rDNA, but instead it shows that each of these populations represents a distinct species. Successful application of the ITS for species differentiation in *Pratylenchus* has been shown by Orui (1996), Waeyenberge *et al*. (2000) and De la Peña *et al*. (2006) with PCR-RFLP. These studies also revealed heterogeneity in the ITS sequences, which resulted in additional bands on gels after restriction of PCR products. These additional bands constitute ~~a~~ complex RFLP profiles, which may complicate diagnostics of *Pratylenchus* species. Our study also revealed heterogeneity in the ITS sequences for all studied *Pratylenchus* species. However, in most cases the phylogenetic analysis of the ITS sequence dataset allowed clear separation of sample populations because, except for *Pratylenchus* spH2 and spH3, all sequences obtained from the same sample clustered together. Although *P. parafloridensis* n. sp. and *Pratylenchus* spH6 formed separate subclades on the ITS trees, relationships between these species based on the D2-D3 remain uncertain. Thus, heterogeneity of ITS rRNA did not preclude species discrimination. Combined with the PCR-RFLP method, sequence and phylogenetic analysis has become a reliable approach for differentiation of *Pratylenchus* species. More detailed analysis of the ITS sequence alignment (Fig. 1) will allow the design of species specific primers and the discovery of appropriate

restriction enzymes for diagnostics of *P. hippeastri*, *P. floridensis* n. sp., *P parafloridensis* n. sp. and closely related species.

THE *PRATYLENCHUS HIPPEASTRI* SPECIES COMPLEX

Phylogenetic and sequence analysis revealed that a complex of sibling species genetically similar to *P. hippeastri* occurs in Florida, USA, South Africa and Russia. In addition to *P. floridensis* and *P. parafloridensis*, we conclude that eight other populations should be considered as putative undescribed species. However, additional molecular, morphological and biological studies are required to clarify the taxonomic status of these eight populations. It is noteworthy that, based on preliminary morphological ~~study~~studies, these populations were identified not only as *P. hippeastri*, but as several other known species. The diagnostic morphological characters for *P. hippeastri* and the newly described *P. floridensis* n. sp. and *P. parafloridensis* n. sp. overlap to a significant degree, requiring careful examination of many specimens for an accurate diagnosis. Thus, identification of the species of *P. hippeastri*-complex is likely to rely increasingly on molecular methods. Two (N1, N2) of the eight putative species from Florida were described herein as new species because of their regulatory significance. The description of the other six Florida putative *P. hippeastri*-complex species (spH1, H4-H8) is currently not of crucial interest for agronomic or regulatory purposes; however, information provided in this paper documents their existence. The description of the putative *P. hippeastri*-complex species from Russia and South Africa (spH2,H3) requires more detailed morphological information.

Opmerking [lw7]: Same remark as before

The fact that species in the *P. hippeastri*-complex were found in Florida, Russia and South Africa suggests their world-wide distribution and a broad host range among monocots. Moreover, our findings suggest the recent evolution in Florida of numerous lesion nematodes including *P. hippeastri.* These species are maleless (spH5, H7 and H8) or amphimictic (*P. floridensis* n. sp., *P. parafloridensis* n. sp., spH1 and H6). All likely have an undivided face with two and occasionally three lip annuli with the exception of the maleless spH4 which has a divided face with two lip annuli and was identified as *P. scribneri* by Hernández *et al.* (2000). This putative *P. scribneri* in the *P. hippeastri*-complex further complicates the taxonomic status of *P. scribneri*. Many lesion nematodes from turf grasses in Florida including spH8 have been identified as *P. zeae*. The inclusion in the *P. hippeastri*-complex of

another putative *P. zeae* population from South Africa casts doubt about the real identity of *P. zeae* and provides evidence that the reports of *P. zeae* in Florida need to be revaluated.

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Table 1. Pratylenchus *species and populations used in this study.*

Identification based on ITS and D2-D3 rDNA sequences	Preliminary identification based on morphology	Host plant	Locality	Collection codes for DNA or nematode cultures	Genbank accession number for ITS	Genbank accession number for D23 of 28S rDNA	Source of materials or Reference
P. hippeastri	*P. hippeastri*	Amaryllis (*Hippeastrum* sp.)	Tampa, Hillsborough County, Florida, USA	PhippTampa	FJ712932-FJ712936	GU214112-GU214113	Dr. L. Duncan
P. hippeastri	*P. hippeastri*	Bromeliads	Goulds, Dade County, Florida, USA	FloridaPH	N554883-N554887	N554879-N554882	Dr. L. Duncan
P. hippeastri	*P. hippeastri*	Amaryllis (*Hippeastrum* sp.)	Gainesville, Alachua County, Florida, USA	FloridaPh	FN554888;FN554889	DQ498829, DQ498831	Inserra *et al.* (2007)
P .parafloridensis n. sp.	*P. loosi/ Pratylenchus N2*	Maidencane (*Panicum hemitomon*)	Lithia, Hillsborough County, Florida, USA	Ploosi Lithia	GQ988377-GQ988378~~Clones 1, 2~~	AF170438 GU214114-GU214115	Dr. L. Duncan
Pratylenchus floridensis n sp.	*P. loosi/ Pratylenchus N1*	Bahia grass (*Paspalum notatum*)	Zolfo Springs, Hardee County, Florida, USA	PloosiZolfoN1	GQ988375-GQ988376~~Clones 1, 2~~	AF170437 GU214116-GU214117	Dr. L. Duncan
Pratylenchus spH1	*P. hippeastri*	Pop ash (*Fraxinus caroliniana*)	Perry, Taylor county, Florida, USA	CD580	GU131132-GU131135	GU131127-GU131129	Dr. R. Inserra
Pratylenchus spH2	*P. zeae*	Unknown	Upington, South-Africa	PzUping	FJ713012-FJ713016	GU214121-GU214122	Dr. E. Van den Berg
Pratylenchus spH3	*P. subranjani*	Grassland	Russia	PsubMi8	GQ988369-GQ988370~~Clones 1, 2~~	-	Dr. A. Ryss
Pratylenchus spH4	*P. scribneri*	Corn	Florida, USA	PscribFloridaUSA	FJ712997-FJ713001	-	Dr. J. Pinochet
Pratylenchus spH5	*P. hippeastri*	Bottlebrush (*Callistemon rigidus*)	Hastings, ST. John County, Florida, USA	CD544	GU131136, GU131137	Clones 1 2	Dr. R. Inserra
Pratylenchus spH6	*Pratylenchus* sp.	St. Augustine grass (*Stenotaphrum secundatum*)	Arcadia, De Soto County, Florida, USA	CD547, CD548	GU131138-GU131141	GU131123-GU131126	Dr. R. Inserra
Pratylenchus spH7	*P. zeae*	Turf	Florida, USA	PzInserra	GQ988371-GQ988372~~Clones 1, 2~~	GU214123-GU214124	Dr. L. Duncan
Pratylenchus spH8	*P. jordanensis/ P.zeae*	Grassland	La Belle, Hendry County, Florida, USA	PjordInserra	GQ988373-GQ988374~~Clones~~	GU214118-GU214120	Dr. L. Duncan

P. jaehni	*P. jaehni*	Citrus	Sao Paolo, Brasil	Pjaehni	~~1, 2~~ FJ712937-FJ712941	AF170426, AF170427	Dr. L. Duncan

Table 2. *Bromeliad species and cultivars sampled in Florida*[a]

Genus	Cultivar
Guzmania	Confetti, Eloy Intro, Indian night, Irene, Marjan, Optima*, Orange, Ostara
Neoregelia	Passion*, Ardie*, Frank*, Inferno*, Martin*, Tricolor*
Vriesea sp.*	-

[a] Bromeliads marked by an asterisk were infected by root lesion nematodes.

