Molecular and morphological characterisation of a new root-lesion nematode, Pratylenchus horti n. sp. (Tylenchomorpha: Pratylenchidae), from Ghent University Botanical Garden

Article *in* Nematology · March 2019

DOI: 10.1163/15685411-00003249

CITATIONS
13

READS
701

6 authors, including:

Tien Nguyen
The Institute Of Ecology and Biological Resources
40 PUBLICATIONS **107** CITATIONS
SEE PROFILE

Phap Trinh
The Institute Of Ecology and Biological Resources
72 PUBLICATIONS **369** CITATIONS
SEE PROFILE

Marjolein Coureur
Ghent University
49 PUBLICATIONS **492** CITATIONS
SEE PROFILE

Rolish Singh
University of Florida
17 PUBLICATIONS **90** CITATIONS
SEE PROFILE

BRILL

Nematology 21 (2019) 739-752

Nematology
brill.com/nemy

Molecular and morphological characterisation of a new root-lesion nematode, *Pratylenchus horti* n. sp. (Tylenchomorpha: Pratylenchidae), from Ghent University Botanical Garden

Huu Tien NGUYEN [1,2], Quang Phap TRINH [1,*], Marjolein COUVREUR [2], Phougeishangbam Rolish SINGH [2], Wilfrida DECRAEMER [2] and Wim BERT [2]

[1] *Institute of Ecology and Biological Resource, Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Cau giay, Hanoi, Vietnam*

[2] *Nematology Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium*

Received: 17 December 2018; revised: 31 January 2019
Accepted for publication: 31 January 2019; available online: 20 March 2019

Summary – Root-lesion nematodes, *Pratylenchus* spp., are one of the most important nematode groups in economic terms. The combination of morphological analyses and molecular analyses based on D2-D3 of 28S rDNA, ITS rDNA, and *COI* mtDNA regions supported the establishment of a new *Pratylenchus* species, making a total of 103 valid *Pratylenchus* species. The females of *P. horti* n. sp. are characterised by the following traits: low labial region with two annuli continuous to the body, *en face* form belonging to group II *sensu* Corbett & Clark (1983) with submedian triangular-shaped segments fused with the oral disc and separated from the lateral segments, lateral field with four incisures at vulval level and lacking areolation, robust stylet 15-17 μm long with rounded knobs, and subcylindrical tail with smooth tail tip. The males are largely similar to the females but differ from the females by the partially areolated lateral field, slightly ventrally arcuate and weakly cephalated spicules (15-19 μm), and ventrally curved elongate conical tail with a poorly protruding, crenate bursa. The new species was recovered from soil and root samples from the rhizosphere of *Hedychium greenii* growing in the Botanical Garden, Ghent University, Belgium.

Keywords – 28S, Belgium, cluster analysis, *COI*, D2-D3, exotic plant, Ginger lily, *Hedychium greenii*, ITS, morphology, morphometrics, mtDNA, new species, plant-parasitic nematode, rDNA, taxonomy, web-based key.

Pratylenchus spp. or root-lesion nematodes are one of the most important nematode groups in economic terms. Plants infected by these migratory endoparasites can display symptoms such as a reduction in root growth, formation of lesions, necrotic areas, browning, and cell death. Such damage creates favourable conditions for secondary attack by other pathogens such as soil fungi or bacteria (Jones *et al*., 2013).

Pratylenchus species are distributed worldwide in Europe, Africa, Asia, North America, South and Central America to Oceania, and even Antarctica. Moreover, they are polyphagous plant-parasitic nematodes with a very wide host range, from monocotyledon to dicotyledon plants (Castillo & Vovlas, 2007). Singh *et al*. (2018) recognised 101 valid species of *Pratylenchus*, including *P. rwandae* Singh, Nyiragatare, Janssen, Couvreur, Decraemer & Bert, 2018. However, the number of *Pratylenchus* spp. in Geraert (2013) utilised by Singh *et al*. (2018) should be 99 because *P. jaehni* Inserra, Duncan, Troccoli, Dunn, dos Santos, Kaplan & Vovlas, 2001 was missing in the list of species in his book. Therefore, the total number of valid species of *Pratylenchus* at that time should have been 102 (Geraert, 2013; Hodda *et al*., 2014; Palomares-Rius *et al*., 2014; Wang *et al*., 2015; Nguyen *et al*., 2017; Singh *et al*., 2018).

The diversity of root-lesion nematodes in Belgium is relatively well investigated with 14 reported species, namely: *P. brzeskii* Karssen, Waeyenberge & Moens, 2000, *P. convallariae* Seinhorst, 1959, *P. crenatus* Loof, 1960, *P. dellatrei* Luc, 1958, *P. dunensis* de la Peña, van Aelst, Moens & Karssen, 2006, *P. fallax* Seinhorst, 1968, *P. flakkensis* Seinhorst, 1968, *P. goodeyi* Sher & Allen, 1953, *P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *P. penetrans* (Cobb, 1917) Filipjev

* Corresponding author, e-mail: tqphap@gmail.com

DOI 10.1163/15685411-00003249

& Schuurmans Stekhoven, 1941, *P. pratensis* (de Man, 1880) Filipjev, 1936, *P. pseudopratensis* Seinhorst, 1968, *P. thornei* Sher & Allen, 1953, and *P. vulnus* Allen & Jensen, 1951 (Steel *et al*., 2014; Janssen *et al*., 2017a).

Identifying *Pratylenchus* spp. is a difficult task due to the great number of valid species and their intraspecific variation. Castillo & Vovlas (2007) developed a very useful tabular identification key for *Pratylenchus* species based on 11 main morphological characteristics. Currently, 18S rDNA, ITS rDNA, D2-D3 of 28S rDNA, and *COI* mtDNA regions are being used extensively as molecular markers to identify to species level and to allow the detection of cryptic species throughout plant-parasitic nematode groups (Blaxter *et al*., 1998; Subbotin *et al*., 1999, 2003, 2007; Subbotin & Moens, 2006; Holterman *et al*., 2009; Janssen *et al*., 2017b). According to Subbotin *et al*. (2008), D2-D3 of 28S rDNA seems to be a better target than partial 18S rDNA for identification at species level in *Pratylenchus*. Therefore, the markers of D2-D3 of 28S rDNA, ITS rDNA, and *COI* mtDNA regions are excellent tools for studying *Pratylenchus* species. However, Janssen *et al*. (2017b) discussed the pitfalls of a molecular-only approach in identifying *Pratylenchus*, including the presence of misassembled, mislabelled, unlabelled or misidentified sequences in GenBank. Consequently, it is both necessary and desirable to include both morphological and molecular approaches when characterising and identifying *Pratylenchus* species.

This aim of this paper was to describe, based on the combination of morphological and molecular analyses, a new *Pratylenchus* species from Belgium associated with an exotic plant.

Materials and methods

Sampling and nematode extraction

After the removal of the detritus layer, the soil and root samples were collected from the upper 30-cm soil layer in the rhizosphere of *Hedychium greenii* W.W.Sm. (Ginger lily) at the Botanical Garden of Ghent University, Belgium (GPS coordinates 51°2′6.7″N, 3°43′22.4″E). The nematodes were extracted from soil and roots by the modified Baermann tray method (Whitehead & Hemming, 1965).

Morphological characterisation

Nematodes were fixed in Trump's fixative (2% paraformaldehyde + 2.5% glutaraldehyde in a 0.1 M Sorenson buffer (sodium phosphate buffer, pH 7.3)) and transferred to anhydrous glycerin to make permanent slides following the method described by Singh *et al*. (2018). Microphotographs and drawings were made from permanent slides using an Olympus BX51 DIC Microscope equipped with a digital camera and a drawing tube. The measurements were calculated based on the obtained pictures using ImageJ 1.51.

Illustrator® CS 3 was used to make the illustrations based on pencil drawings and scanning electron microscopy (SEM) pictures. For SEM, nematodes in Trump's fixative were subsequently washed three times in 0.1 M Sorenson buffer and twice in double-distilled water (10 min each). In the next step, they were dehydrated by passing through a graded ethanol concentration series of 30, 50, 75, 95% and 3 × 100% (20 min each). In the last step, the specimens were critical point-dried with liquid CO_2, mounted on stubs with carbon tabs and coated with gold (25 nm, JEOL 1200jfc) before observation with a JSM-840 EM (JEOL) at 12 kV.

