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Morphological and molecular characterisation of *Pratylenchus rwandae* n. sp. (Tylenchida: Pratylenchidae) associated with maize in Rwanda

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Summary – *Pratylenchus rwandae* n. sp., a root-lesion nematode associated with maize (*Zea mays*) from Rwanda, is described. This new species is characterised by females of medium to large size (469-600 μm) having an offset lip region with three annuli, stylet of 13-14.6 μm long with prominent rounded or anteriorly concave knobs, short to long pharyngeal gland overlap of 10.9-34.7 μm long, variable number of lateral lines (4-14) in different regions of the body, lateral field consisting of smooth bands, oval to slightly rounded spermatheca, vulva located at 75-80% of the total body length, post-vulval uterine sac (PUS) 20.3-26.5 μm long, tail subcylindrical to conoid with variation in tail tip shape from rounded to truncate or indented with generally smooth tip, and male unknown. The results of the phylogenetic analyses based on sequences of the D2-D3 expansion regions of 28S, partial 18S and ITS of rDNA and *COI* of mitochondrial DNA indicate that *P. rwandae* n. sp. is a species within the *Penetrans* group and appears as a sister species to a group comprising *P. convallariae*, *P. dunensis*, *P. fallax*, *P. oleae*, *P. penetrans*, *P. pinguicaudatus*, and three other unidentified species. A comparison of important morphological characters of the closely related *Pratylenchus* spp. is provided.

Keywords – 18S rDNA, 28S rDNA, *COI*, ITS rDNA, morphometrics, new species, phylogeny, plant-parasitic nematode, root-lesion nematode, SEM, taxonomy, *Zea mays*.

Root-lesion nematodes of the genus *Pratylenchus* Filipjev, 1936 (Tylenchida, Pratylenchidae) are migratory endoparasites that are ranked as the third most important group of plant-parasitic nematodes after root-knot nematodes (*Meloidogyne*) and cyst nematodes (*Heterodera* and *Globodera*) (Castillo & Vovlas, 2007) in terms of economic loss in agriculture and horticulture. They have a wide geographical distribution ranging from cool temperate regions, including Antarctica (Ryss *et al.*, 2005), to tropical regions, occurring both in natural and agricultural ecosystems. *Pratylenchus* species can parasitise a wide range of host plants (for a detailed list, see Castillo & Vovlas, 2007) and are also widely distributed in maize fields, often associated with poor plant growth and yield reduction (Todd & Oakley, 1996; De Waele *et al.*, 1998; Kimenju *et al.*, 1998; Koenning *et al.*, 1999; Arim *et al.*, 2006; Talwana *et al.*, 2008). In tropical and subtropical areas, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941, *P. zeae* Graham, 1951, and *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 are among the most commonly distributed species (see Egunjobi, 1974; De Waele & Jordaan, 1988; Youssef, 2013). Other *Pratylenchus* species associated with maize plants are *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941, *P. delattrei* Luc, 1958, *P. goodeyi* Sher & Allen, 1953, *P. hexincisus* Taylor & Jenkins, 1957, *P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *P. pratensis* (de Man, 1880) Filipjev, 1936, *P. sefaensis* Fortuner, 1973, and *P. thornei* Sher & Allen,

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1953 (see Loof, 1978; Karamura *et al.*, 1996; Inomoto *et al.*, 1998; Van Biljon & Meyer, 2000). Within *Pratylenchus*, 98 species were recognised by Geraert (2013), and since then four additional species have been described: *P. oleae* Palomares-Rius, Guesmi, Horrigue-Raouani, Cantalapiedra-Navarrete, Liébanas & Castillo, 2014, *P. quasitereoides* Hodda, Collins, Vanstone, Hartley, Wanjura & Kehoe, 2014, *P. parazeae* Wang, Zhuo, Ye & Liao, 2015, and *P. haiduongensis* Nguyen, Le, Nguyen, Nguyen, Liébanas & Trinh, 2017 (see Hodda *et al.*, 2014; Palomares-Rius *et al.*, 2014; Wang *et al.*, 2015; Nguyen *et al.*, 2017). *Pratylenchus lentis* Troccoli, De Luca, Handoo & Di Vito, 2008 was considered a junior synonym of *P. pratensis* by Janssen *et al.* (2017a) and *P. arlingtoni* Handoo, Carta & Skantar, 2001 was considered to be a *species inquirenda* (Janssen *et al.*, 2017b), bringing the total species number to 100.

Although 100 *Pratylenchus* species have been described, the morphological delimitation of *Pratylenchus* species has always been challenging. There is often bias during species delimitation due to a low number of diagnostic characters and morphological plasticity of several of these characters, such as the shape of lip region, female tail terminus, stylet knobs and spermatheca, structure of the post-vulval uterine sac (PUS) and lateral field (Loof, 1991; Inserra *et al.*, 1998). Such morphological plasticity may be due to environmental factors, geographical origin or type of host (Tarte & Mai, 1976; Duncan *et al.*, 1999; Doucet *et al.*, 2001; Lax *et al.*, 2004; Loubama *et al.*, 2007). This has resulted in an underestimation of the intraspecific variability of certain morphological characters used in species diagnosis and the synonymy of some species (Siddiqi, 2000; Castillo & Vovlas, 2007; Janssen *et al.*, 2017b).

Despite the lack of a consensual species identification approach for *Pratylenchus*, there have been several approaches that have been shown to be successful in discriminating important *Pratylenchus* spp. (Janssen *et al.*, 2017b). In an attempt to facilitate identification based on morphological and morphometric features for the large number of nominal *Pratylenchus* species, Castillo & Vovlas (2007) developed tabular keys based on 11 reliable diagnostic features (A-K). These features can be used as either main diagnostic traits or pragmatic characters to differentiate the species into groups. These keys remain useful and are employed in many works, including herein.

In the study of multi-gene phylogeny using nuclear ribosomal and mitochondrial gene sequences in combination with molecular species delineation analysis by Janssen *et al.* (2017b), a *Pratylenchus* population recovered from Rwanda associated with *Zea mays* ("*Pratylenchus* sp. 3") was revealed as a separate taxonomic entity. To obtain a more precise characterisation of this species, a combination of morphological features (based on light microscopy and scanning electron microscopy) and sequences (18S of rDNA, D2-D3 of 28S rDNA and ITS of rDNA and *COI* of mitochondrial DNA) are analysed in the current paper. Thus, this study aims at describing the new *Pratylenchus* species from Rwanda and differentiating it from other phenotypically similar species and to analyse further its phylogenetic relationship within the genus.

Materials and methods

Sample collection and nematode extraction

Pratylenchus rwandae n. sp. was isolated from the rhizosphere and roots of maize from a field at Nyamata Sector, Bugesera district, located in the Eastern province of Rwanda with GPS coordinates 2°08′50.8″S 30°05′03.4″E. The samples were collected with a shovel from the upper 30 cm of soil and roots and stored at 4°C until extraction and further processing. Nematodes were extracted from the soil and root material separately by using, respectively, a Baermann tray (Hooper, 2005) and a mistifier (Viglierchio & Schmitt, 1983). For later molecular work, some nematodes were stored in DESS (0.25 M disodium EDTA at pH 8.0, 20% dimethyl sulfoxide and saturated NaCl). DESS-preserved materials can be stored for months at room temperature and routine molecular analyses can be performed on DESS-stored individual specimens (Yoder *et al.*, 2006).