Table 3. *Morphometrics of* Pratylenchus hippeastri *from Florida. All measurements are in* µm *and in the form: mean* ± *standard deviation (range).*

Character	Population from bromeliads (present study)			Population from amaryllis (Inserra *et al*, 2007)
	Female	Female	Male	Female
n	12 (live)	10 (fixed)	5 (fixed)	21 (fixed)
L	527 ± 48.5 (447-616)	614 ± 22.4 (585-651)	421 ± 32.3 (370-452)	590 ± 21.8 (545-627)
a	28.3 ± 1.6 (24.6-30.7)	25.2 ± 2.0 (16.4-23.3)	30.6 ± 3.3 (25.8-33.9)	25.5 ± 1.2 (23.2-27.9)
b	5.8 ± 0.4 (5.2-6.6)	6.6 ± 0.4 (5.9-7.2)	5.5 ± 0.2 (5.3-5.8)	6.5 ± 0.4 (5.7-7.1)
b’	3.7 ± 0.3 (3.3-4.2)	4.5 ± 0.4 (4.0-5.3)	3.5 ± 0.3 (3.2-3.8)	4.4 ± 0.3 (3.9-5.1)
c	17.9 ± 1.2 (15.7-19.9)	18.6 ± 2.0 (16.4-23.3)	18.4 ± 1.4 (16.1-19.3)	16.1 ± 1.0 (14.6-18.7)
c’	2.4 ± 0.2 (2.0-2.6)	2.2 ± 0.2 (1.8-2.5)	2.4 ± 0.1 (2.3-2.5)	2.6 ± 0.2 (22-2.9)
Stylet length	15.4 ± 0.3 (15.1-15.8)	15.8 ± 0.4 (15.3-16.7)	14.3 ± 0.3 (14.0-14.7)	15.5 ± 0.4 (15-16)
DGO from stylet base	3.4 ± 0.3 (3.0-3.8)	2.5 ± 0.3 (2.0-2.7)	2.2 ± 0.4 (1.7-2.7)	2.9 ± 0.2 (2.5-3.0)
o	22.0 ± 1.6 (19.2-25.2)	15.7 ± 1.7 (12.8-17.4)	15.4 ± 3.1 (11.6-19.0)	19 ± 1.2 (16-20)
Anterior end to: centre of metacorpus	58 ± 2.6 (55-64)	62 ± 1.9 (58-65)	50.6 ± 0.7 (50-51)	63 ± 1.9 (59-66)
cardia	89.5 ± 3.0 (85-95)	93.0 ± 5.3 (87-106)	78.5 ± 2.0 (76.7-81.3)	92 ± 3.3 (83-98)
end of pharyngeal gland lobe	139 ± 9.7 (128-153)	137 ± 8.8 (123-147)	126 ± 7.0 (117-132)	134 ± 6.6 (116-145)
secretory/excretory pore	87.6 ± 5.5 (77.4-95)	94 ± 2.9 (89-99)	72.7 ± 4.2 (66-76)	91 ± 2.5 (85-95)
Pharyngeal overlap	49.5 ± 8.0 (38.5-61.5)	44.5 ± 7.6 (33-58)	47.5 ± 8.9 (35-55)	43 ± 5.4 (32-51)
Max. body diam.	18.5 ± 1.5 (15.6-21.5)	24.4 ± 0.7 (23.3-25.7)	13.8 ± 0.8 (12.7-14.5)	23.2 ± 1.4 (21-27)
Vulval body diam.	17.3 ± 1.5 (14.2-20.5)	21.6 ± 1.6 (19.3-24)	–	20.5 ± 1.1 (18.0-23.0)
Anal body diam.	12.1 ± 1.2 (10.7-14.7)	15.3 ± 0.4 (14.7-16)	9.5 ± 0.3 (9.3-10)	14.4 ± 0.8 (13-16)
Vulva to anus distance	86.4 ± 10.9 (70.5-103)	103 ± 5.4 (92-109)	–	98 ± 6.1 (88-112)
V or T	77.6 ± 1.3 (75.6-79.6)	77.7 ± 1.2 (75.7-79.4)	46.0 ± 2.8 (42.5-49.0)	77 ± 0.8 (75-78)
Anterior genital tract length	132.7 ± 60.3 (108-170)	268 ± 60.3 (200-387)	194 ± 20.4 (165-220)	254 ± 47.2 (181-360)
PUS	23.2 ± 3.2 (18.6-29.4)	34.5 ± 3.0 (30-39.3)	–	30 ± 4.9 (21-45)
Tail length	29.5 ± 2.5 (27.2-35.7)	33.3 ± 3.0 (28-37.3)	22.9 ± 0.6 (22.0-23.7)	36.8 ± 2.2 (32.0-42.0)
Spicule length	–	–	18.6 ± 0.6 (18.0-19.3)	–
Gubernaculum length	–	–	5.3 ± 0.6 (4.7-6.0)	–
No. of tail annuli	24 ± 1.9 (21-26)	20 ± 2.6 (17-25)	–	22 ± 2.1 (19-26)

Table 4. *Comparison of selected morphometrics of* Pratylenchus floridensis *n. sp. (Zolfo Springs population) and* P. parafloridensis *n. sp. (Lithia population).*

Characters	*P. floridensis* n. sp.		*P. parafloridensis* n. sp.	
	Females	Male	Females	Males
n	9	1	10	5
L	450 ± 31.5 (387-507)*	457	532 ± 41.6 (475-603)§	448 ± 37.9 (414-494)
Stylet length	15.0 ± 0.6 (14.0-15.8)	22	15.4 ± 0.6 (14.5-16.0)	13.7 ± 0.3 (13.5-14.0)
V or T (%)	78 ± 1.1 (77-80)*	52	77 ± 1.2 (75-79)§	46 ± 7.9 (35-52)
Anterior end to center of metacorpus	54 ± 4.1 (48-59)	53	59 ± 3.6 (52-64)	54 ± 4.3 (51-61)
end of pharyngeal gland lobe	118 ± 10.5 (104-140)	113	134 ± 6.8 (127-145)	128 ± 15 (118-145)
secretory/excretory pore	78 ± 6.6 (66-86)	75	88 ± 5.3 (81-96)	76 ± 3.6 (72-81)
Spermatheca (longitudinal diam.)	17 ± 3.9 (13-24.5)	–	24 ± 8.0 (15-38)	–
Spermatheca (cross diam.)	11.5 ± 1.4 (9.5-13)	–	12.6 ± 2.2 (9.5-16)	–
P.U.S.	27 ± 3.5 (23-32)	–	28 ± 5.2 (21-37)	–
Tail length	27.6 ± 1.4 (25-29)	31.3	32.1 ± 2.7 (28-35)	24.9 ± 5.4 (22-33)
Anal body diam.	10.9 ± 1.2 (9.0-12.0)	10.7	11.2 ± 0.7 (10.5-12.5)	8.8 ± 1.7 (6.7-10.7)
Spicules	–	19	–	18.5 ± 0.6 (17.8-19)
Gubernaculum	–	6	–	5.3 ± 0 (5.3)
a	26.4 ± 1.8 (20.7-30.1)	25.4	29 ± 3.3 (25.2-37)	29.7 ± 3.8 (25 -35.3)
b	5.6 ± 0.3 (5.3-5.9)	6.1	5.9 ± 0.3 (5.3-6.3)	5.6 ± 0.4 (5.3-6.0)
b’	3.9 ± 0.3 (3.4-4.3)	4.1	4.0 ± 0.4 (3.5-4.6)	3.7 ± 0.4 (3.4-4.0)
c	16.8 ± 0.8 (15.3-17.8)	14.6	16.8 ± 1.4 (14.9-18.5)	17.9 ± 2.0 (15-19.1)
c’	2.6 ± 0.3 (2.2-3.1)	2.9	2.9 ± 0.3 (2.6-3.3)	2.8 ± 0.4 (2.4-3.3)

* Measurements taken on 18 specimens.