Molecular characterisation

The living nematodes were used to make temporary slides (one specimen per slide) for taking digital light microscope pictures as morphological vouchers. In the next step, the single nematode was taken out of the temporary slide, washed with distilled water for 10 min, cut into 2-3 pieces and put together into an Eppendorf tube with 20 μl of WLB (50 mM KCl;10 mM Tris pH 8.3; 2.5 mM $MgCl_2$; 0.45% NP-40 (Tergitol Sigma); 0.45% Tween-20). Subsequently, the samples were incubated at −20°C for at least 10 min, followed by adding 1 μl proteinase K (1.2 mg ml^{-1}) before incubation in a PCR machine for 1 h at 65°C and 10 min at 95°C and centrifugation for 1 min at 20 800 *g*. Finally, the samples were stored at −20°C before running PCR (Singh *et al*., 2018).

The primers DP391/501 were used to amplify the 5′-end of the D2-D3 of 28S rDNA region (Nadler *et al*., 2006) with the PCR reaction started at 94°C for 4 min, followed by 5 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 2 min. This step was followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min and finished at 12°C for 10 min. For amplifying ITS rDNA region, the primers Vrain2F/Vrain2R were used (Nguyen *et al*., 2017) with the PCR reaction started at 94°C for 4 min, followed by 50 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 2 min. The cytochrome c oxidase subunit 1 (*COI*) gene fragment was amplified using the

primers JB3/JB4 following the protocol of Derycke *et al*. (2010). The PCR reactions were checked by gel electrophoresis. After that, the successful PCR reactions were purified and sequenced commercially by Macrogen Inc. (Europe).

The consensus sequences were obtained by assembling forward and backward sequences using Geneious R11 (www.geneious.com). The BLAST search was used to check for closely related sequences of other species on GenBank (Altschul *et al*., 1997). *Meloidogyne enterolobii* Yang & Eisenback., 1983 (accession no. KX823403) and *M. ichinohei* Araki, 1992 (accession no. EF029862) were chosen as out-groups for D2-D3 of 28S rDNA sequences, *M. mali* Itoh, Ohshima & Ichinohe, 1969 (accession no. KR535971) and *M. africana* Whitehead, 1960 (accession no. KY433429) were chosen as out-groups for ITS rDNA sequences, and *M. haplanaria* Eisenback, Bernard, Starr, Lee & Tomaszewski, 2004 (accession no. KU174206) and *M. enterolobii* (accession no. KT936633) were chosen as out-groups for *COI* mtDNA sequences. Multiple alignments were made from selected sequences by using MUSCLE in MEGA 7 (Barry, 2011). The poorly aligned regions of the alignments were eliminated using Gblocks v0.91b (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks) (Castresana, 2000; Dereeper *et al*., 2008). The best fit models were selected by using MEGA7 based on BIC criterion (Barry, 2011). HKY + G model was chosen for all the datasets. The phylogenetic trees were created by using MrBayes 3.2.6 Add-in in Geneious R11. The Markov chains were set with 1×10^6 generations, four runs, 20% burn-in, and subsampling frequency of 500 generations (Huelsenbeck & Ronquist, 2001).

Cluster analysis and web-based key

The Hierarchical Cluster analysis in Primer 6 was used to cluster the 102 valid species and *P. horti* n. sp. into small groups of similar specimens. This analysis is based on the Bray-Curtis similarity measure, the percent similarity between species being defined by the average of multiple characters. Eleven main characters were analysed according to Castillo & Vovlas (2007): A) Lip annuli: 1: two, 2: three, 3: four; B) Male: 1: absent, 2: present; C) Stylet length: 1: stylet <13 μm, 2: stylet 13-15.9 μm, 3: stylet 16-17.9 μm, 4: stylet 18-20 μm, 5: stylet >20 μm; D) Shape of spermatheca: 1: absent or reduced, 2: rounded to spherical, 3: oval, 4: rectangular; E) Vulva position, ratio V: 1: V $\leqslant$ 75, 2: V = 75-79.9, 3: V = 80-85, 4: V $\geqslant$ 85; F) Post-vulval uterine sac (PUS): 1: <16 μm, 2: 16-19.9 μm, 3: 20-24.9 μm, 4: 25-29.9 μm, 5: 30-35 μm, 6: >35μm; G) Female tail shape: 1: cylindrical, 2: subcylindrical, 3: conoid; H) Female tail tip: 1: smooth, 2: striated, 3: pointed, 4: with ventral projection; I) Pharyngeal overlap length: 1: <30 μm, 2: 30-39.9 μm, 3: 40-50 μm, 4: >50 μm; J) Lateral field lines at vulval region: 1: four, 2: five, 3: six to eight; and K) Lateral field structure at vulval region: 1: smooth bands, 2: partially or completely areolated bands.

To facilitate the Hierarchical Cluster analysis, we created a web-based key for quick morphological identification of *Pratylenchus* spp. The domain of this website was obtained from https://www.awardspace.com. Notepad ++ v7.5.6 was used to design the interface of the web-based key and the algorithm for this web-based key was based on the Bray-Curtis similarity measure.

Results

Pratylenchus horti* **n. sp.**
(Figs 1, 2)

Measurements

See Table 1.

Description

Female

Body habitus slightly curved ventrally. Body annulation prominent. Lateral field with four incisures at vulva level, lacking areolation but sometimes with oblique strokes. Outline of outer bands becoming indented towards tail end, between phasmid and tail tip. Low labial region with two annuli, continuous with body contour. *En face* view with submedian segments triangular-shaped fused to oral disc and separated from lateral segments, amphidial apertures slit-like, laterally bordering oral disc. Stylet rather long, robust, conus *ca* 0.5 stylet length long, stylet shaft slender, basal knobs prominent, rounded. Pharyngeal procorpus narrowing just anterior to small, oval metacorpus with conspicuous valve, isthmus elongate, slender, encircled by nerve ring, gland lobe overlapping intestine ventrally for *ca* 45 μm. Secretory-excretory pore located just posterior to hemizonid, at pharyngo-intestinal

* The name of the new species is derived from the Latin word *hortus*, meaning garden, and referring to the Botanical Garden of Ghent University.