Morphological characterisation

Morphological and morphometric characterisation was done based on fresh and fixed nematodes. A small suspension of nematodes in an embryo glass block were killed and fixed by adding a few drops of Trump's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M Sorenson buffer (sodium phosphate buffer at pH 7.5)). The embryo glass block was then heated in a microwave (700 W) for 5 s, left to rest for 1 h at 20°C and then left for 24 h at 4°C for maximum penetration of the fixative. Following this, the nematodes were gradually transferred to anhydrous glycerin for permanent slides, following the protocol of Seinhorst (1959a) and mounted on glass

and Cobb slides for light microscopy study. Nematodes were examined, photographed, measured and drawn using an Olympus BX51 DIC Microscope (Olympus Optical), equipped with an Olympus C5060Wz camera and a drawing tube. Although no significant differences in the morphometrics were recorded between the fresh and the fixed nematodes, several structures were more clearly visible from the fresh material.

For scanning electron microscopy (SEM), specimens fixed in Trump's fixative were washed in 0.1 M phosphate buffer (pH 7.5) and dehydrated in a graded series of ethanol solutions, critical point-dried with liquid CO_2, mounted on stubs with carbon tabs (double conductive tapes), coated with gold of 25 nm, and photographed with a JSM-840 EM (JEOL) at 12 kV.

Molecular characterisation

The DESS-preserved nematodes were individually picked and washed in 200 μl of double distilled water three times each for 10 min and mounted on temporary glass slides to record all necessary morphological and morphometric data by taking pictures and measurements as described above. DNA extraction was done by cutting an individual specimen and transferring it to a PCR tube with 20 μl of worm lysis buffer (50 mM KCl, 10 mM Tris at pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40 (Tergitol Sigma), 0.45% Tween-20) followed by incubation at -20°C (10 min), adding 1 μl proteinase K (1.2 mg ml^{-1}), incubation at 65°C (1 h) and 95°C (10 min) and finally centrifuging the mixture at 14000 *g* for 1 min. PCR amplification (Taq DNA Polymerase, Qiagen) of 18S rDNA sequence was done using the primers, G18S4 (GCT TGT CTC AAA GAT TAA GCC) and 18P (TGA TCC WKC YGC AGG TTC AC) with internal sequencing primers 4F (CAA GGA CGA WAG TTW GAG G) and 4R (GTA TCT GAT CGC CKT CGA WC) according to Bert *et al.* (2008), with the following thermal profile: touch-down amplification with an initial step of 94°C for 4 min, followed by 5 cycles of 94°C for 30 s, annealing temperatures starting at 58°C for 30 s (decreasing by 1°C per cycle), and 72°C for 2 min for extension. 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 10°C for 10 min. The PCR products were sequenced by Macrogen (https://dna.macrogen.com) and the newly obtained sequence was submitted to GenBank. Other sequences, including D2-D3 of 28S and ITS of rDNA and *COI* of mitochondrial DNA, were made available for *Pratylenchus* sp. 3 by Janssen *et al.* (2017b).

The obtained sequences for the amplified gene were assembled using Chromas 2.01 and analysed with other relevant sequences available in GenBank. Multiple alignments of the different sequences of each gene were made using ClustalX 1.8 with default parameters for gap extension and gap opening penalties. The aligned sequences were trimmed using GeneDoc. The phylogenetic analysis were restricted to Bayesian Inference (BI) as this method has several advantages over other phylogenetic analysis methods (Ronquist & Huelsenbeck, 2012). The BI was performed with MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) under general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR + I + G). For each gene, a random starting tree was run for chains of 1×10^6 generations. The Markov chains were sampled at intervals of 1000 generations. Two independent runs of four chains were done for each analysis and, after discarding the burn-in sample of 25%, 50% majority rule consensus trees were generated using topology. Posterior probabilities (PP) are given on appropriate clades. Trees were visualised and rooted using FIGTREE v1.4.

Results

Pratylenchus rwandae* **n. sp.**

= *Pratylenchus* sp. 3 *apud.* Janssen *et al.*, 2017b

(Figs 1, 2)

Measurements

See Table 1.

Description

Female

Body showing various postures when killed by heat, from straight, slightly curved ventrally, C-shaped to irregular. Lateral field width occupying 25-50% of body diam. with variable number of lateral lines in different body regions giving three smooth bands and five smooth bands at level of pharynx and mid-body, respectively. Two outer ridges broader than inner ridges and at vulval region more than six low, finer ridges split prevulvar and fused postvulvar producing *ca* 10-14 lines be-

* Named after Rwanda, the country from where the new species was collected. The epithet takes the genitive form *rwandae*.

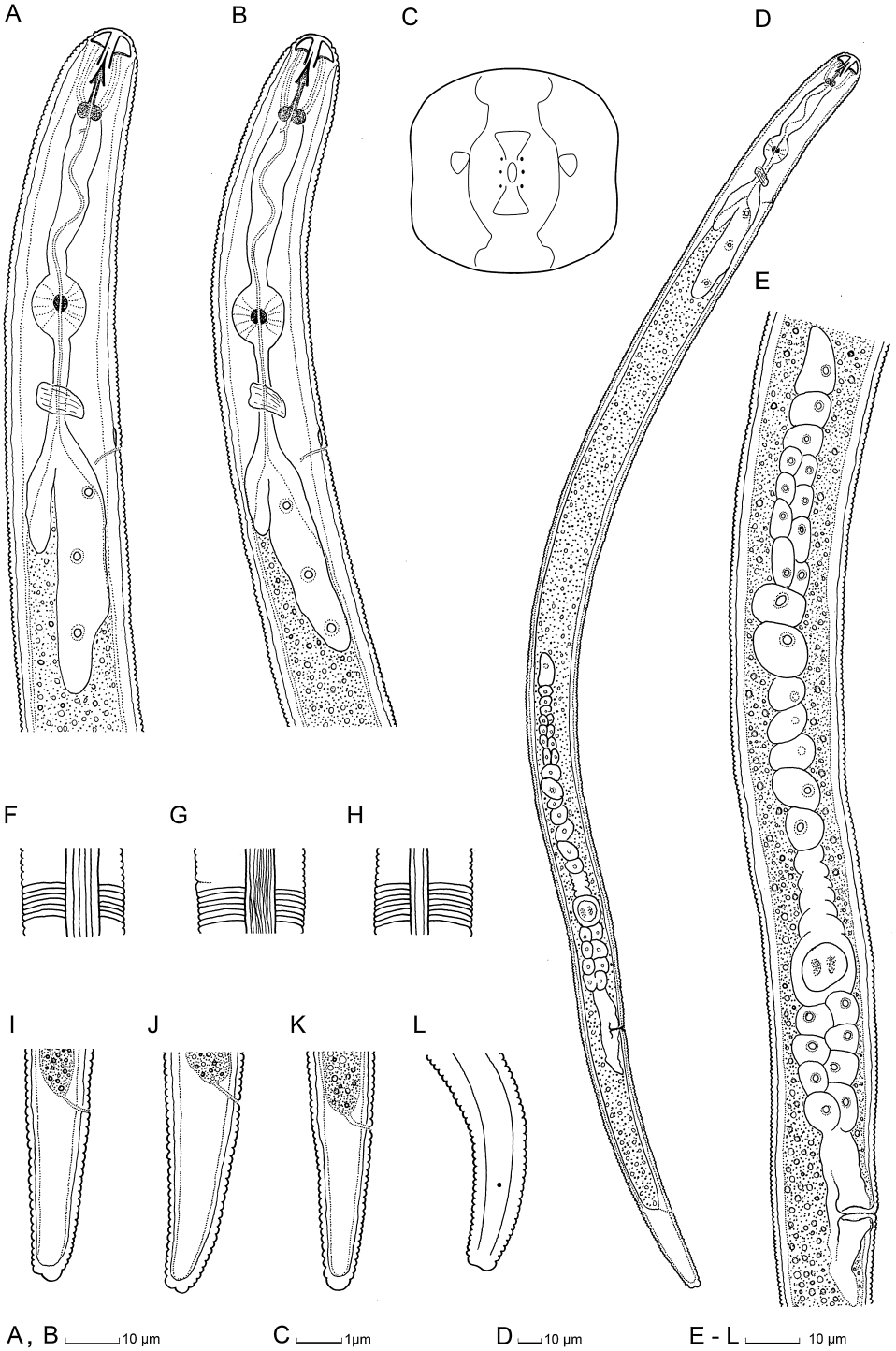

Fig. 1. *Pratylenchus rwandae* n. sp. in lateral view. A, B: Female anterior region (A: Holotype female); C: *En face* view; D: Entire body of female holotype; E: Female reproductive system; F-H: Variation in lateral line number in different regions of female body (F: Mid-body region; G: Vulval region; H: Pharynx region); I-L: Variation in female tail shape.

