BRILL

Nematology 23 (2021) 381-413

Nematology
brill.com/nemy

The taxonomic status of *Aphelenchoides besseyi* Christie, 1942 (Nematoda: Aphelenchoididae) populations from the southeastern USA, and description of *Aphelenchoides pseudobesseyi* sp. n.

Sergei A. SUBBOTIN [1,2,*], Clemen J. OLIVEIRA [3], Sergio ÁLVAREZ-ORTEGA [4], Johan A. DESAEGER [3], William CROW [5], Charles OVERSTREET [6], Robert LEAHY [7], Silvia VAU [8] and Renato N. INSERRA [8]

[1] *Plant Pest Diagnostic Centre, California Department of Food and Agriculture, Sacramento, CA 95832-1448, USA*
[2] *Centre of Parasitology of A.N Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow 117071, Russia*
[3] *University of Florida, GCREC, Wimauma, FL 33598, USA*
[4] *Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, Campus de Móstoles, 28933-Madrid, Spain*
[5] *University of Florida, Department of Entomology and Nematology, P.O. Box 110620, Gainesville, FL 32611-0620, USA*
[6] *Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA*
[7] *USDA-APHIS-PPQ-CAPS, P.O. Box 147100, Gainesville, FL 32614-7100, USA*
[8] *Florida Department of Agriculture and Consumer Services, DPI, Nematology Section, P.O. Box 147100, Gainesville, FL 32614-7100, USA*

Received: 7 June 2020; revised: 14 July 2020
Accepted for publication: 14 July 2020; available online: 8 September 2020

Summary – Populations reviously identified as *Aphelenchoides besseyi* were studied. Using an integrated approach, the *A. besseyi* species complex contains several cryptic species: *A. besseyi sensu stricto*, *A. oryzae*, *A. pseudobesseyi* sp. n. and other putative undescribed species. A population from Florida strawberry morphologically fits the *A. besseyi* of both Christie and Allen and is considered the only representative of this species. A Louisiana rice population fitted the descriptions of *A. oryzae* of both Yokoo and Fortuner; PUS length was consistently less than one-third of VA. *Aphelenchoides oryzae*, parasitising rice and other monocots, was re-established based on morphological and molecular datasets. Three populations from Florida ornamental plants (*Dryopteris erythrosora*, *Echinacea* sp. and *Farfugium japonicum*) differed from those of the two above-mentioned species and are described as *A. pseudobesseyi* sp. n. Populations previously identified as '*A. besseyi*' from several countries were considered representatives of this new species, which usually had a large and conspicuous PUS, 8-14 μm wide and with a length greater than one-third of VA in 40-70% of studied specimens. Morphological variability made separation of *A. pseudobesseyi* sp. n. from *A. oryzae* and *A. besseyi* unreliable without the examination of numerous specimens and molecular analysis.

Keywords – *Aphelenchoides oryzae*, cryptic species, *Dryopteris erythrosora*, *Echinacea* sp., *Farfugium japonicum*, *Fragaria* × *Ananassa*, molecular analysis, morphology, *Oryza sativa*, phylogenetic analysis, rice white tip nematode, summer crimp nematode, taxonomy.

Aphelenchoides besseyi Christie, 1942 is a well known foliar nematode that parasitises rice (*Oryza sativa* L.), strawberry (*Fragaria* × *ananassa* Duchesne) and several other plants in many countries (Franklin & Siddiqi, 1972; De Waele, 2002; Anon., 2017). In the USA, infestations of *A. besseyi* on rice and ornamental plants have been reported to occur frequently in southeastern and some northeastern states such as Arkansas, Delaware,

* Corresponding author, e-mail: sergei.a.subbotin@gmail.com

DOI 10.1163/15685411-bja10048

Florida, Louisiana, Maryland, Mississippi, Texas, and Virginia (Allen, 1952; Christie, 1959; Duncan & Moens, 2013; Desaeger & Noling, 2017; Subbotin & Chitambar, 2018). In Florida, damage induced by the nematode to strawberry was prevalent from 1930 to 1950 but became uncommon afterwards for almost 60 years. Foliar nematode infestations on strawberry appeared again in 2016, likely due to the withdrawal of methyl bromide resulting in foliar and other nematode infestations on propagative strawberry runners produced in nurseries, and consequently in strawberries planted in production fields in Florida (Desaeger & Noling, 2017).