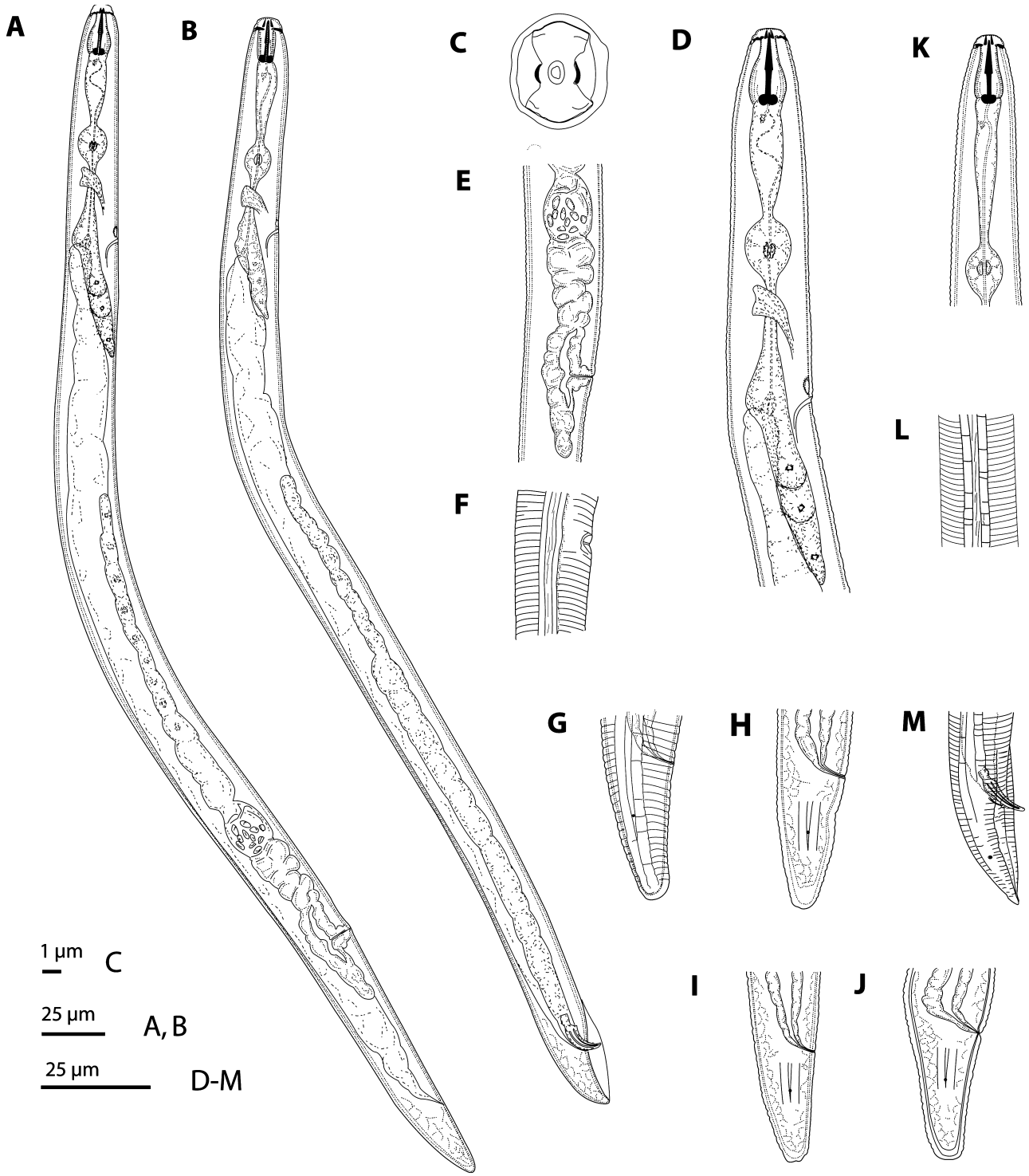

Fig. 1. *Pratylenchus horti* n. sp. Female: A: Entire body; C: *En face* view; D: Pharyngeal region; E: Vulval region; F: Lateral field at vulval region; G-J: Tail variations. Male: B: Entire body; K: Head region; L: Lateral field at mid-body; M: Tail region. (D, H: holotype, others based on paratypes.).

Fig. 2. LM and SEM of *Pratylenchus horti* n. sp. A-N: female. A, B: Pharyngeal region; C: Lateral field at vulval region; D: Head region, slightly oblique lateral view; E: Vulval region, ventral view; F: Entire body; G: Vulval region (vulva indicated by arrow); H: *En face* view; I-N: Tail region (I = ventral view). O-U: male. O: Pharyngeal region; P: *En face* view; Q: Head region; R: Lateral field; S, T: Tail region lateral view; U: Tail region ventral view. (A, F, L: female holotype; others from paratypes.).

Table 1. Morphometric data of fixed specimens of *Pratylenchus horti* n. sp. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Female		Male
	Holotype	Paratypes	Paratypes
n	–	15	10
L	527	510 ± 14.1 (497-527)	504 ± 22.1 (475-534)
a	21.9	19.9 ± 3.8 (13.3-22.6)	23.7 ± 2.6 (21.1-28.1)
b′	4.5	4.1 ± 0.4 (3.8-4.6)	4.1 ± 0.2 (3.8-4.3)
c	18.8	19.0 ± 2.0 (16.6-22.0)	19.8 ± 1.8 (17.6-21.8)
c′	2.0	1.8 ± 0.3 (1.4-2.1)	1.9 ± 0.2 (1.6-2.3)
V	77.4	78 ± 1.3 (76-79)	277 ± 32.4 (248-327)
Lip height	2.2	2.8 ± 0.4 (2.0-3.0)	2.7 ± 0.8 (2.0-4.0)
Lip diam	8.7	8.4 ± 0.5 (8.0-9.0)	7.7 ± 0.5 (7.0-8.0)
Stylet length	17	16 ± 0.7 (15-17)	15.5 ± 0.5 (15-16)
Conus length	8.9	7.6 ± 0.5 (7.0-8.0)	7.8 ± 0.4 (7.0-8.0)
Shaft length	6.0	5.6 ± 0.5 (5.0-6.0)	5.7 ± 0.5 (5.0-6.0)
Knob height	2.1	2.8 ± 0.4 (2.0-3.0)	2.2 ± 0.4 (2.0-3.0)
Dorsal gland opening from stylet base	2.3	2.6 ± 0.5 (2.0-3.0)	2.7 ± 0.8 (2.0-4.0)
Anterior end to secretory-excretory pore	80	84 ± 8.1 (70-90)	85 ± 7.3 (72-92)
Anterior end to nerve ring	74	66 ± 7.8 (55-75)	59 ± 25.1 (8-74)
Anterior end to end of pharyngeal gland	117	124 ± 8.2 (115-131)	123 ± 4.8 (115-129)
Pharyngeal gland overlap	34	45 ± 12.3 (34-65)	36 ± 5.9 (28-43)
Post-uterine sac	25	30 ± 6.5 (22-36)	–
Max body diam.	24	27 ± 6.5 (22-38)	21 ± 1.5 (19-23)
Vulval body diam.	21	25 ± 5.4 (21-34)	–
Anal body diam.	14	15.6 ± 2.7 (13-20)	13.3 ± 1.0 (12-15)
Tail length	28	27 ± 2.2 (24-30)	26 ± 1.6 (23-27)
Hyaline length	4.0	4.2 ± 0.8 (3.0-5.0)	–
Tail annuli number	22	20 ± 2.2 (17-22)	–
Spicule length (arc)	–	–	17.0 ± 1.4 (15-19)
Spicule width (mid-way)	–	–	3.0 ± 0.6 (2.0-4.0)
Gubernaculum length	–	–	5.5 ± 0.8 (5.0-7.0)

junction level. Reproductive system monodelphic, ovary outstretched, oocytes arranged in one or two rows, spermatheca small, *ca* 14 × 20 μm, round to (frequently) oval, full of sperm, PUS *ca* 1.0-1.5 vulval body diam. long, vulva slightly protruding from body. Tail subcylindrical, tapering towards tail tip, tail terminus variable in shape, from truncate (rarer) to smooth rounded margin, phasmids located at mid-tail.

Male

Largely similar to female except for sexual features. However, anterior part of body more slender than in female and outer bands of lateral field partially areolated. Testis outstretched, short. Spicules paired, weakly cephalate, slightly ventrally arcuate, gubernaculum slightly curved. Tail conical, elongate, bent on ventral side, enveloped by a poorly protruding, crenate peloderan bursa.

Type host and locality

Recovered from soil and root samples from the rhizosphere of *Hedychium greenii* growing outside in the Botanical Garden, Ghent University, Belgium (GPS coordinates 51°2′6.7″N, 3°43′22.4″E; altitude: 15 m a.s.l.).

Type material

Slide number UGMD 104366 (comprising the holotype female and two paratype females) is deposited at the Ghent University Museum, Zoology Collections. Additional paratypes, two females (UGnem-179) and two males (UGnem-232) are available in the UGent Nematode Collection of the Nematology Research Unit, Department of Biology, Ghent University, Ghent, Belgium.

DIAGNOSIS AND RELATIONSHIPS

The females of *P. horti* n. sp. are characterised by the following traits: labial region bearing two annuli and continuous with body contour, *en face* structure, as seen in SEM, belonging to group II according to Corbett & Clark (1983), characterised by the fusion of triangular-shaped submedian segments and oral disc with slit-like amphidial apertures located in the inner edges of lateral segments, lateral field with four incisures at vulval level and lacking areolation, middle band smooth (but sometimes with oblique strokes), spermatheca is small, round to (frequently) oval when filled with sperm, tail subcylindrical with truncate to (frequently) smooth margin. The males have a partially areolated lateral field, outstretched testis, weakly cephalate, ventrally arcuate spicules, and a poorly protruding, crenate bursa. The matrix code (Castillo & Vovlas, 2007) for this species is: A1, B2, C3, D3, E2, F5, G2, H1, I3, J1, K1.