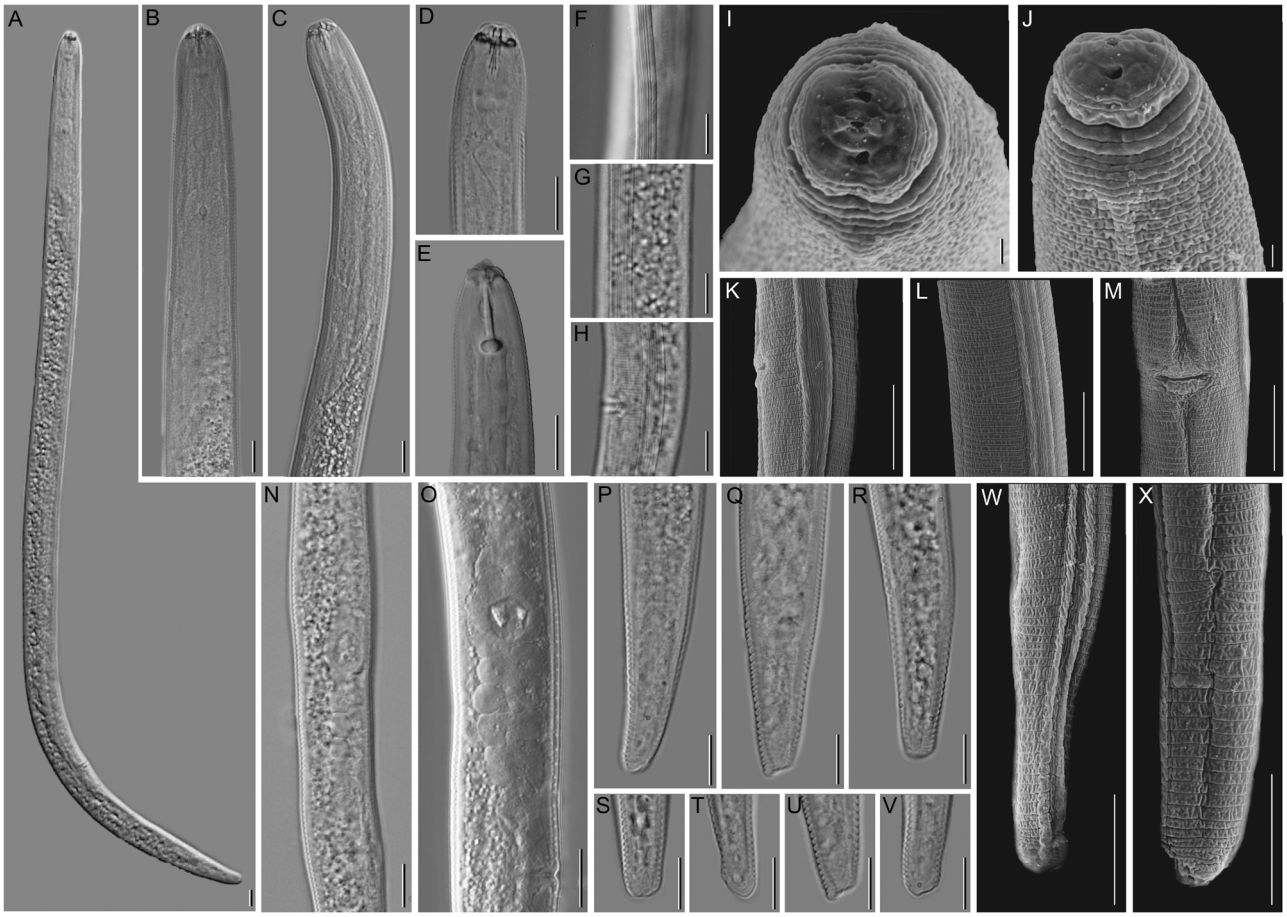

Fig. 2. Photomicrographs and SEM photographs of *Pratylenchus rwandae* n. sp. paratypes. A: Entire female body; B-E: Female anterior and neck regions; F-H: Lateral field in female mid-body regions and vulval region; I, J: SEM images of female head region; K-M: SEM images of lateral field in vulval region and mid-body region and vulva; N, O: Female reproductive system; P-V: Variation in tail shape and tail tip of female; W, X: SEM images of female tail. (Scale bars = 1 μm for SEM images and 10 μm for photomicrographs.)

tween outer two ridges. Labial region flattened to low rounded, offset by a constriction from rest of body, with three annuli, first of which fused with oval oral disc under SEM. *En face* view showing an oval oral aperture surrounded by six inner labial sensilla. Submedian sectors fused to oral disc, slightly separated from lateral sectors, and narrower at their inner extremity but widening towards outer edge of face and with amphidial apertures being on their inner edges (Group 2 according to Corbett & Clark, 1983). Cephalic framework strongly sclerotised, extending toward anterior border of first body annulus. Well-developed strong stylet, sclerotised conus and shaft with strongly developed rounded to slightly anteriorly concave basal knobs. Pharynx with dorso-pharyngeal gland opening located at 2.7-3.6 μm posterior to stylet base. Round to slightly oval medium bulb occupying almost half of corresponding body diam. with strong conspicuous valve 1.7-2.6 μm wide. Hemizonid immediately anterior to secretory-excretory pore or at *ca* 3 annuli anterior. Secretory-excretory pore and canal located posterior to, or at level of, pharyngo-intestinal junction, pharyngeal glands with their nuclei in tandem, strongly to slightly overlapping intestine ventrally. Reproductive system monodelphic with long, outstretched ovary, not reaching the pharyngeal gland overlap, oocytes arranged in a single row, oviduct narrow, spermatheca oval to rounded, empty and in some specimens, including holotype, unknown secretions found in lumen. Vulva in form of a transverse slit in ventral view, with cuticular folding at its edges, PUS 20.3-26.5 μm long. Tail with 18-28 annuli,