The taxonomic status of foliar nematodes with a stellate tail terminus has been a complicated issue since the description by Christie (1942) of a strawberry population of the foliar nematode, *A. besseyi*, from Willard, North Carolina. Several years later, Yokoo (1948) described another representative of *Aphelenchoides*, a rice nematode from Kyushu and Hokkaido in Japan, and named it as *A. oryzae* Yokoo, 1948. However, the overlap in many morphological characters of *A. besseyi* and *A. oryzae* prompted Allen (1952) to consider *A. oryzae* a junior synonym of *A. besseyi*. This synonymy has been accepted by all taxonomists for more than 60 years and has not been challenged due to a lack of molecular data on these species.

The subsequent redescriptions and morphological studies of *A. besseyi* have been based mainly on rice populations because rice is a more common and widespread crop than strawberry. The most accurate redescription and illustrations of the characters of the rice populations of *A. besseyi* were published by Fortuner (1970). The combination of these characters has been used as a criterion for the morphological identification of *A. besseyi* in all subsequent studies conducted on this nematode (Franklin & Siddiqi, 1972; Huang & Huang, 1974; Hunt, 1993; Shahina, 1996; Hockland, 2001; Lin *et al*., 2004; Tzeng & Lin, 2005; Khan *et al*., 2012; De Jesus *et al*., 2016; Oliveira *et al*., 2019).

Fortuner (1970) emphasised the importance of the variations in the stylet length, ranging from 11 to 13 μm, and in the position of the excretory pore at a level with, or anterior to the nerve ring. He included also the characteristics of the female spermatheca: prominent and packed with sperm; the post-vulval uterine sac (PUS) that is usually empty of sperm and short (one-fourth of the distance between vulva and anus); and the stellate tail mucron. The shape of the male spicules, which lack a condylus and have an inconspicuous rostrum, was another important differential character for the identification of this species. Based on these taxonomic studies, the foliar nematode populations with a stellate tail terminus on strawberry, rice and ornamental plants have been identified in the USA for decades as *A. besseyi* during routine nematological analyses for plant problems, certification, regulatory and nematode management purposes.

The results of recent molecular and phylogenetic analysis, however, using Florida populations of foliar nematodes from strawberry and other crops in China, have provided evidence that *A. besseyi* is indeed a species complex consisting of several cryptic species that are not well delimited morphologically (Oliveira *et al*., 2019; Xu *et al*., 2020). In these studies, phylogenetic relationships among these Florida and other populations from distant geographical areas, as inferred from analyses of 18S rRNA, 28S rRNA and partial *COI* gene sequences, indicated that populations of *A. besseyi sensu lato* from strawberry and those from rice and other ornamental plants grouped in separated clades in the phylogenetic trees. These findings support the idea for establishing the strawberry, rice and ornamental plant populations as separate species.

A species delimitation among these *A. besseyi s.l.* populations is very important both for nematode management and phytosanitary purposes and for the selection of crop cultivars resistant to the nematode. The separation of these populations as species requires a reconsideration of the taxonomic status of the rice white tip nematode, *A. oryzae*, and its reinstatement as a valid species that differs from the summer crimp nematode of strawberry, *A. besseyi sensu stricto*. It is important to note that delimiting these new species allows for the separation of a major important and quarantine-listed nematode parasite of rice, *A. oryzae*, from morphologically similar species of lesser or major economic importance. In addition, delimiting these species provides the means to classify other foliar nematode populations damaging ornamental plants and agronomic crops, such as cotton and soybean, as a new species that we name as *A. pseudobesseyi* sp. n.