Pratylenchus horti n. sp. differs from all other species according to the dichotomous keys of Castillo & Vovlas (2007) and Geraert (2013), as well as following the comparison with the more recent species of Hodda *et al*. (2014), Palomares-Rius *et al*. (2014), Wang *et al*. (2015), Nguyen *et al*. (2017), and Singh *et al*. (2018). A comparison of *P. horti* n. sp. with 102 other species using the matrix codes *sensu* Castillo & Vovlas (2007), facilitated by a Cluster analysis, showed that *P. horti* n. sp. is most similar to *P. mulchandi* Nandakumar & Khera, 1970, *P. hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 and *P. paraflorideńsis* De Luca, Troccoli, Duncan, Subbotin, Waeyenberge, Moens & Inserra, 2010 (Fig. 3; Table 2). These species are more

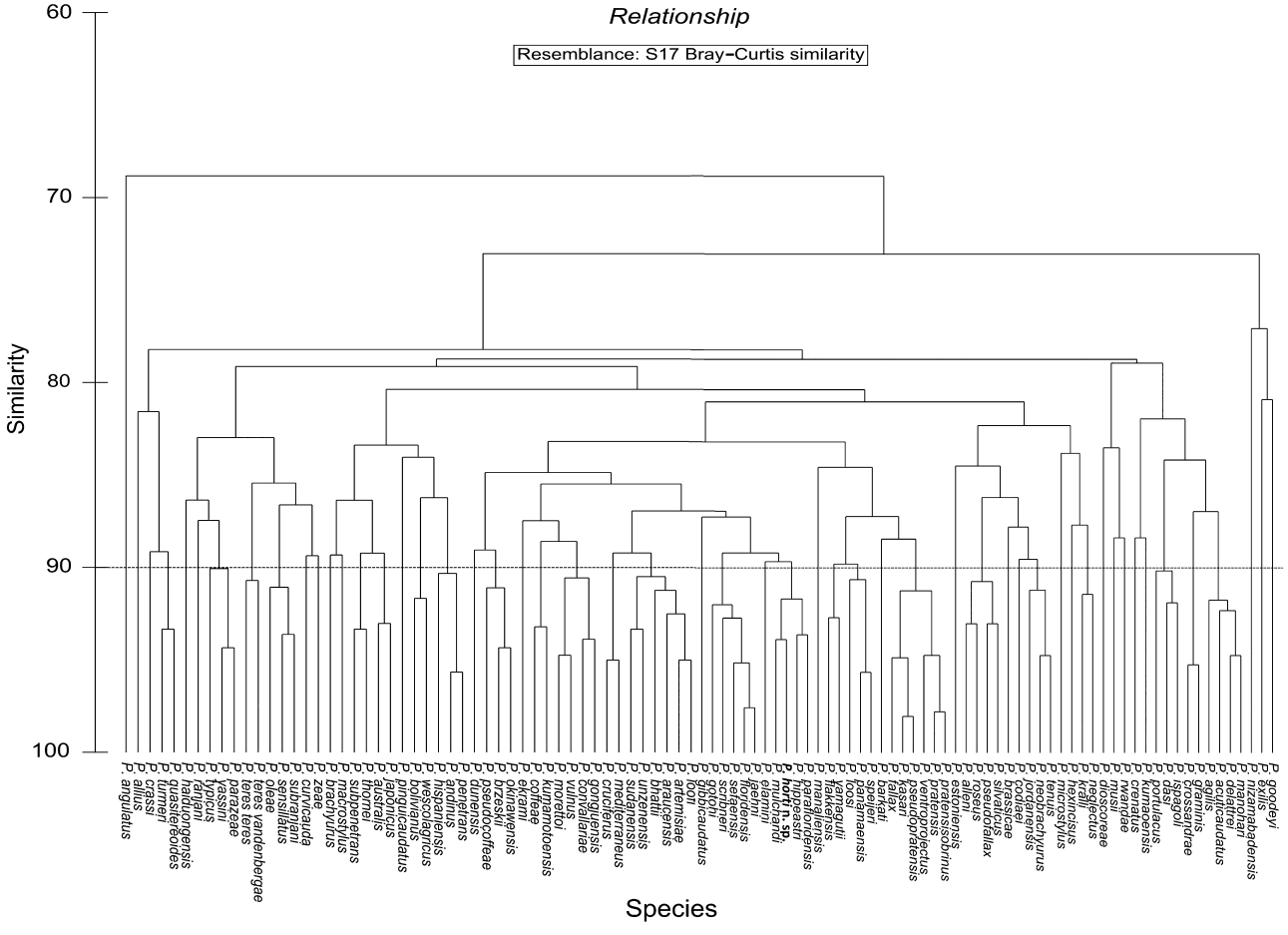

Fig. 3. Cluster analysis of 103 *Pratylenchus* species (included *P. horti* n. sp.) based on the Bray-Curtis similarity measure of 11 ranked features. *Pratylenchus horti* n. sp. is in bold. The dotted line indicates 90% similarity.

Table 2. Matrix code comparison of *Pratylenchus horti* n. sp. with similar *Pratylenchus* species.

Species	Code											
	A	B	C	D	E	F	G	H	I	J	K	L*
P. horti n. sp.	1	2	3	3	2	5	2	1	3	1	1	2
P. mulchandi	2	2	3	3	2	5	3	1	2	1	1	1
P. hippeastri	1	1	2	4	2	5	3	1	3	1	1	1
P. parafloridensis	1	2	2	2	2	5	3	1	3	1	1	1
P. pseudocoffeae	1	2	2	3	3	4	2	1	4	1	1	2
P. speijeri	1	2	3	3	3	3	2	2	3	1	1	1

**En face* group *sensu* Corbett & Clark (1983): 1 = group I; 2 = group II.

than 90% similar to each other and are less than 90% similar compared to all other species (Fig. 3). The species in this group share the following common traits: V = 75-79 (E2); post-uterine sac 30-35 μm long (F5); smooth female tail tip (H1); and four lateral lines at vulval level (J1) with smooth bands (K1). *Pratylenchus horti* n. sp. can be differentiated from these former species by the features compared below.

Although the molecular data for *P. mulchandi* are unavailable, *P. horti* n. sp. clearly differs from *P. mulchandi* by morphological features and by three out of 11 features of the matrix codes according to Castillo & Vovlas (2007): A: lip annuli (two *vs* three); G: female tail shape (subcylindrical *vs* conoid); and I: Pharyngeal overlap (40-50 *vs* 30-39.9 μm). Furthermore, the females of *P. horti* n. sp. have a different *en face* (group II *vs* group I), slightly shorter body length (510 (497-527) *vs* 540 (470-600) μm), smaller a value (19.9 (13.3-22.6) *vs* 30 (26-32)), larger c value (19 (16.6-22.0) *vs* 16 (15-19)), and larger V ratio (78 (76-79) *vs* 75 (72-77)). The males of *P. horti* n. sp. have a smaller a value (23.7 (21.1-28.1) *vs* 33), smaller c′ value (1.9 (1.6-2.3) *vs* 2.4), shorter tail (26 (23-27) *vs* 34 μm), and longer spicules (17 (15-19) *vs* 14 μm).

Pratylenchus horti n. sp. can be distinguished from *P. hippeastri* by four out of 11 features in the matrix codes: B: males (present *vs* absent); C: stylet length (between 16-17.9 *vs* 13-15.9 μm); D: spermatheca shape (oval *vs* rectangular); and G: female tail shape (subcylindrical *vs* conoid). Moreover, the females of *P. horti* n. sp. have a different *en face* structure (group II *vs* group I), shorter body length (510 (497-527) *vs* 590 (550-630) μm), smaller a value (19.9 (13.3-22.6) *vs* 25.5 (23.2-27.9)), larger c value (19.0 (16.6-22) *vs* 16.1 (14.6-18.7)), and smaller c′ value (1.8 (1.4-2.1) *vs* 2.6 (2.2-2.9)).