Table 1. Morphometric data for female *Pratylenchus rwandae* n. sp. from glycerin-fixed specimens. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Holotype	Paratypes
n	–	14
L	600	531 ± 44 (469-600)
a	22.0	24.5 ± 3.1 (19.8-29.6)
b	6.1	5.7 ± 0.4 (5.2-6.8)
b'	4.4	4.8 ± 0.6 (4.0-6.2)
c	17	16.3 ± 1.9 (12.8-20.1)
c'	2.5	2.4 ± 0.3 (1.8-2.7)
V	77.4	77.6 ± 1.4 (75.3-79.7)
m	40.3	47.0 ± 3.5 (41.2-52.2)
o	28	22.7 ± 2 (19.4-28.7)
S	0.8	0.8 ± 0.07 (0.75-1.0)
MB	60.4	61.2 ± 3.6 (54.4-68.5)
Distance from head to vulva	464	409 ± 40 (338-464)
Max. body diam.	27	22 ±1.8 (19-25)
Anterior end to centre of metacorpus	60	55 ± 2.7 (51-60)
Anterior end to nerve ring	75	70 ± 4.6 (61-78)
Anterior end to secretory-excretory pore	84	76 ± 12.2 (50-90)
Anterior to pharyngo-intestinal junction	99	91 ± 6.6 (82-107)
Pharyngeal overlap	26	22 ± 6.6 (11-35)
Body diam. at metacorpus	22	19 ± 1.6 (17-22)
Body diam. at vulval region	23	19 ± 1.8 (15-23)
Anterior genital tract length	205	142 ± 25.3 (95-206)
Post-vulval uterine sac	26	23 ± 1.8 (20-26)
Spermatheca-vagina	54	45 ± 9.2 (31-59)
Spermatheca length	15.3	13.3 ± 2.1 (9.7-17.8)
Spermatheca width	11.1	10.3 ± 1.9 (8.0-14.9)
Vulva to anus distance	99	80 ± 11.4 (64-101)
Anal body diam.	14.4	13.6 ± 1.7 (10.9-18)
Tail length	36	32 ± 2.9 (26-37)
Lateral field width at vulva	5.6	6.1 ±1.3 (4.7-9.4)
Dorsal gland opening from stylet base	3.7	3.1 ± 0.3 (2.7-3.9)
Stylet length	13.2	13.6 ± 0.5 (13.0-14.6)
Conus length	5.5	5.8 ± 0.3 (5.3-6.4)
Shaft length	5.3	5.5 ± 0.3 (5.1-6.1)
Stylet knob height	2.5	2.3 ± 0.3 (1.9-2.7)
Stylet knob width	4.7	4.7 ± 0.5 (3.4-5.1)
Body diam. at conus	13.6	14.2 ± 1.02 (13.0-16.1)
Labial disc diam.	2.7	2.7 ± 0.3 (2.3-3.2)
Lip region diam.	9.1	9.4 ± 0.5 (8.5-10.4)
Lip region height	3.7	4.0 ± 0.6 (3.1-5.0)

subcylindrical and conical towards tip. Tail tip smooth, varying in shape from rounded to truncate or indented. Phasmid at or posterior to mid-tail level.

Male

Absent and no sperm seen in female genital tract.

TYPE HOST AND LOCALITY

Pratylenchus rwandae n. sp. was collected from the rhizosphere and root of maize in a harvested field at Nyamata sector, Bugesera District, Eastern province of Rwanda, during the drought season, the period that covers mid-June to mid-October (vernacularly called

"impeshyi"). This location has a tropical climate with an average temperature of 20-30°C, an altitude of 1360 m a.s.l. and an average precipitation of 900 mm. The GPS coordinates of the location are 2°08′50.8″S 30°05′03.4″E.

Type material

Holotype female, two female and three juvenile paratypes, all in one slide, are deposited at the Ghent University Museum, Zoology Collections, collection number UGMD 104346. Additional paratypes (three females and three juveniles in one slide) are available in the UGent Nematode Collection (slide UGnem-176) of the Nematology Research Unit, Department of Biology, Ghent University, Ghent, Belgium. The new species name has been registered in ZooBank (http://www.zoobank.org/References/CD0103C0-E60C-4800-BE0F-A30144FFE164).

Diagnosis and relationships

Pratylenchus rwandae n. sp. is characterised by a combination of the following traits. Relatively long body (531 ± 44 (469-600) μm), labial region *en face* view flat with an amalgamated oval oral disc and the first annulus, followed by other two annuli, the third annulus thicker than the other two. Lip region offset by a deep constriction from the rest of the body, short to long robust stylet (13.6 ± 0.5 (13.0-14.6) μm) with prominent rounded or anteriorly concave knobs. Short to long pharyngeal gland overlap. Four lateral lines at pharynx level, six or more at mid-body and vulval regions. Spermatheca oval to rounded and generally empty or with unknown secretions. Tail subcylindrical to conoid with highly variable tail tip. Males were not found. Matrix code for the tabular key proposed by Castillo & Vovlas (2007): A2, B1, C2, D3(2), E2, F4(3), G2(3), H1, I1(2), J3, K1 (variation in characters is given in brackets). In Janssen *et al.* (2017b), the number of lateral lines in the vulval region of the same species was described as four with the code J(1) and a reduced spermatheca with the code D(1). However, a closer look revealed the presence of two outer broad ridges with numerous narrow ridges in between at the vulval region and also the presence of a relatively well-developed spermatheca (Fig. 2).

Based on the tabular key of Castillo & Vovlas (2007), *P. rwandae* n. sp. belongs to Group 2 of *Pratylenchus* species with three lip annuli (feature A) among which only four species, *P. arlingtoni* (= *species inquirenda*), *P. crenatus* Loof, 1960, *P. manaliensis* Khan & Sharma, 1992, and *P. teres* Khan & Singh, 1974 have more than six lateral lines at the vulval region (feature J) (Table 2). They also have smooth lateral field lines at the vulval region (feature K) and either a subcylindrical or conoid female tail shape (feature H). *Pratylenchus rwandae* n. sp. can be differentiated from all of them based on the presence of smooth tail tip *vs* generally striated tail tip in all four species and vulva position (feature E) (V =

Table 2. Comparison of matrix codes of tabular key for *Pratylenchus* spp. identification, proposed by Castillo & Vovlas (2007), between *Pratylenchus rwandae* n. sp. (asterisk) and its morphologically and molecularly related species.

Species	A	B	C	D	E	F	G	H	I	J	K
P. convallariae	2	2	3	2	2	6	2	2	3	1	1
P. crenatus	2	2	3	1	3	3	2	2	1	3	1
P. dunensis	1	2	3	2	2	4	1	1	4	1	2
P. fallax	2	2	3	2	2	3	3	2	2	1	1
P. manaliensis	2	2	2	4	3	3	3	2	4	3	1
P. oleae	2	1	2,3	2	2,3	3,4,5	2,3	2	1,2	1	2
P. penetrans	2	2	3	2	3	4	2	1	3	1	1
P. pinguicaudatus	2	1	3	1	3	1	2	1	4	1	2
P. rwandae n. sp.*	2	1	2	2,3	2	3,4	2,3	1	1,2	3	1
P. teres	2	1	2	1	1	3	3	2	3	3	1
Pratylenchus sp. 1	2	2	3	2	3	4	2	1	4	1	1
Pratylenchus sp. 2	2	1	2	1	2	1	2	1	3	1	1
Pratylenchus sp. 4	2	1	3	2	2	4	1	1,2	3	1	1

Three undescribed species, which were morphologically and molecularly characterised by Janssen *et al.* (2017b), have been added to the table. The codes A-K represent various morphological features and the numbers represent variations of the characters (for detailed interpretation, refer to Castillo & Vovlas, 2007).