§ Measurements taken on 19 specimens.

Figure Legends

Fig. 1. *Sequence alignment of partial 18S, complete ITS1, 5.8S , ITS2 and partial 28S rRNA for the P. hippeastri species complex. The 18S, 5.8S and 28S rRNA gene sequences are marked in bold, the primers sequences are underline. Consensus sequence is given for each species only. Single letter code recommended by NC-IUB was used to specify nucleotide, if two or more bases were permitted at a particular position in a species subalignment. Lower case symbols indicate presence of one or several gaps in a particular position in sequences for a species subalignment.*

Fig. 2. *The 50% majority rule consensus tree from Bayesian analysis generated from the ITS sequence dataset for the* P. hippeastri *species complex using the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades.*

Fig. 3. *The 50% majority rule consensus tree from Bayesian analysis generated from the D2-D3 of 28S rRNA gene sequence dataset for the* P. hippeastri *species complex using the GTR + I + G model. Posterior probability is given for appropriate clades.*

Fig. 4. *Morphometric relationships among 20* Pratylenchus *species studied by Duncan et al. (1999), and* P. hippeastri *from bromiliads (XXX) and amaryllis (YYY). Note the similarity of these populations to those of* P. floridensis *n. sp. (N1) and* P. parafloridensis *n. sp. (N2).*

Fig. 5. Camera lucida *line drawings of male of* Pratylenchus hippeastri. *A: Entire body; B:* En face *view showing the oral disc fused with median and lateral lip sectors; C: Pharyngeal region; D: Anterior end; E, F: Tail region.*

Fig. 6. *Light micrographs of male of* Pratylenchus hippeastri. *A: Entire body; B: Pharyngeal region (live specimen); C: Anterior end; D: Lateral field at mid-body; E-G: Tail region at different foci. (Scale bars: A = 50 μm; B-G = 20 μm).*

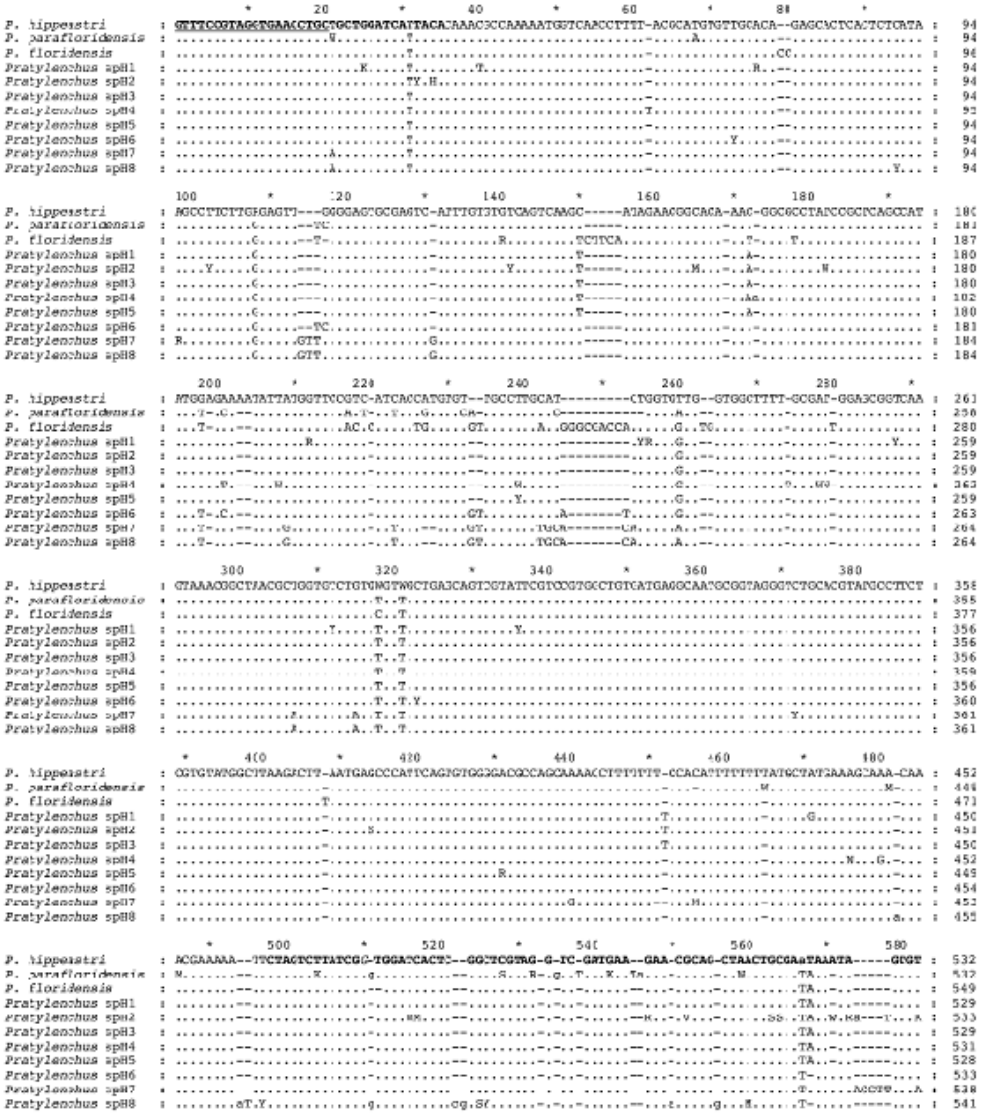
Fig. 7. *SEM morphology of* Pratylenchus hippeastri *from bromeliads in Florida: A, B: Female; C, D: Male. A, B: Undivided face pattern with all labial sectors fused together and with the oral disc. Note the second lip annulus thicker than the first; C, D: Undivided face pattern similar to that of the female, but with broader amphidial apertures.*

Fig. 8. Camera lucida *line drawings of* Pratylenchus floridensis *n. sp. (A-E) and* P. parafloridensis *n. sp. (F-Y). A, N: Female pharyngeal region; B, O: Male pharyngeal region; C, P: Female posterior region; D, Q: Female anterior end; E, M: Female entire body; F, R: Female vulval region with spermatheca; G, S: Male tail; H-L, T-Y: Female tail.*

Fig. 9. *Light micrographs of* Pratylenchus floridensis *n. sp. (A-D) and* P. parafloridensis *n. sp. (E-H). A, E: Female pharyngeal region; B, F: Female anterior end; C, G: Female vulval region (brackets indicate the position and the extent of the spermatheca); D, H: Female posterior body portion (spermatheca in brackets).*

Fig. 10. *Comparative light micrographs of tail region of* Pratylenchus floridensis *n. sp. and* P. parafloridensis *n. sp. (the broken line shows the anus level).*

Fig. 11. *Comparison of SEM head morphology of* Pratylenchus floridensis *n. sp. (A-C) and* P. parafloridensis *n. sp. (D-F). A, B: Female face view showing undivided patterns with all labial sectors fused together and partially with the oval oral disc. C: Male face pattern. D-F: Female undivided face patterns with all labial sectors fused together and partially with the round oral disc. Note the second lip annulus larger and thicker than the first in both species.*