Pratylenchus horti n. sp. differs from *P. parafloridensis* by three out of 11 features in the matrix codes including: C: stylet length (between 16-17.9 *vs* 13-15.9 μm); D: spermatheca shape (round to (frequently) oval *vs* rounded to spherical); and G: female tail shape (subcylindrical *vs* conoid). The females of *P. horti* n. sp. also differ from *P. parafloridensis* by having a different *en face* structure (group II *vs* group I), smaller a value (19.9 (13.3-22.6) *vs* 29 (25.2-37)), larger c value (19.0 (16.6-22.0) *vs* 16.8 (14.9-18.5)), larger c′ value (1.8 (1.4-2.1) *vs* 2.9 (2.4-3.3)), larger anal body diam. (16 (13-20) *vs* 11 (10.5-13) μm), and shorter tail (27 (24-30) μm *vs* 32 (28-35) μm). The males of *P. horti* n. sp. differ from *P. parafloridensis* by having a different *en face* structure (group II *vs* group I), larger body length (504 (475-534) *vs* 448 (414-494) μm), smaller a value (23.7 (21.1-28.1) *vs* 29.7 (25-35.3)), larger b′ value (4.1 (3.8-4.3) *vs* 3.7 (3.4-4.0)), larger c value (19.8 (17.6-21.8) *vs* 17.9 (15-19.1)), smaller c′ value (1.9 (1.6-2.3) *vs* 2.8 (2.4-3.3)), and shorter spicules (17 (15-19) *vs* 18.5 (17.8-19) μm).

To facilitate the comparison with the tabular key of Castillo & Vovlas (2007), we have created a freely available web-based key for identification of *Pratylenchus* spp. (http://nematodeidentification.mypressonline.com/category/identification-tool/). This key performs a cluster analysis based on Bray-Curtis similarity using the matrix codes of Castillo & Vovlas (2007) and more recent descriptions. The user can simply fill in the matrix codes of their species under question and the expected Bray-Curtis similarity index to check for the most closely related species among the currently 103 known *Pratylenchus* species (*i.e.*, including *P. horti* n. sp.).

Molecular characterisation

The molecular analyses based on D2-D3 of 28S rDNA, ITS rDNA, and *COI* mtDNA regions indicated *P. horti* n. sp. as a unique lineage clearly different from all other species. The ITS rDNA sequences of *P. horti* n. sp.

are most similar to the sequences of *P. pseudocoffeae* Mizukubo, 1992, while the D2-D3 of 28S rDNA, and *COI* mtDNA sequences of *P. horti* n. sp. are most similar to the sequences of *P. speijeri* De Luca, Troccoli, Duncan, Subbotin, Waeyenberge, Coyne, Brentu, Inserra, 2012. *Pratylenchus horti* n. sp. can also be differentiated from both species by morphological features.

Pratylenchus horti n. sp. is distinguished from *P. speijeri* by three out of 11 features in the matrix codes: E: V ratio (78 (76-79) *vs* 80 (78-82)); F: Post-uterine sac length (between 30-35 *vs* 20-24.9 μm); and H: tail tip (smooth *vs* striated). Furthermore, the females of *P. horti* n. sp. differ from *P. speijeri* by having a different *en face* structure (group II *vs* group I), smaller a value (19.9 (13.3-22.6) *vs* 27.7 (23.4-32.7)), and larger anal body diam. (16 (13-20) *vs* 12.5 (11.0-14.5) μm). The males of *P. horti* n. sp. can be differentiated from *P. speijeri* by having a smaller a value (23.7 (21.1-28.1) *vs* 29.7 (27.1-33.0)), larger c value (19.8 (17.6-21.8) *vs* 16.2 (14.2-17.6)), larger c′ value (1.9 (1.6-2.3) *vs* 3.2 (2.9-3.5)), and shorter tail (26 (23-27) *vs* 30 (27-34) μm).

Pratylenchus horti n. sp. can be differentiated from *P. pseudocoffeae* by four out of 11 features in the matrix codes including: C: stylet length (between 16-17.9 *vs* between 13-15.9 μm); E: V ratio (between 75-79.9 *vs* 80-85); F: PUS length (between 30-35 *vs* 25-29.9 μm); and I: Pharyngeal overlap (between 40-50 *vs* > 50 μm). In addition, the females of *P. horti* n. sp. differ from *P. pseudocoffeae* by having a smaller a value (19.9 (13.3-22.6) *vs* 27.5 (22.6-32.1)), and larger b′ value (4.1 (3.8-4.6) *vs* 3.1 (2.6-3.4)). The males of *P. horti* n. sp. differ from *P. pseudocoffeae* by having smaller a value (23.7 (21.1-28.1) *vs* 30.6 (25.6-37.0)), larger b′ value (4.1 (3.8-4.3) *vs* 3.3 (2.9-3.8)), shorter tail (26 (23-27) *vs* 43 (38-48) μm), and longer stylet length (16 (15-16) *vs* 15 (14.0-15.5) μm).

Molecular phylogeny

D2-D3 of 28S rDNA

Five new sequences of the 5′-end region of 28S rDNA were obtained, 1004-1066 bp long. The resulting MUSCLE alignment included 153 nucleotide sequences and 1146 positions, 691 positions were retained in the final dataset by Gblocks. The D2-D3 of 28S rDNA sequences of *P. horti* n. sp. were 99-100% similar to each other with differences on 0-1 position. These sequences differ by 27-127 nucleotides (81-96% similar) from all other *Pratylenchus* species in this study. They were most similar (95-96%) to the sequences of *P. speijeri* with 27-30 different positions. The Bayesian interference phylogenetic tree based on the D2-D3 of 28S rDNA sequences showed that the sequences of *P. horti* n. sp. were placed in a maximally supported clade with a sister relation to a clade (0.77 PP) including *P. scribneri* Steiner *in* Sherbakoff & Stanley, 1943, *P. hexincisus* Taylor & Jenkins, 1957, *P. agilis* Thorne & Malek, 1968, *P. pseudocoffeae*, *P. alleni* Ferris, 1961, *P. gutierrezi* Golden, López & Vílchez, 1992, *P. panamaensis* Siddiqi, Dabur & Bajaj, 1991, *P. hippeastri*, *P. parafloridensis*, *P. floridensis* De Luca, Troccoli, Duncan, Subbotin, Waeyenberge, Moens & Inserra, 2010, *P. araucensis* Múnera, Bert & Decraemer, 2009, *P. loosi* Loof, 1960, *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941, and *P. speijeri* (Fig. 4).

ITS rDNA

Three ITS rDNA sequences were obtained, 961 to 1049 bp in length, and with a variation in 1-3 positions (99-99.7% similar). The MUSCLE alignment comprised 94 sequences and was 1296 bp long, only 280 positions were retained by Gblocks. The ITS rDNA sequences of *P. horti* n. sp. differed 14-46 nucleotides compared to other studied *Pratylenchus* species (84-96% similar), and were most similar to the sequence of *P. pseudocoffeae* with 14-16 different positions (95-96% similar). According to the ITS rDNA tree topology, *P. horti* n. sp. is sister to a poorly supported clade (0.5 PP), including *P. jaehni*, *P. araucensis*, *P. scribneri*, *P. agilis*, *P. floridensis*, *P. parafloridensis*, *P. hippeastri*, *P. gutierrezi*, *P. loosi*, *P. alleni*, *P. japonicus* and *P. pseudocoffeae*. Compared to the D2-D3 tree, *Pratylenchus horti* n. sp. is sister to *P. japonicus*, but not *P. coffeae* and *P. speijeri* in the ITS tree (Fig. 5).