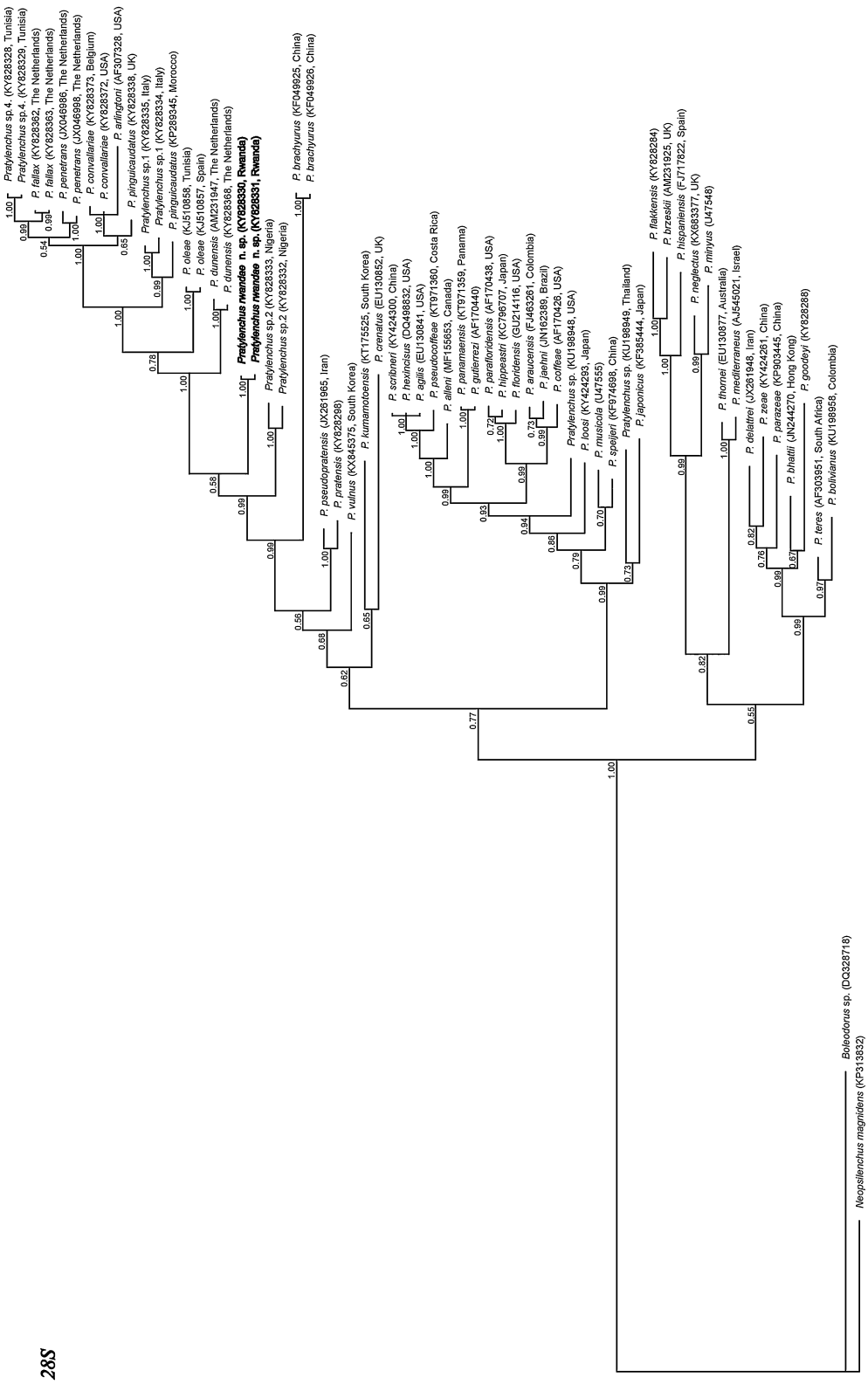

Fig. 3. Phylogenetic relationships of *Pratylenchus rwandae* n. sp. with 50 *Pratylenchus* spp. Bayesian 50% majority consensus tree as inferred from D2-D3 expansion segments of 28S rDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The newly described species is highlighted in bold.

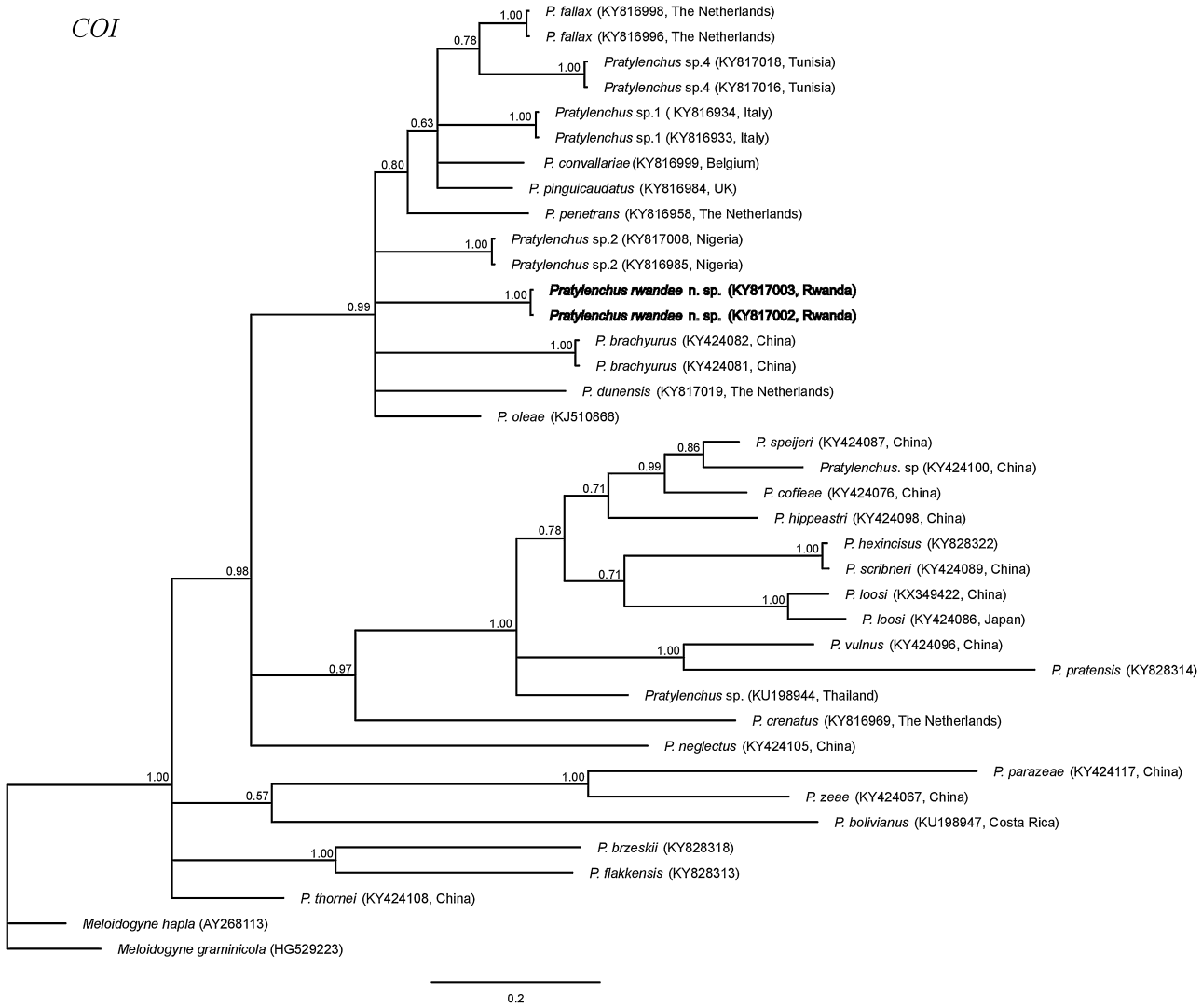

Fig. 4. Phylogenetic relationships of *Pratylenchus rwandae* n. sp. with 29 *Pratylenchus* spp. Bayesian 50% majority consensus tree as inferred from *COI* of mitochondrial DNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The newly described species is highlighted in bold.

77.6 ± 1.4 (75.3-79.7) *vs* 80-85) for *P. crenatus* and *P. manaliensis* and V ⩽ 75 for *P. teres*. Additionally, in this group of species, which includes our new species, only *P. manaliensis* has a rectangular spermatheca.

Pratylenchus rwandae n. sp. is also clearly different from its molecularly closely related species, including *P. convallariae* Seinhorst, 1959b, *P. dunensis* de la Peña, Moens, van Aelst & Karssen, 2006, *P. fallax* Seinhorst, 1968, *P. oleae*, *P. penetrans*, and *P. pinguicaudatus* Corbett, 1969. They all have a stylet length (feature C) of 13-18 μm, a vulva position of 75-85%, and either a smooth or striated female tail tip. Among them, *P. oleae* has eight out of 11 features in common with the new species (Table 2). *Pratylenchus dunensis* differs from all these species by the presence of three lip annuli and a conoid or subcylindrical tail. *Pratylenchus rwandae* n. sp. can be differentiated from all of the above species based on the lateral field and ridges around the vulval region. Table 2 gives a comparison of the matrix codes of the tabular key proposed by Castillo & Vovlas (2007) for the above-mentioned morphologically and molecularly related *Pratylenchus* species.