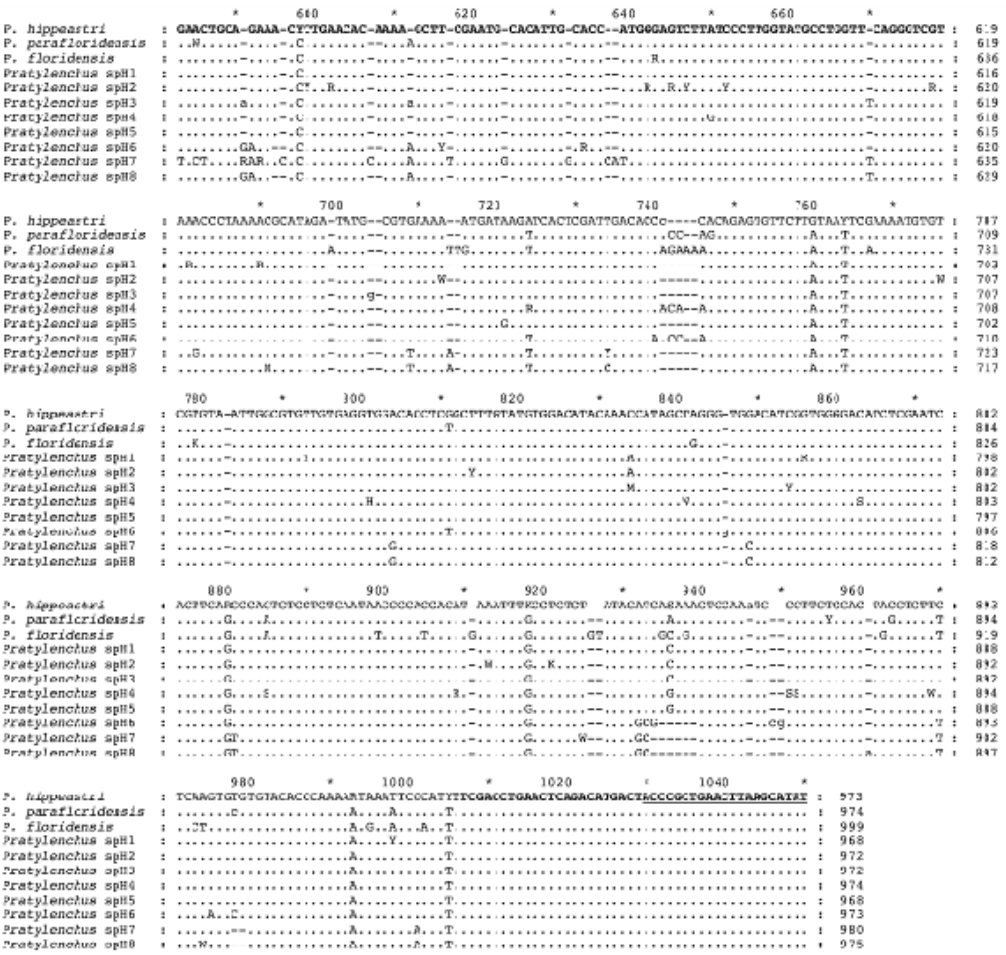
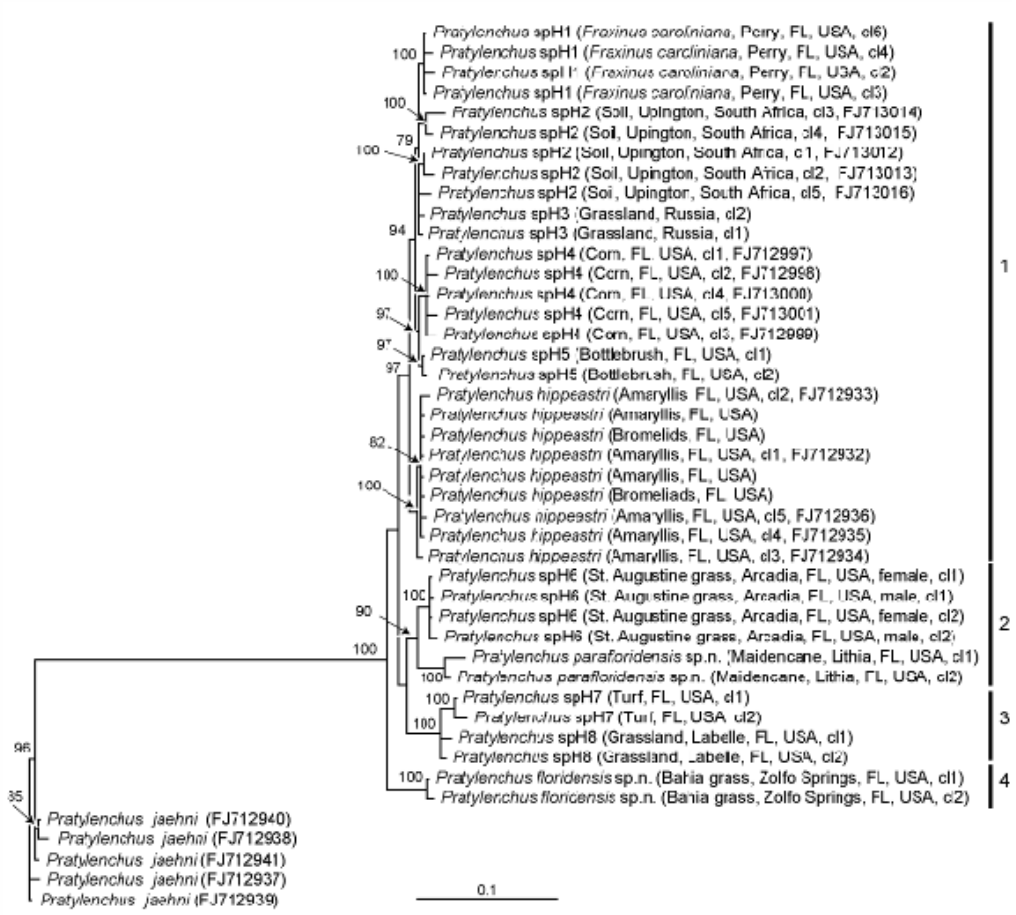