COI mtDNA

The eight obtained *COI* gene fragments were 436-443 bp long. The MUSCLE alignment comprised 69 nucleotide sequences and was 825 positions long, 389 positions were retained by Gblocks. The *COI* mtDNA sequences of *P. horti* n. sp. were identical and thus without intraspecific variation. These sequences were highly different to other *Pratylenchus* species, 85-167 different nucleotides (64-79% similar), and were most similar to *P. speijeri* with 78% similarity (85 different positions). The phylogenetic tree based on the *COI* mtDNA sequences revealed a sister relationship (0.66 PP) of *P. horti* n. sp. with a poorly supported clade (0.63 PP), including *P. scribneri*, *P. hexincisus*, *P. loosi*, *P. coffeae*, *P. speijeri* and *P.*

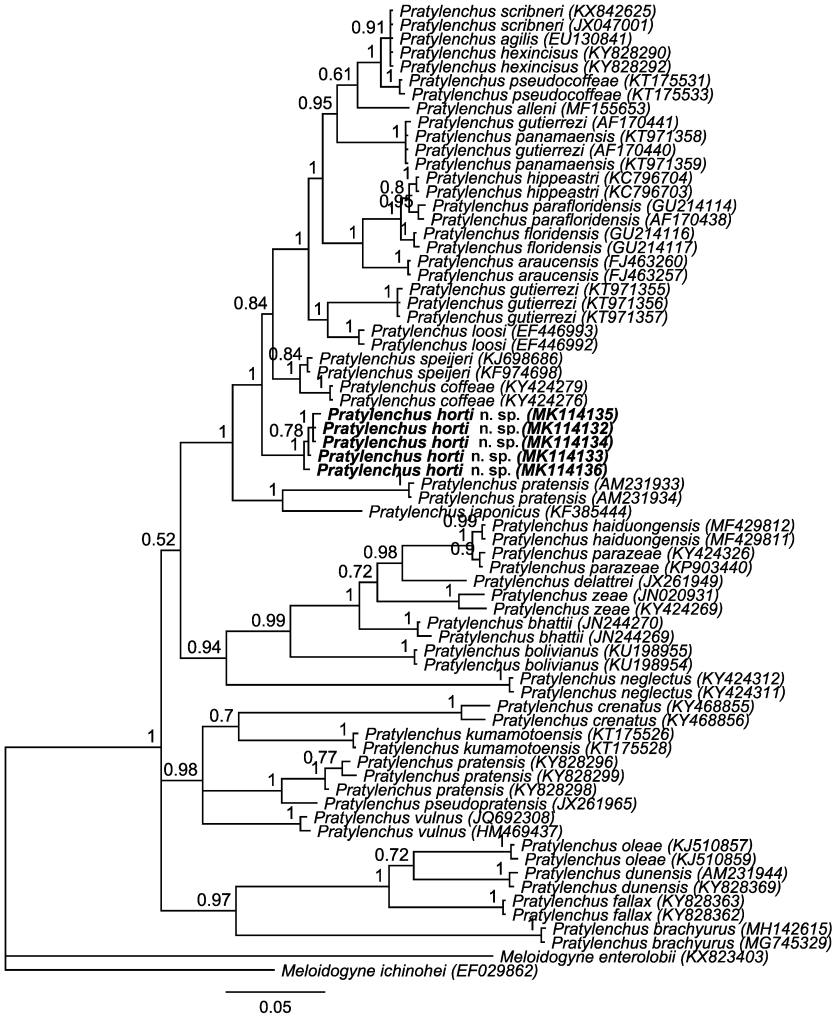

Fig. 4. BI phylogenetic tree generated from the D2-D3 of 28S rDNA sequences dataset with the HKY + G model. Bayesian posterior probabilities are given next to each node. Sequences of *Pratylenchus horti* n. sp. are in bold.

hippeastri. This clade together with *P. horti* n. sp., *P. vulnus* and *P. pratensis* formed a maximally supported clade (Fig. 6).

Discussion

Cluster analysis based on the matrix tabular key of Castillo & Vovlas (2007) in this study was able to cluster *Pratylenchus* spp. into small groups with a high number of similarities. Cryptic species or species complexes without significant morphological differences, such as *P. teres teres* Khan & Singh, 1974 and *P. teres vandenbergae* Carta, Handoo, Skantar, van Biljon & Botha, 2002 or *P. parafloridensis* and *P. hippeastri*, were grouped together, thus supporting the reliability of this analysis. In a manual comparison using a dichotomous or polytomous key, two very similar species (even those displaying only one different feature) could be considered to be quite different depending on the starting point used for comparison. For example, *P. horti* n. sp. would never be considered as the most similar species to *P. mulchandi* if a comparison was based on the number of labial annuli (two *vs* three) as in Castillo & Vovlas (2007). Cluster analysis, based on Bray-Curtis similarity, not only helps to avoid a biased selection of species to compare with, but it can also facilitate the use of tabular keys to minimise mistakes made in manual comparisons, as well as speeding up the identi-

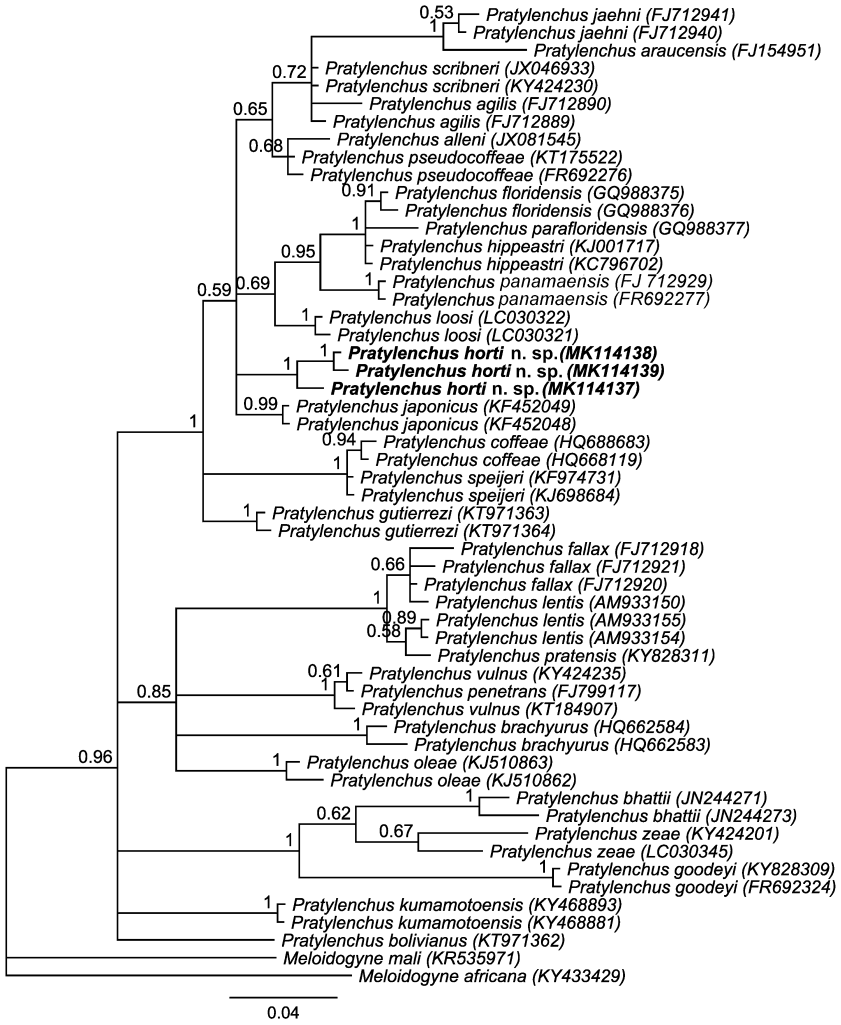

Fig. 5. BI phylogenetic tree generated from ITS rDNA sequences with the HKY + G model. Bayesian posterior probabilities are given next to each node. Sequences of *Pratylenchus horti* n. sp. are in bold.

fication process, especially for a huge dataset. The drawback of the Cluster analysis is that the data of the tabular key needs to be imported at the first time use. For greater convenience, a web-based key for identification of *Pratylenchus* spp. was created (http://nematodeidentification.mypressonline.com/category/identification-tool/), which can be used as an alternative method to Cluster analysis in identifying *Pratylenchus* species. A similar key has also been created for *Rotylenchus* spp. (Nguyen *et al*., unpubl.).