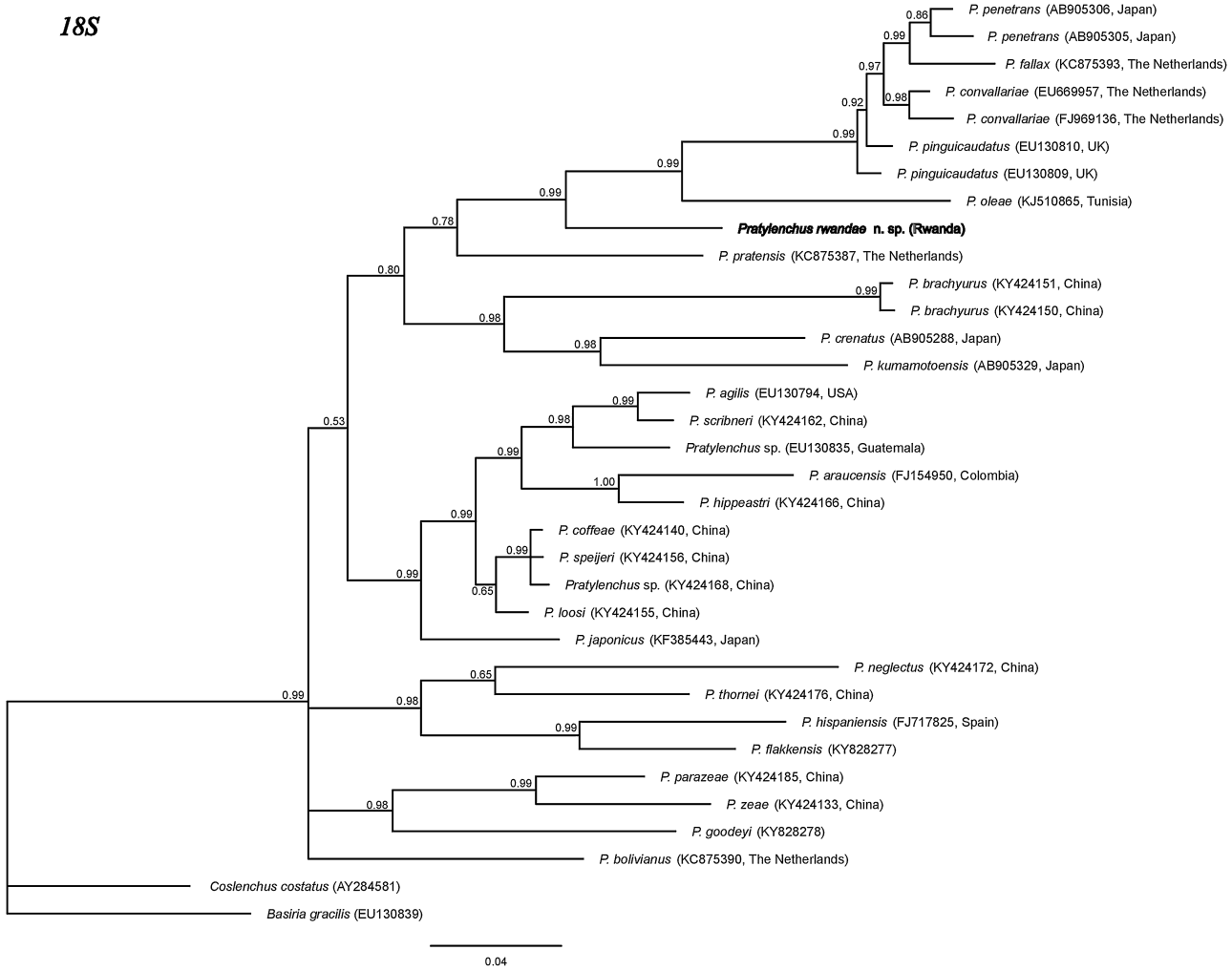

Fig. 5. Phylogenetic relationships of *Pratylenchus rwandae* n. sp. with 28 *Pratylenchus* spp. Bayesian 50% majority consensus tree as inferred from 18S of rDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The newly described species is highlighted in bold.

MOLECULAR ANALYSES

In this study, we analysed the phylogenetic relationships of *P. rwandae* n. sp. using four different genetic markers: the D2-D3 of 28S rRNA gene with an alignment of 61 *Pratylenchus* sequences from 50 species (Fig. 3); *COI* of the mitochondrial gene with an alignment of 36 *Pratylenchus* sequences from 29 species (Fig. 4); the 18S of rRNA gene with an alignment of 32 *Pratylenchus* sequences from 28 species (Fig. 5); and the ITS of rRNA gene with an alignment of 55 *Pratylenchus* sequences from 42 species (Fig. 6). The pairwise comparison of 18S of rDNA sequence (MG835610) of *P. rwandae* n. sp. with other 18S rDNA *Pratylenchus* sequences from GenBank gave the highest sequence similarity of 90.6% with *P. pinguicaudatus* (EU130809). Similarly, for D2-D3 of 28S rDNA sequence (KY828330), *COI* of mitochondrial DNA sequence (KY817002) and ITS of rDNA sequence (KY828259), the highest sequence similarity were seen with *Pratylenchus* sp. 2 (87%; KY828333), with *P. oleae* (82.6%; KJ510866), and with *P. dunensis* (70.5%; KY828245), respectively. Thus, the sequences of the new species are clearly different to all known sequences. All the phylogenetic trees obtained were found to have congruent tree topologies except for the positions of several weakly supported clades. In the D2-D3 phylogeny,