Fig. 1.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Pratylenchus spH1 (Fraxinus caroliniana, Perry, FL, USA, cl4)
Pratylenchus spH1 (Fraxinus caroliniana, Perry, FL, USA, cl2)
Pratylenchus spH1 (Fraxinus caroliniana, Perry, FL, USA, cl3)
Pratylenchus spH2 (Soil, Upington, South Africa, cl3, FJ713014)
Pratylenchus spH2 (Soil, Upington, South Africa, cl4, FJ713015)
Pratylenchus spH2 (Soil, Upington, South Africa, cl1, FJ713012)
Pratylenchus spH2 (Soil, Upington, South Africa, cl2, FJ713013)
Pratylenchus spH2 (Soil, Upington, South Africa, cl5, FJ713016)
Pratylenchus spH3 (Grassland, Russia, cl2)
Pratylenchus spH3 (Grassland, Russia, cl1)
Pratylenchus spH4 (Corn, FL, USA, cl1, FJ712997)
Pratylenchus spH4 (Corn, FL, USA, cl2, FJ712998)
Pratylenchus spH4 (Corn, FL, USA, cl4, FJ713000)
Pratylenchus spH4 (Corn, FL, USA, cl5, FJ713001)
Pratylenchus spH4 (Corn, FL, USA, cl3, FJ712999)
Pratylenchus spH5 (Bottlebrush, FL, USA, cl1)
Pratylenchus spH5 (Bottlebrush, FL, USA, cl2)
Pratylenchus hippeastri (Amaryllis, FL, USA, cl2, FJ712933)
Pratylenchus hippeastri (Amaryllis, FL, USA)
Pratylenchus hippeastri (Bromelids, FL, USA)
Pratylenchus hippeastri (Amaryllis, FL, USA, cl1, FJ712932)
Pratylenchus hippeastri (Amaryllis, FL, USA)
Pratylenchus hippeastri (Bromeliads, FL, USA)
Pratylenchus hippeastri (Amaryllis, FL, USA, cl5, FJ712936)
Pratylenchus hippeastri (Amaryllis, FL, USA, cl4, FJ712935)
Pratylenchus hippeastri (Amaryllis, FL, USA, cl3, FJ712934)
Pratylenchus spH6 (St. Augustine grass, Arcadia, FL, USA, female, cl1)
Pratylenchus spH6 (St. Augustine grass, Arcadia, FL, USA, male, cl1)
Pratylenchus spH6 (St. Augustine grass, Arcadia, FL, USA, female, cl2)
Pratylenchus spH6 (St. Augustine grass, Arcadia, FL, USA, male, cl2)
Pratylenchus paraforidensis sp.n. (Maidencane, Lithia, FL, USA, cl1)
Pratylenchus paraforidensis sp.n. (Maidencane, Lithia, FL, USA, cl2)
Pratylenchus spH7 (Turf, FL, USA, cl1)
Pratylenchus spH7 (Turf, FL, USA, cl2)
Pratylenchus spH8 (Grassland, Labelle, FL, USA, cl1)
Pratylenchus spH8 (Grassland, Labelle, FL, USA, cl2)
Pratylenchus floridensis sp.n. (Bahia grass, Zolfo Springs, FL, USA, cl1)
Pratylenchus floridensis sp.n. (Bahia grass, Zolfo Springs, FL, USA, cl2)
Pratylenchus jaehni (FJ712940)
Pratylenchus jaehni (FJ712938)
Pratylenchus jaehni (FJ712941)
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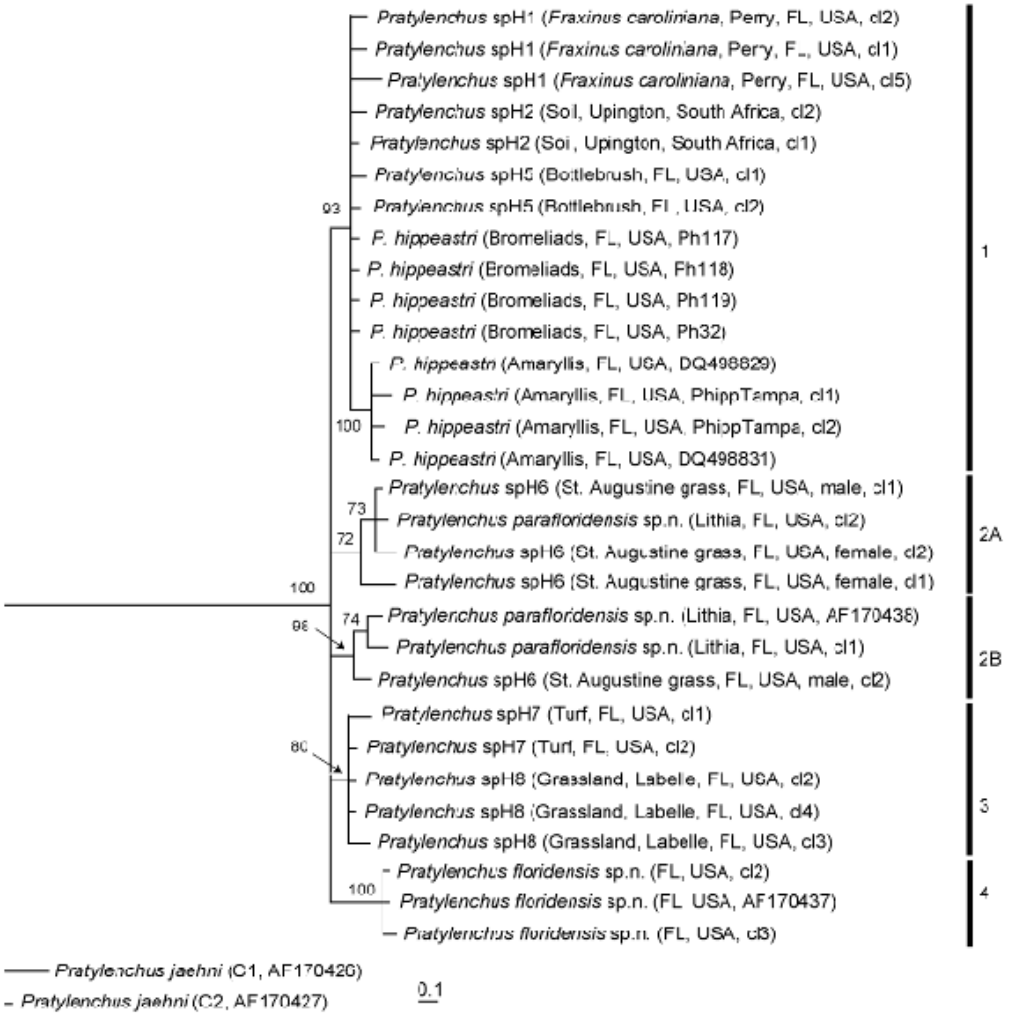
2
3
4
0.1

Fig. 2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Pratylenchus spH2 (Soil, Upington, South Africa, cl2)
Pratylenchus spH2 (Soil, Upington, South Africa, cl1)
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P. hippeastri (Amaryllis, FL, USA, PhippTampa, cl2)
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Pratylenchus parafloridensis sp.n. (Lithia, FL, USA, cl2)
Pratylenchus spH6 (St. Augustine grass, FL, USA, female, cl2)
Pratylenchus spH6 (St. Augustine grass, FL, USA, female, cl1)
Pratylenchus parafloridensis sp.n. (Lithia, FL, USA, AF170438)
Pratylenchus parafloridensis sp.n. (Lithia, FL, USA, cl1)
Pratylenchus spH6 (St. Augustine grass, FL, USA, male, cl2)
Pratylenchus spH7 (Turf, FL, USA, cl1)
Pratylenchus spH7 (Turf, FL, USA, cl2)
Pratylenchus spH8 (Grassland, Labelle, FL, USA, cl2)
Pratylenchus spH8 (Grassland, Labelle, FL, USA, cl4)
Pratylenchus spH8 (Grassland, Labelle, FL, USA, cl3)
Pratylenchus floridensis sp.n. (FL, USA, cl2)
Pratylenchus floridensis sp.n. (FL, USA, AF170437)
Pratylenchus floridensis sp.n. (FL, USA, cl3)
93
100
73
72
100
98
74
80
100
1
2A
2B
3
4
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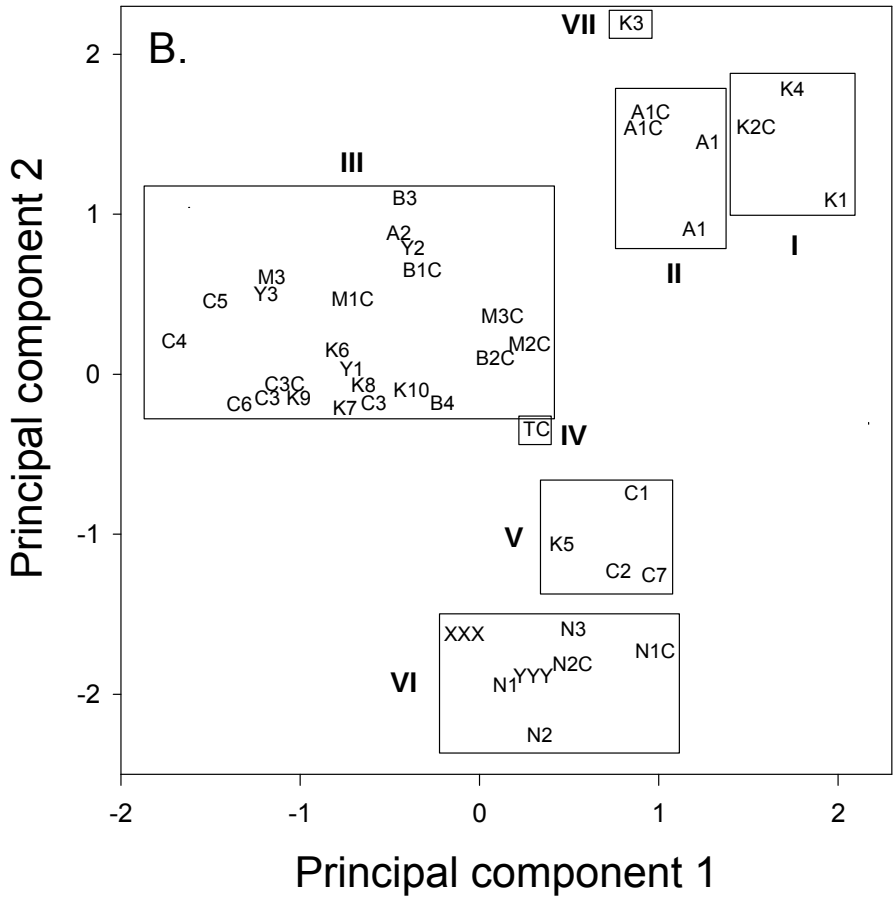
Pratylenchus jaehni (C2, AF170427)
0.1