The *en face* structure of *P. horti* n. sp. clearly belongs to group II *sensu* Corbett & Clark (1983). By linking molecular data and *en face* structures, Subbotin *et al*. (2008) evaluated lip patterns as one of the most informative features to group *Pratylenchus* species. In this study, the *en face* structure was also used to support the species delimitation. It is recommended to add the *en face* feature, if available, to the tabular key of Castillo & Vovlas (2007).

Strikingly, some sequences of the same species did not gather together in one clade on our phylogenetic trees. For example, the sequences of *P. gutierrezi* in the D2-D3 of 28S rDNA tree were placed in two different clades; the first clade included the sequences of *P. gutierrezi* (AF170441, AF170440) and *P. panamaensis* (KT971358, KT971359) with maximal support (1 PP), and the second clade the sequences of *P. gutierrezi* (KT971355, KT971356, KT971357) with 1 PP. The ITS rDNA tree also comprises two distantly related maximally sup-

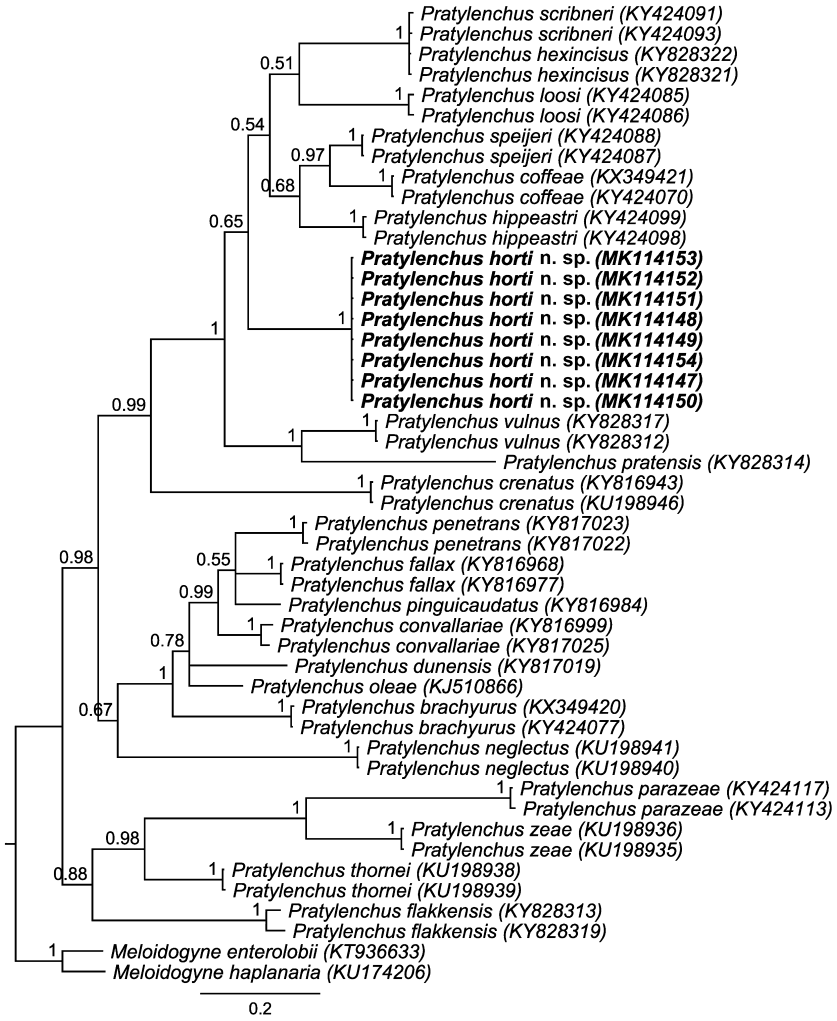

Fig. 6. BI phylogenetic tree generated from *COI* sequences with the HKY + G model. Bayesian posterior probabilities are given next to each node. Sequences of *Pratylenchus horti* n. sp. are in bold.

ported clades of *P. gutierrezi* (the first clade: FJ712929, FR692277; the second clade: KT971363, KT971364). After a study of *P. gutierrezi* and *P. panamaensis* topotypes, Araya *et al*. (2016) concluded that the D2-D3 of 28S rDNA sequences of *P. gutierrezi* (AF170441, AF170440) and the ITS rDNA sequence of *P. gutierrezi* (FR692277) should be considered as conspecific with *P. panamaensis*. Combining the results of our study and Araya *et al*. (2016), we suggest that the ITS rDNA sequence of *P. gutierrezi* (FJ712929) also needs to be considered as conspecific with *P. panamaensis*. The D2-D3 of 28S rDNA sequences of *P. pratensis* are separated into two clades with 100% support on the phylogenetic tree (the first clade: AM231933, AM231934, AM231931, AM231930; the second clade: KY828296, KY828299, KY828298). These arrangements imply that these sequences have either been generated from cryptic species, or that they are mislabelled or misidentified sequences.

The identification of *Pratylenchus* spp. should always take into account both morphological and molecular aspects to provide the most precise identification; sequencing of topotype material is often the only way to confidently connect DNA sequences to formerly described morphospecies (Duncan *et al*., 1999; Inserra *et al*., 2007; De Luca *et al*., 2010; Troccoli *et al*., 2016). In this study, the combination of the morphological and molecular data of *P. horti* n. sp. provides a good reference for the com-

parison of morphological features as well as for DNA barcoding.

Pratylenchus horti n. sp. was found on *H. greenii*, an exotic plant imported from the Himalayan Mountains, and growing in the Botanical Garden of Ghent University, Belgium. Although the origin of this plant is probably a cold region (the Himalayas), the exact native weather conditions remain unknown. This specimen was planted several years ago and has lived outside under Belgian climatic conditions. Interestingly, the aerial parts of this plant were cut down and used together with other chopped wood to cover its growing area during winter time, which may have created slightly different soil and root conditions compared to normal Belgian conditions. To our knowledge, this is the first report of the plant-parasitic nematode, *Pratylenchus horti* n. sp., on *H. greenii*. It remains to be investigated whether *P. horti* n. sp was introduced together with the *H. greenii* specimen, or whether it is a native species that also occurs on other host plants in Belgium.

Acknowledgements

Huu Tien Nguyen was supported by a VLIR-UOS ICP scholarship and Phougeishangbam Rolish Singh was supported by a special research fund, UGent BOF 01D05918.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25, 3389-3402.

Araya, T.Z., Padilla, W.P., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Liébanas, G., Palomares-Rius, J.E. & Castillo, P. (2016). Root-lesion nematodes of the genus *Pratylenchus* (Nematoda: Pratylenchidae) from Costa Rica with molecular identification of *P. gutierrezi* and *P. panamaensis* topotypes. *European Journal of Plant Pathology* 145, 973-998.

Barry, G.H. (2011). *Phylogenetic trees made easy: a how-to manual*. Sunderland, MA, USA, Sinauer Associates.

Blaxter, M., De Ley, P., Garey, J., Liu, L., Scheldeman, P., Vierstraete, A., Vanfleteren, J., Mackey, L., Dorris, M., Frisse, L. *et al.* (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* 392, 71-75. DOI: 10.1038/32160

Castillo, P. & Vovlas, N. (2007). Pratylenchus *(Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management*. *Nematology Monographs and Perspectives 6* (Series Editors: Hunt, D.J. & Perry, R.N.). Leiden, The Netherlands, Brill.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17, 540-552. DOI: 10.1093/oxfordjournals.molbev.a026334

Corbett, D.C.M. & Clark, S.A. (1983). Surface features in the taxonomy of *Pratylenchus* species. *Revue de Nématologie* 6, 85-98.