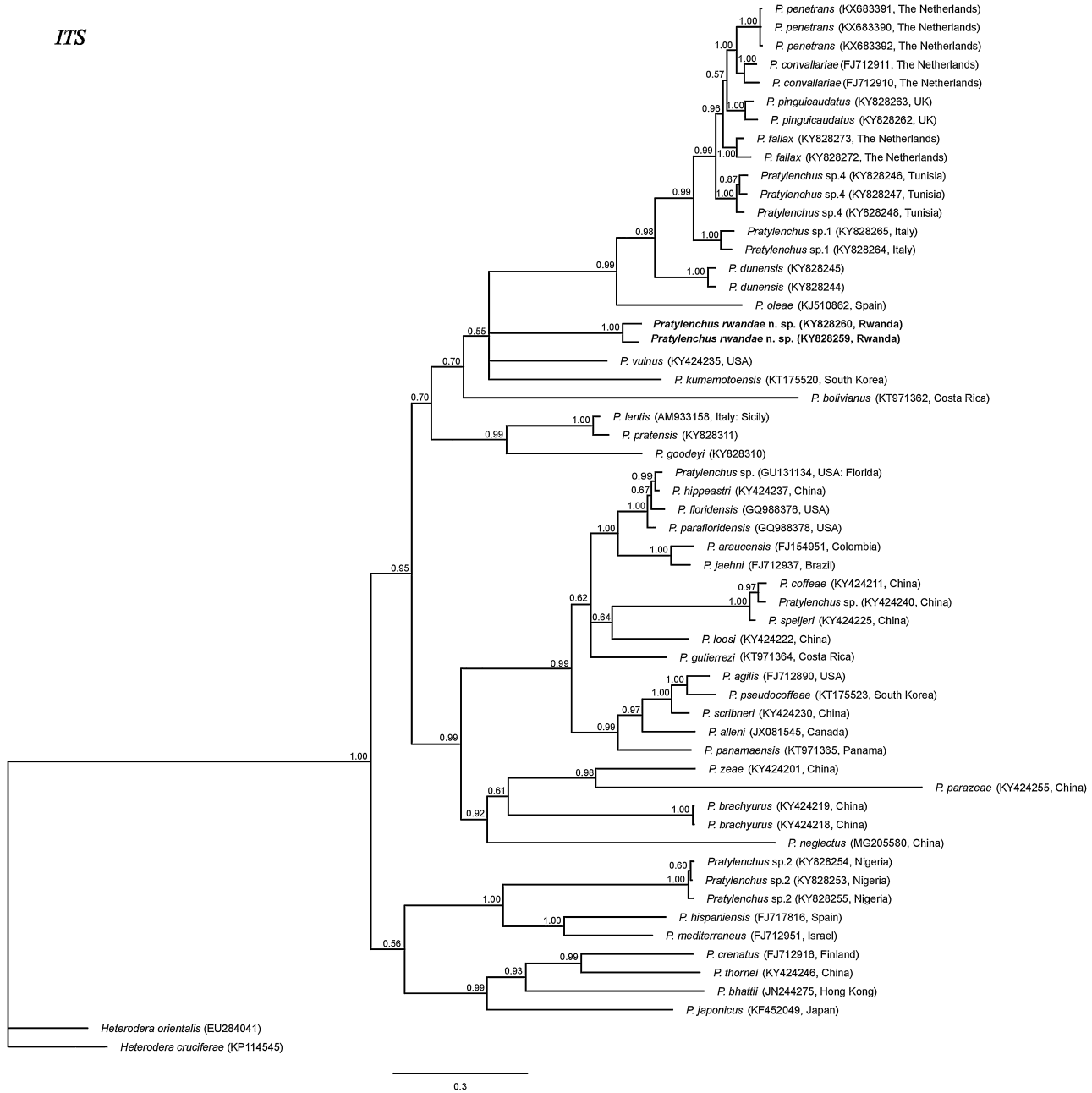

Fig. 6. Phylogenetic relationships of *Pratylenchus rwandae* n. sp. with 42 *Pratylenchus* spp. Bayesian 50% majority consensus tree as inferred from ITS of rDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The newly described species is highlighted in bold.

P. rwandae n. sp. formed a monophyletic group with *P. convallariae*, *P. dunensis*, *P. fallax*, *P. oleae*, *P. penetrans*, *P. pinguicaudatus*, and three undescribed species (*Pratylenchus* sp. 1, 2, 4), a clade previously referred to as the *Penetrans* group (Janssen *et al.*, 2017b). A similar clade was also seen from the *COI* tree, but with unresolved positions of several species. The clade was well supported (PP = 0.99) and the position of *P. rwandae* n. sp. was well resolved in the 18S tree. The ITS tree did not provide a clear resolution of the position of our new species.

Taken all together, our analyses indicated that *P. rwandae* n. sp. occupied a sister position to a clade comprising *P. convallariae*, *P. dunensis*, *P. fallax*, *P. oleae*, *P. penetrans*, *P. pinguicaudatus*, and three undescribed species. Our tree topologies are also in agreement with the phylogenetic trees of *Pratylenchus* based on D2-D3 of 28S rDNA, ITS of rDNA and *COI* of mitochondrial DNA in Janssen *et al.* (2017b).

Discussion

Pratylenchus is an economically important genus of plant-parasitic nematode with a world-wide distribution. *Pratylenchus rwandae* n. sp. was found in soil samples from a maize field in Rwanda in moderate numbers along with other plant-parasitic nematodes such as *Rotylenchus* Filipjev, 1936 and *Scutellonema* Andrássy, 1958 but with no other *Pratylenchus* spp. The impact of this new root-lesion nematode on its hosts is still unknown and remains to be investigated.

Although *Pratylenchus* are notorious for the difficulty in identifying them at species level, this species could be differentiated from other described *Pratylenchus* species by combined efforts of morphological and molecular analyses. Out of all the related species, *P. oleae* seems to be the closest relative of *P. rwandae* n. sp., both morphologically and molecularly. Their discrimination was possible using the tabular and dichotomous keys of Castillo & Vovlas (2007) according to which they can be distinguished based on female tail tip and the number of lateral lines in the vulval region. The new species is also similarly related to and distinguished from *P. penetrans* and *P. fallax* using these aforementioned morphological characters. Remarkably, *P. dunensis* appears to be molecularly closely related to *P. rwandae* n. sp., but has distinct morphological differences in the number of lip annuli, female tail shape, length of pharyngeal overlap and the number of lateral lines in the vulval region. On the other hand, the molecularly distantly related *P. teres* was found to share certain morphological features, including the presence of three lip annuli and more than six lateral lines in the vulval region, *etc.* However, the molecular information for *P. teres* is limited (only 28S sequences are available on GenBank) and therefore more sequence information is needed to draw a better conclusion of its relationship with the new species.

The usefulness of morphological characters in nematode species discrimination is still evident from our current study. However, morphological characters should be used with extreme care and these features alone are often not sufficient as several *Pratylenchus* species are known that are morphologically very similar and yet molecularly distantly related and *vice versa*. Thus, the combination of both morphological and molecular studies is preferred to provide a more substantiated species discrimination. Especially important for *Pratylenchus* is the link between DNA sequences and morphological characters, this being of crucial importance in order to prevent misidentifications and mislabelling sequences in public databases (Janssen *et al.*, 2017a).

Our trees were found to be consistent with the analyses by Janssen *et al.* (2017b) based on 28S and ITS of rDNA and *COI* of mitochondrial DNA sequences. *Pratylenchus* sp. 3 (= *P. rwandae* n. sp.) was also put forward by Janssen *et al.* (2017b) as one of the early-branching asexual lineages of the *Penetrans* group (clade IV in Subbotin *et al.*, 2008; clade IV without *P. brachyurus* in Palomares-Rius *et al.*, 2014). In this paper, only the 18S rDNA-based analyses resolved the position of *P. rwandae* n. sp. Hence, although all the markers analysed could discriminate *Pratylenchus* spp., 18S of rDNA sequence appears to be superior in terms of tree resolution for *Pratylenchus* in our study. The superior resolution of 18S-based phylogeny in some nematode groups has also been demonstrated by various other works (Holterman *et al.*, 2006; Qing *et al.*, 2017).

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References

Arim, O., Waceke, J., Waudo, S. & Kimenju, J. (2006). Effects of *Canavalia ensiformis* and *Mucuna pruriens* intercrops on *Pratylenchus zeae* damage and yield of maize in subsistence agriculture. *Plant and Soil* 284, 243-251. DOI: 10.1007/s11104-006-0053-9

Bert, W., Leliaert, F., Vierstraete, A.R., Vanfleteren, J.R. & Borgonie, G. (2008). Molecular phylogeny of the Tylenchina and evolution of the female gonoduct (Nematoda: Rhabditida). *Molecular Phylogenetics and Evolution* 48, 728-744. DOI: 10.1016/j.ympev.2008.04.011

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.

Corbett, D.C.M. (1969). *Pratylenchus pinguicaudatus* n. sp. (Pratylenchinae: Nematoda) with a key to the genus *Pratylenchus*. *Nematologica* 15, 550-556. DOI: 10.1163/187529269X00885

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

de la Peña, E., Moens, M., van Aelst, A. & Karssen, G. (2006). Description of *Pratylenchus dunensis* sp. n. (Nematoda: Pratylenchidae), a root-lesion nematode associated with the dune grass *Ammophila arenaria* (L.) link. *Nematology* 8, 79-88. DOI: 10.1163/156854106776179917

De Waele, D. & Jordaan, E. (1988). Plant-parasitic nematodes on field crops in South Africa. 1. Maize. *Revue de Nématologie* 11, 65-74.