Fig. 3


B.
VII
K3
I
K4
K2C
K1
A1C
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A1
A1
II
III
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Y2
B1C
M3
Y3
C5
M1C
M3C
C4
K6
M2C
B2C
Y1
C3C
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Principal component 2
Principal component 1

Fig 4

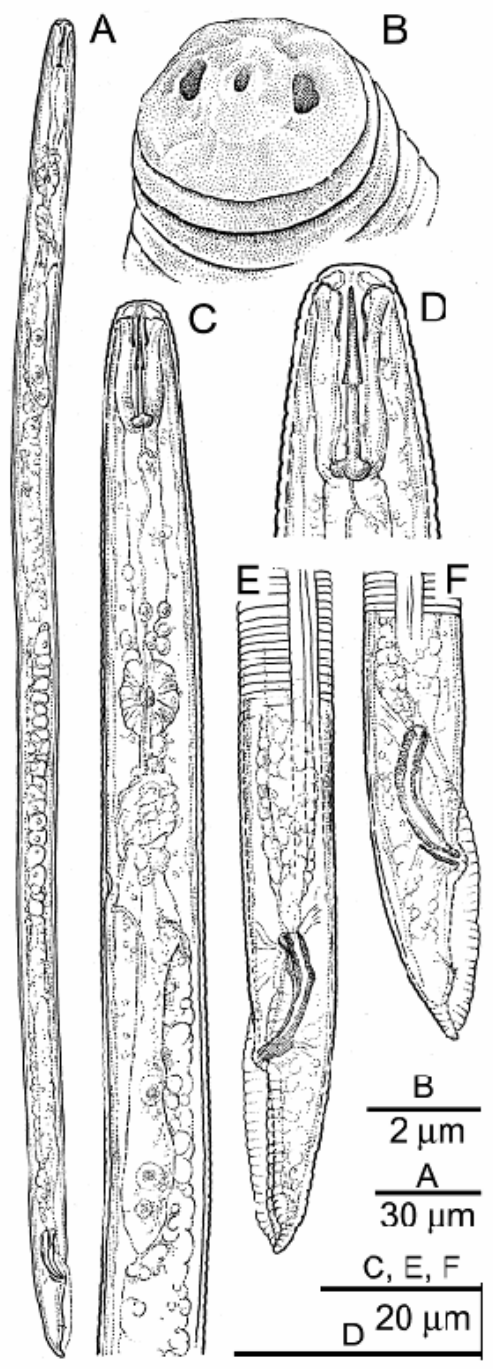

Fig. 5

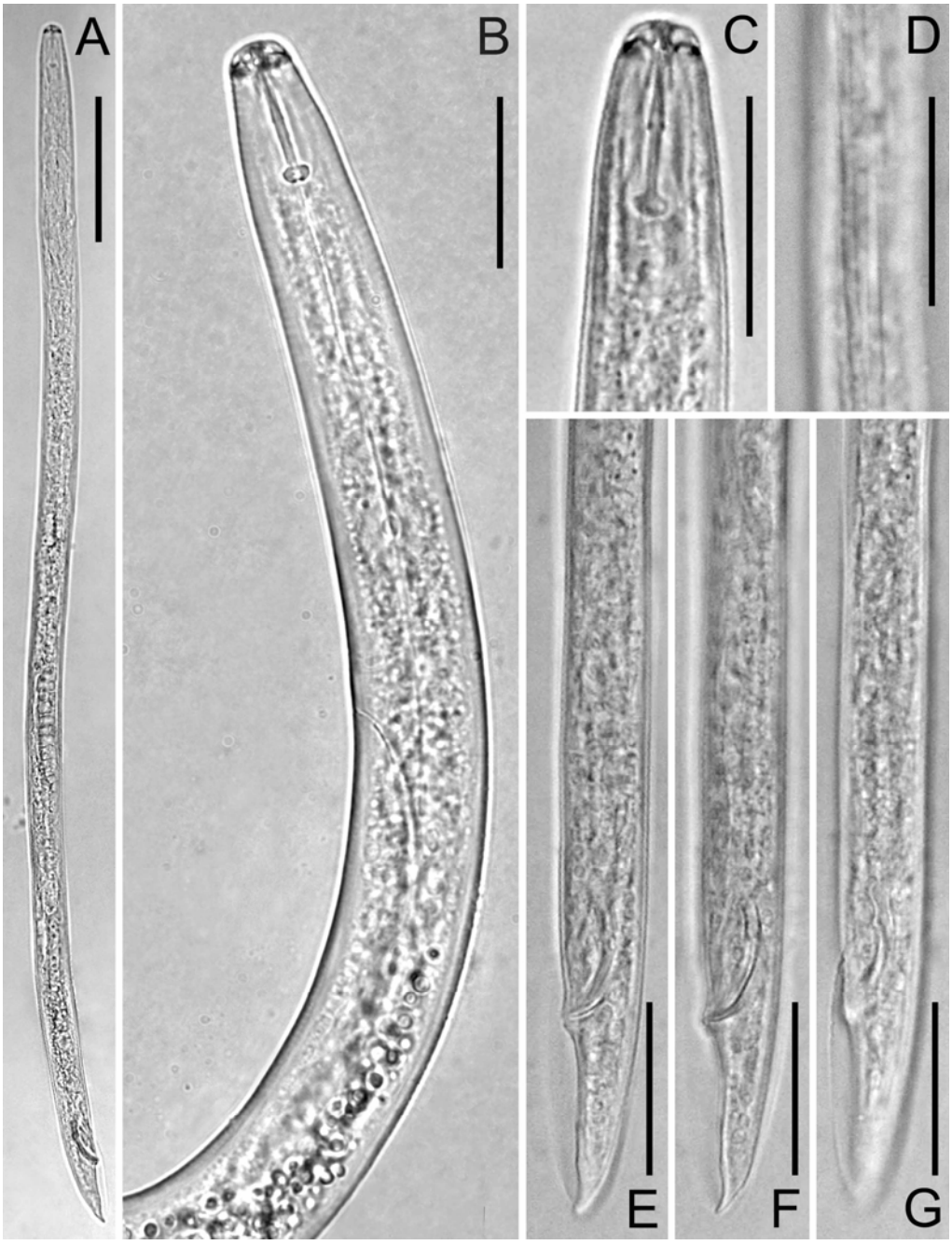

Fig. 6

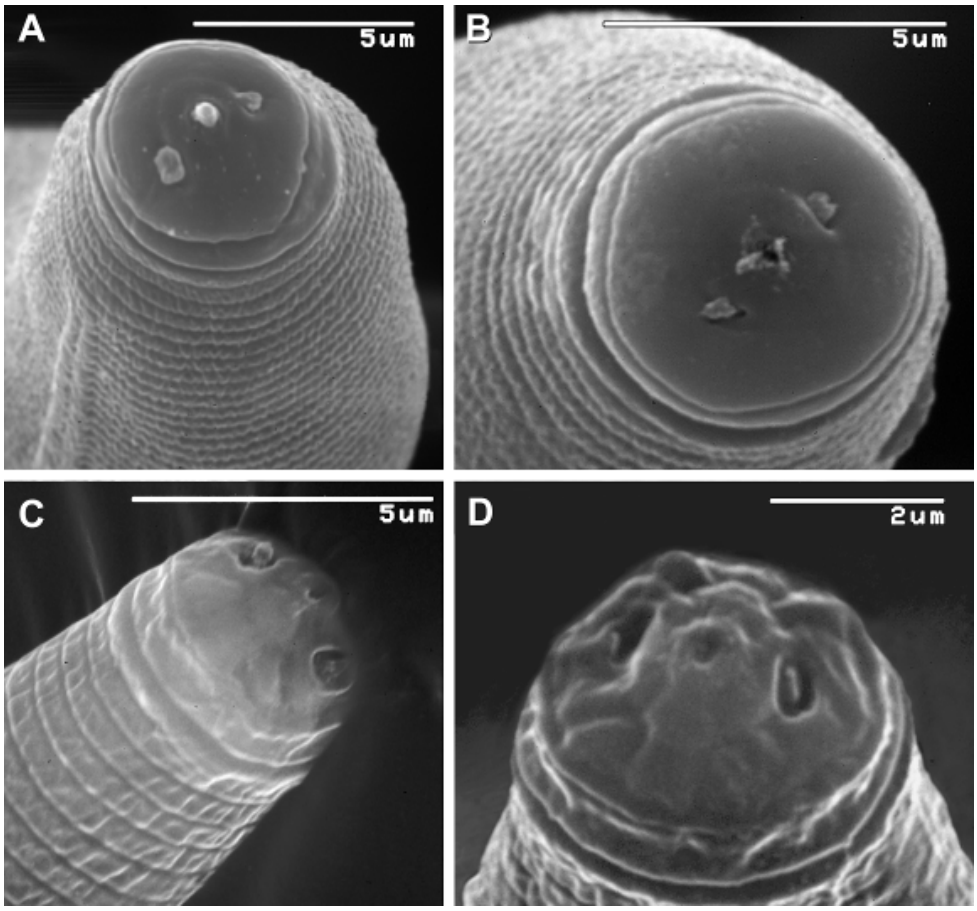