De Luca, F., Troccoli, A., Duncan, L., Waeyenberge, L., Subbotin, S., Inserra, R. & Moens, M. (2010). Characterisation of a population of *Pratylenchus hippeastri* from bromeliads and description of two related new species, *P. floridensis* n. sp. and *P. parafloridensis* n. sp., from grasses in Florida. *Nematology* 12, 847-868. DOI: 10.1163/138855410X495809

De Luca, F., Inserra, R.N., Duncan, L.W., Coyne, D.L., Brentu, F.C., Troccoli, A., Waeyenberge, L. & Subbotin, S.A. (2012). *Pratylenchus speijeri* n. sp. (Nematoda: Pratylenchidae), a new root-lesion nematode pest of plantain in West Africa. *Nematology* 14, 987-1004. DOI: 10.1163/156854112X638424

de Man, J.G. (1880). Die Einheimischen, frei in der reinen Erde und im sussen Wasser lebenden Nematoden Vorlaufiger Bericht und descriptiv-systematischer Theil. *Tijdschrift der Nederlandsche Dierkundige Vereeniging* 5, 1-104.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort, V., Lescot, M. *et al.* (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* 36, 465-469. DOI: 10.1093/nar/gkn180

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T. & Moens, T. (2010). Exploring the use of cytochrome oxidase c subunit 1 (*COI*) for DNA barcoding of free-living marine nematodes. *PLoS ONE* 5, e13716. DOI: 10.1371/journal.pone.0013716

Duncan, L.W., Inserra, R.N., Thomas, W., Dunn, D., Mustika, L., Frisse, L., Mendes, M.L., Morris, R. & Kaplan, D.T. (1999). Molecular and morphological analysis of *Pratylenchus coffeae* and closely related species. *Nematropica* 29, 61-80.

Geraert, E. (2013). *The Pratylenchidae of the world: identification of the family Pratylenchidae (Nematoda: Tylenchida)*. Ghent, Belgium, Academia Press.

Hodda, M., Collins, S.J., Vanstone, V.A., Hartley, D., Wanjura, W. & Kehoe, M. (2014). *Pratylenchus quasitereoides* n. sp. from cereals in Western Australia. *Zootaxa* 3866, 277-288. DOI: 10.11646/zootaxa.3866.2.6

Holterman, M., Karssen, G., van den Elsen, S., van Megen, H., Bakker, J. & Helder, J. (2009). Small subunit rDNA-based phylogeny of the Tylenchida sheds light on relationships among some high-impact plant-parasitic nematodes and the evolution of plant feeding. *Phytopathology* 99, 227-235. DOI: 10.1094/PHYTO-99-3-0227

Huelsenbeck, J.P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754-755. DOI: 10.1093/bioinformatics/17.8.754

Inserra, R., Bernard, E., Dunn, D., Duncan, L., Troccoli, A. & Gozel, U. (2007). *Pratylenchus hippeastri* n. sp. (Nematoda: Pratylenchidae) from amaryllis in Florida with notes on *P. scribneri* and *P. hexincisus*. *Nematology* 9, 25-42. DOI: 10.1163/156854107779969754

Janssen, T., Karssen, G., Orlando, V., Subbotin, S.A. & Bert, W. (2017a). Molecular characterization and species delimiting of plant-parasitic nematodes of the genus *Pratylenchus* from the *penetrans* group (Nematoda: Pratylenchidae). *Molecular Phylogenetics and Evolution* 117, 30-48. DOI: 10.1016/j.ympev.2017.07.027

Janssen, T., Karssen, G., Couvreur, M., Waeyenberge, L. & Bert, W. (2017b). The pitfalls of molecular species identification: a case study within the genus *Pratylenchus* (Nematoda: Pratylenchidae). *Nematology* 19, 1-17. DOI: 10.1163/15685411-00003117

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M.L. *et al.* (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14, 946-961. DOI: 10.1111/mpp.12057

Mizukubo, T. (1992). Morphological and statistical differentiation of *Pratylenchus coffeae* complex in Japan (Nematoda: Pratylenchidae). *Applied Entomology and Zoology* 27, 213-224. DOI: 10.1303/aez.27.213

Nadler, S.A., De Ley, P., Mundo-Ocampo, M., Smythe, A.B., Stock, S.P., Bumbarger, D., Adams, B.J., De Ley, I.T., Holovachov, O. & Baldwin, J.G. (2006). Phylogeny of Cephalobina (Nematoda): molecular evidence for recurrent evolution of probolae and incongruence with traditional classifications. *Molecular Phylogenetics and Evolution* 40, 696-711. DOI: 10.1016/j.ympev.2006.04.005

Nandakumar, C. & Khera, S. (1970). A new nematode species, *Pratylenchus mulchandi*, from millets of Rajasthan. *Indian Phytopathology* 22, 359-363.

Nguyen, T.D., Le, T.M.L., Nguyen, H.T., Nguyen, T.A.D., Liébanas, G. & Trinh, Q.P. (2017). Morphological and molecular characteristics of *Pratylenchus haiduongensis* sp. n., a new species of root-lesion nematode associated with carrot in Vietnam. *Journal of Nematology* 49, 276-285.

Palomares-Rius, J.E., Guesmi, I., Horrigue-Raouani, N., Cantalapiedra-Navarrete, C., Liébanas, G. & Castillo, P. (2014). Morphological and molecular characterisation of *Pratylenchus oleae* n. sp. (Nematoda: Pratylenchidae) parasitizing wild and cultivated olives in Spain and Tunisia. *European Journal of Plant Pathology* 140, 53-67. DOI: 10.1007/s10658-014-0443-4

Singh, P.R., Nyiragatare, A., Janssen, T., Couvreur, M., Decraemer, W. & Bert, W. (2018). Morphological and molecular characterisation of *Pratylenchus rwandae* n. sp. (Tylenchida: Pratylenchidae) associated with maize in Rwanda. *Nematology* 20, 781-794. DOI: 10.1163/15685411-00003175

Steel, H., Coomans, A., Decraemer, W., Moens, T. & Bert, W. (2014). Nematodes from terrestrial, freshwater and brackish water habitats in Belgium: an updated list with special emphasis on compost nematodes. *Zootaxa* 3765, 143-160. DOI: 10.11646/zootaxa.3765.2.3

Subbotin, S.A. & Moens, M. (2006). Molecular taxonomy and phylogeny. In: Perry, R.N. & Moens, M. (Eds). *Plant nematology*. Wallingford, UK, CAB International, pp. 33-58.

Subbotin, S.A., Halford, P. & Perry, R. (1999). Identification of populations of potato cyst nematodes from Russia using protein electrophoresis, rDNA-RFLP and RAPDS. *Russian Journal of Nematology* 7, 57-63.

Subbotin, S.A., Sturhan, D., Rumpenhorst, H.J. & Moens, M. (2003). Molecular and morphological characterisation of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). *Nematology* 5, 515-538. DOI: 10.1163/156854103322683247

Subbotin, S.A., Sturhan, D., Vovlas, N., Castillo, P., Tanyi, T., Moens, M., Baldwin, J. & Debenham, N. (2007). Application of the secondary structure model of rRNA for phylogeny: D2-D3 expansion segments of the LSU gene of plant-parasitic nematodes from the family Hoplolaimidae Filipjev, 1934. *Molecular Phylogenetics and Evolution* 43, 881-890. DOI: 10.1016/j.ympev.2006.09.019

Subbotin, S.A., Ragsdale, E.J., Mullens, T., Roberts, P.A., Mundo-Ocampo, M. & Baldwin, J.G. (2008). A phylogenetic framework for root-lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18S and D2-D3 expansion segments of 28S ribosomal RNA genes and morphological characters. *Molecular Phylogenetics and Evolution* 48, 491-505. DOI: 10.1016/j.ympev.2008.04.028

Troccoli, A., Subbotin, S.A., Chitambar, J.J., Janssen, T., Waeyenberge, L., Stanley, J.D., Duncan, L.W., Agudelo, P., Munéra-Uribe, G.E., Franco, J. *et al.* (2016). Characterisation of amphimictic and parthenogenetic populations of *Pratylenchus bolivianus* Corbett, 1983 (Nematoda: Pratylenchidae) and their phylogenetic relationships with closely related species. *Nematology* 18, 651-678. DOI: 10.1163/15685411-00002981

Wang, H., Zhuo, K., Ye, W. & Liao, J. (2015). Morphological and molecular charaterisation of *Pratylenchus parazeae* n. sp. (Nematoda: Pratylenchidae) parasitizing sugarcane in China. *European Journal of Plant Pathology* 143, 173-191. DOI: 10.1007/s10658-015-0674-z

Whitehead, A.G. & Hemming, J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, 25-38. DOI: 10.1111/j.1744-7348.1965.tb07864.x