De Waele, D., Mc Donald, A., Jordaan, E., Orion, D., Van den Berg, E. & Loots, G. (1998). Plant-parasitic nematodes associated with maize and pearl Millet in Namibia. *African Plant Protection* 4, 113-117.

Doucet, M., Lax, P., Di Rienzo, J.A., Pinochet, J. & Baujard, P. (2001). Temperature-induced morphometric variability in an isolate of *Pratylenchus vulnus* Allen & Jensen, 1951 (Nematoda: Tylenchida). *Nematology* 3, 1-8. DOI: 10.1163/156854101300106829

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

Egunjobi, O.A. (1974). Nematodes and maize growth in Nigeria I. Population dynamics of *Pratylenchus brachyurus* in and about the roots of maize and its effects on maize production at Ibadan. *Nematologica* 20, 181-186.

Filipjev, I.N. (1936). On the classification of the Tylenchinae. *Proceedings of the Helminthological Society of Washington* 3, 80-82.

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

Handoo, Z.A., Carta, L.K. & Skantar, A.M. (2001). Morphological and molecular characterisation of *Pratylenchus arlingtoni* n. sp., *P. convallariae* and *P. fallax* (Nematoda: Pratylenchidae). *Nematology* 3, 607-618. DOI: 10.1163/156854101753389220

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., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J. & Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* 23, 1792-1800. DOI: 10.1093/molbev/msl044

Hooper, D.J. (2005). Extraction and processing of plant and soil nematodes. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*, 2nd edition. Wallingford, UK, CAB International, pp. 45-68.

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

Inomoto, M., Oliveira, C., Mazzafera, P. & Gonçalves, W. (1998). Effects of *Pratylenchus brachyurus* and *P. coffeae* on seedlings of *Coffea arabica*. *Journal of Nematology* 30, 362-367.

Inserra, R., Duncan, L., Dunn, D., Kaplan, D. & Porazinska, D. (1998). *Pratylenchus pseudocoffeae* from Florida and its relationship with *P. gutierrezi* and *P. coffeae*. *Nematologica* 44, 683-712. DOI: 10.1163/005725998X00087

Janssen, T., Karssen, G., Couvreur, M., Waeyenberge, L. & Bert, W. (2017a). The pitfalls of molecular species identification: a case study within the genus *Pratylenchus* (Nematoda: Pratylenchidae). *Nematology* 19, 1179-1199. DOI: 10.1163/15685411-00003117

Janssen, T., Karssen, G., Orlando, V., Subbotin, S.A. & Bert, W. (2017b). Molecular characterisation 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

Karamura, E., Namanya, P., Gowen, S. & Namaganda, J. (1996). Studies on host range of the banana lesion nematode, *Pratylenchus goodeyi*. In: *International Conference on Banana and Plantain for Africa*, p. 540.

Khan, E. & Singh, D.B. (1975). Five new species of *Pratylenchus* (Nematoda: Pratylenchidae) from India. *Indian Journal of Nematology* 4, 199-211.

Khan, M.L. & Sharma, N.K. (1992). A description of *Pratylenchus manaliensis* sp. n. associated with apple in India (Nematoda: Pratylenchinae). *Nematologia Mediterranea* 19, 275-277.

Kimenju, J., Waudo, S., Mwang'ombe, A., Sikora, R.A. & Schuster, R. (1998). Distribution of lesion nematodes associated with maize in Kenya and susceptibility of maize cultivars to *Pratylenchus zeae*. *African Crop Science Journal* 6, 367-375.

Koenning, S.R., Overstreet, C., Noling, J.W., Donald, P.A., Becker, J.O. & Fortnum, B.A. (1999). Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. *Journal of Nematology* 31, 587-618.

Lax, P., Doucet, M., Di Rienzo, J.A., Pinochet, J. & Baujard, P. (2004). Inter-population variability in *Pratylenchus vulnus* Allen & Jensen, 1951 (Nematoda: Tylenchida). *Nematology* 6, 257-260. DOI: 10.1163/1568541041217906

Loof, P.A.A. (1960). Taxonomic studies on the genus *Pratylenchus* (Nematoda). *Tijdschrift ober Plantenziekten* 66, 29-90.

Loof, P.A.A. (1978). The genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae): a review of its anatomy, morphology, distribution, systematics and identification. *Sveriges Landbruksuniversiteit Växtskyddsrapporter* 5, 1-12.

Loof, P.A.A. (1991). The family Pratylenchidae Thorne, 1949. In: Nickle, W.R. (Ed.). *Manual of agricultural nematology*. New York, NY, USA, Marcel Dekker, pp. 363-421.

Loubama, P.M., Sarah, J.L., Sakwe, P., Mavounghou, J.F., Gnonhouri, P., Boisseau, M. & Foure, E. (2007). Study of the genetic diversity of plant parasitic nematodes on bananas and plantains in central and west Africa. *African Crop Science* 8, 783-786.

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

Qing, X., Decraemer, W., Claeys, M. & Bert, W. (2017). Molecular phylogeny of *Malenchus* and *Filenchus* (Nematoda: Tylenchidae). *Zoologica Scripta*. DOI: 10.1111/zsc.12236

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. & Huelsenbeck, J. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539-542. DOI: 10.1093/sysbio/sys029

Ryss, A., Boström, S. & Sohlenius, B. (2005). Tylenchid nematodes found on the Nunatak Base, East Antarctica. *Annales Zoologici* 55, 315-324.

Seinhorst, J.W. (1959a). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4, 67-69. DOI: 10.1163/187529259X00381

Seinhorst, J.W. (1959b). Two new species of *Pratylenchus*. *Nematologica* 4, 83-86. DOI: 10.1163/187529259X00417

Seinhorst, J.W. (1968). Three new *Pratylenchus* species with a discussion of the structure of the cephalic framework and of the spermatheca in the genus. *Nematologica* 14, 497-510. DOI: 10.1163/187529268X00183

Siddiqi, M.R. (2000). *Tylenchida parasites of plants and insects*, 2nd edition. Wallingford, UK, CABI Publishing.

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

Talwana, H., Butseya, M. & Tusime, G. (2008). Occurrence of plant parasitic nematodes and factors that enhance population build-up in cereal-based cropping systems in Uganda. *African Crop Science Journal* 16, 119-131.

Tarte, R. & Mai, W.F. (1976). Morphological variation in *Pratylenchus penetrans*. *Journal of Nematology* 8, 185-195.

Todd, T.C. & Oakley, T.R. (1996). Seasonal dynamics and yield relationships of *Pratylenchus* spp. in corn roots. *Journal of Nematology* 28, 676-681.

Troccoli, A., De Luca, F., Handoo, Z.A. & Di Vito, M. (2008). Morphological and molecular characterization of *Pratylenchus lentis* n. sp. (Nematoda: Pratylenchidae) from Sicily. *Journal of Nematology* 40, 190-196.

Van Biljon, E. & Meyer, A. (2000). Reproduction of *Pratylenchus zeae* and *P. delattrei* on crops resistant to *Meloidogyne javanica* and *M. incognita* races 2 and 4. *African Plant Protection* 6, 43-45.

Viglierchio, D. & Schmitt, R.V. (1983). On the methodology of nematode extraction from field samples: Baermann funnel modifications. *Journal of Nematology* 15, 438.

Wang, H.H., Zhuo, K., Ye, W.M. & Liao, J.L. (2015). Morphological and molecular characterisation 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

Yoder, M., De Ley, I.T., King, I.W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L. & De Ley, P. (2006). DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* 8, 367-376. DOI: 10.1163/156854106778493448

Youssef, M. (2013). Yield of maize as influenced by population densities of the root lesion nematode, *Pratylenchus zeae*. *Archives of Phytopathology and Plant Protection* 46, 483-486.