Fig. 7

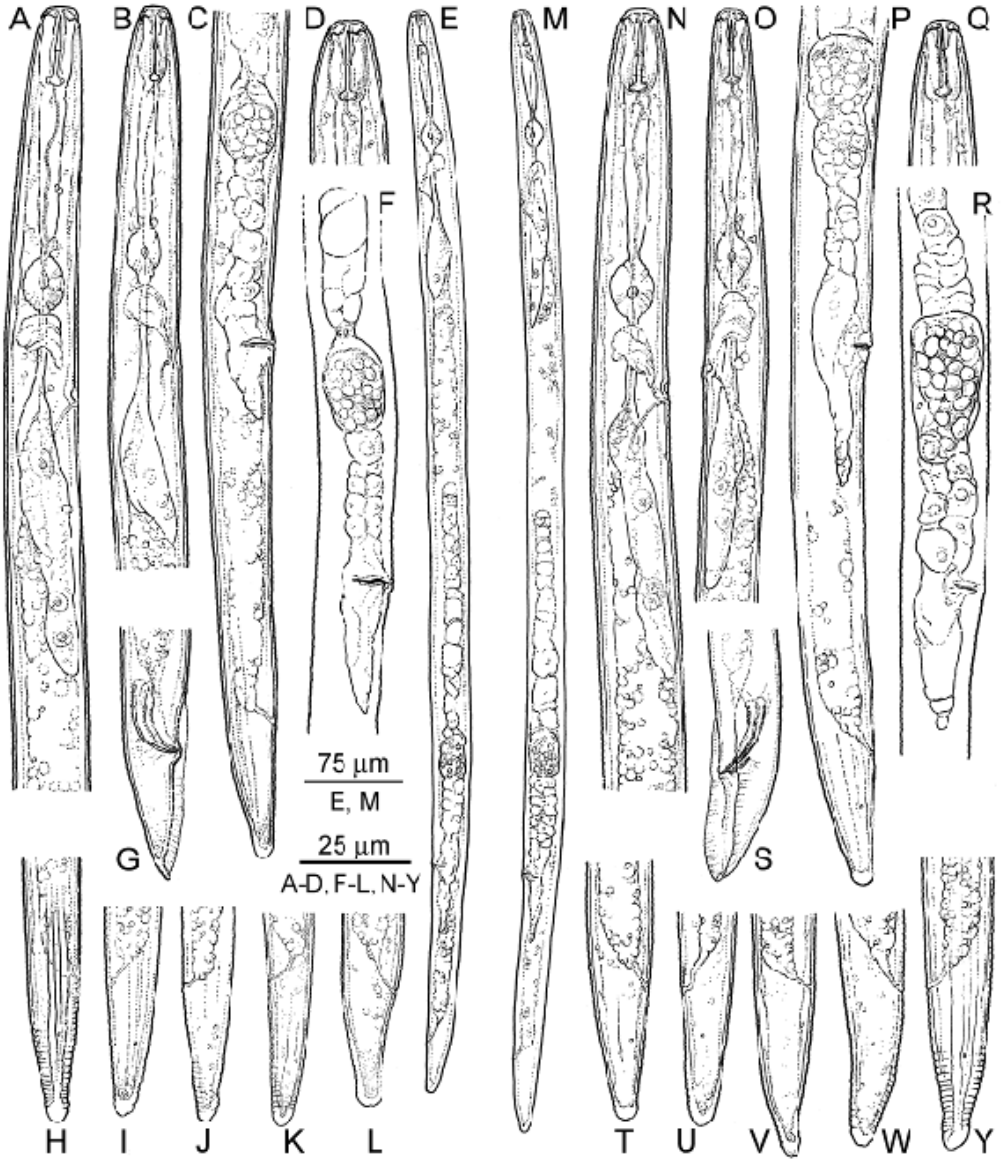

Fig. 8

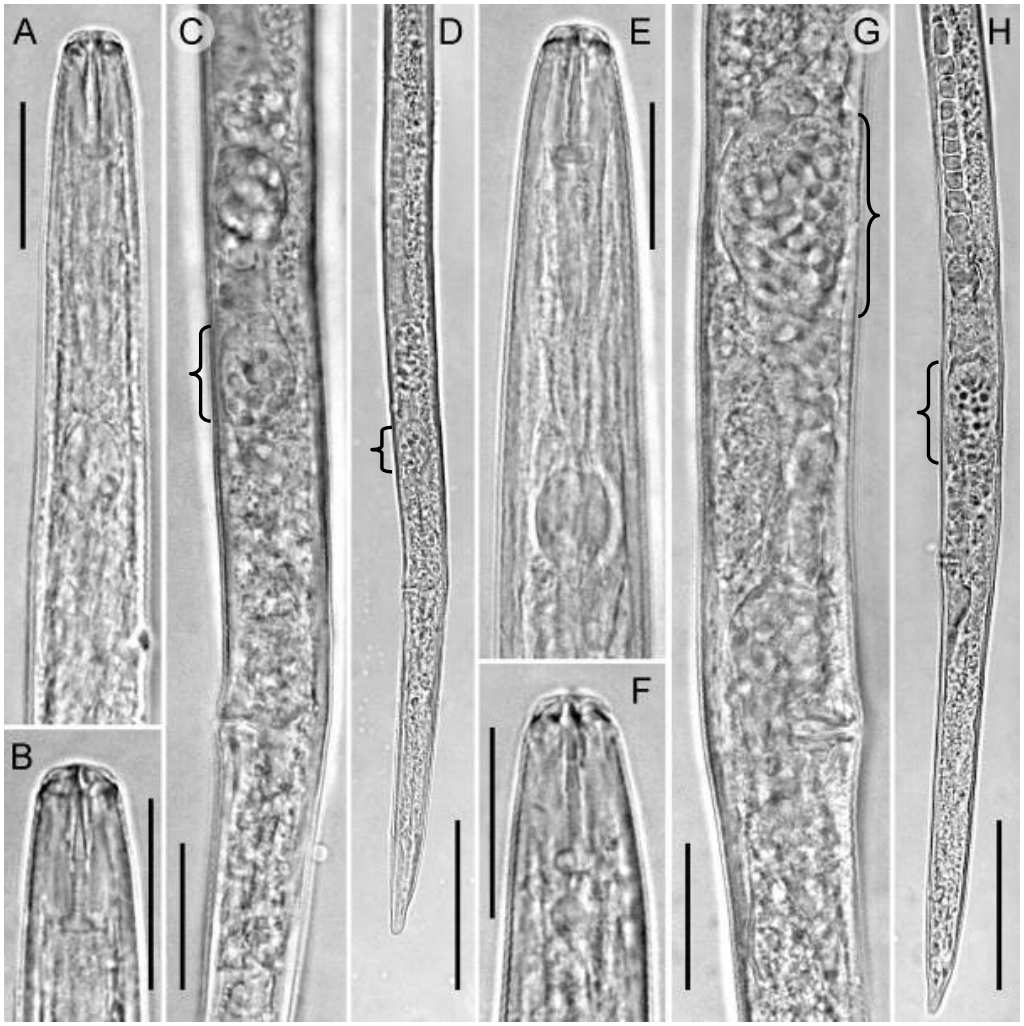

Fig. 9

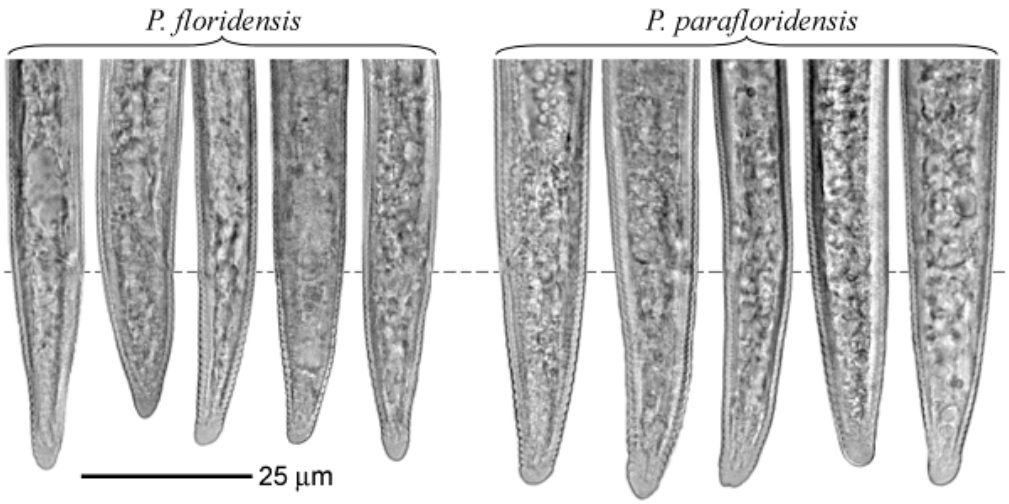

Fig. 10

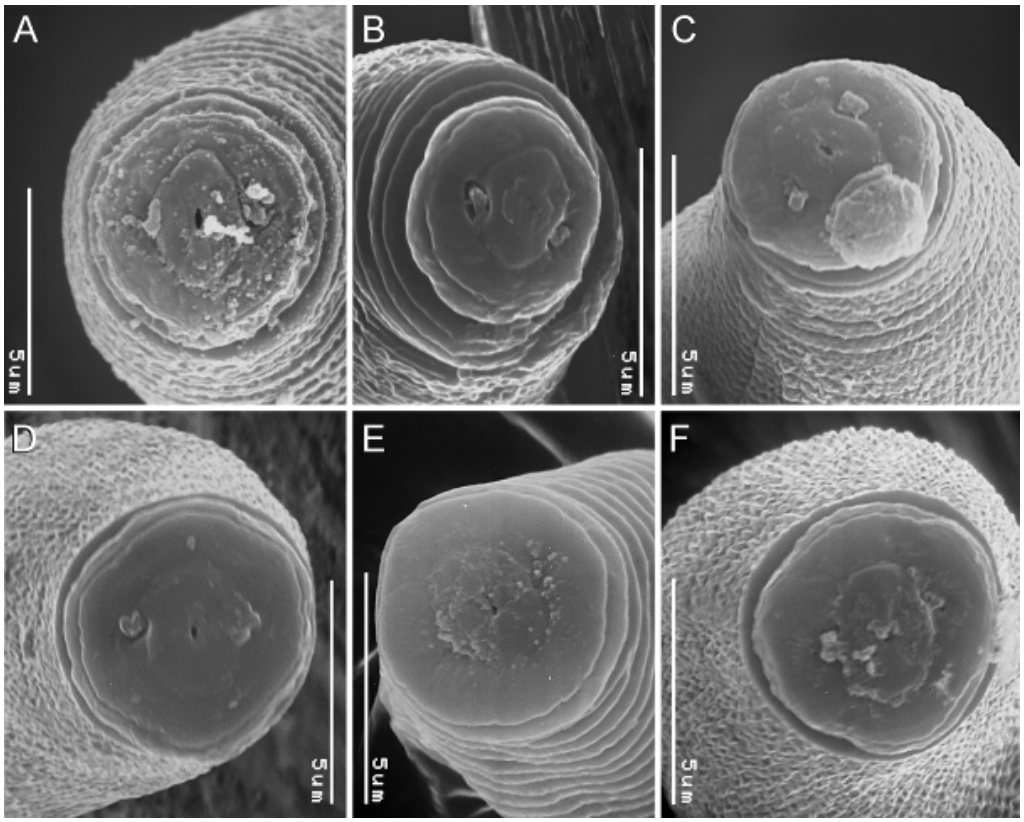

Fig. 11