In the USA, studies on the biology of *A. besseyi s.l.* indicate that rice is the only agronomic crop in the country known to be damaged by the nematode (Christie, 1959; Godoy *et al*., 2019). It is not known whether USA populations of this species complex parasitise agronomic crops other than rice, such as soybean (*Glycine max* (L.) Merr.), which is seriously damaged by the nematode in Brazil (Meyer *et al*., 2017).

The objectives of this study were: *i*) to delimit species boundaries among USA nematode samples previously identified as a *A. besseyi s.l.* by comparing their morphometrics, morphology including reproductive characteristics and molecular data; *ii*) to provide additional molecular characterisation and phylogenetic relationships within the *A. besseyi* species complex using the D2-D3 expansion segments of 28S rRNA, ITS rRNA and partial *COI* gene sequence analysis; *iii*) to differentiate populations damaging to ornamental plants and several agronomic crops detected and characterised in the USA (Florida) and other countries as a new species named *A. pseudobesseyi* sp. n.; and *iv*) to determine the ability of a population of *A. pseudobesseyi* sp. n. from the ornamental leopard plant (*Farfugium japonicum* (L.) Kitam.) to parasitise soybean seedlings in a growth chamber.

Materials and methods

Taxonomic samplings and morphological studies

Nematode populations used in this study were obtained from several sources in southeastern states of the USA (Table 1). Specimens were extracted from leaves and buds by incubating foliar and bud tissue fragments removed from nematode-infested plants for at least 3 h in Petri dishes. Nematode-infested rice and coneflower seeds were also incubated in tap water in Petri dishes for 1-8 h, respectively. The strawberry population studied by Oliveira *et al*. (2019) was re-measured and re-examined by using additional specimens that were maintained on infested strawberry plants kept in a glasshouse. A rice population was obtained from Mer Rouge, Louisiana,

Table 1. Species and populations of foliar nematodes of *Aphelenchoides* characterised in the present study.

Species	Location	Host	Sample codes	GenBank accession number			Source
				D2-D3 of 28S rRNA gene	ITS rRNA gene	*COI* mtDNA gene	
A. besseyi	USA, Florida	*Fragaria* × *ananassa* Duchesne	N17-00341, CD2415	MK564627, MK294342	MT271861	MK559497, MK303401	C. Oliveira, Oliveira *et al.* (2019)
A. oryzae	USA, Louisiana, Morehouse Parish, Mer Rouge	Rice (*Oryza sativa* L.)	N17-00400, CD2471	MT271867	MT271862, MT271863	MT267787	C. Overstreet
A. pseudobesseyi sp. n.	USA, Florida, Sumter County, Sumterville	Wood fern (*Dryopteris erythrosora* (D.C. Eat.) O. Kuntze)	N18-00052, CD2704	MT271871	MT271866	MT267790	W. Crow
A. pseudobesseyi sp. n.	USA, Florida, Alachua County, Gainesville	Cone-flower (*Echinacea* sp.)	N17-00754, CD2491	MT271868	MT271864	MT267789	W. Crow
A. pseudobesseyi sp. n.	USA, Florida, St. Johns County, St. Augustine	Leopard plant (*Farfugium japonicum* (L.) Kitam.)	N17-01199, CD2540 N17-01064, CD2541	MT271869, MT271870	MT271865	MT267791	R. Leahy
A. pseudobesseyi sp. n.	USA, North Carolina, Jackson County, Cullowhee	Soil	N19-01266, CD3097	MT271872	–	MT267788	C. Oliveira

where it was used in a host study conducted by Godoy *et al*. (2019). Specimens of this population were measured twice on 20 April 2017, and again after 28 months, in August 2019, after incubating rice seeds from the same stock population that were kept in a refrigerator. Two Florida populations from coneflower and leopard plant were also measured twice after 8 and 12 months from the first measurements using the same seeds and plant source. The repeated measurements were taken to verify the variability in the morphological characters of these populations. The Florida population from wood fern was measured once. An additional 19 specimens of the population from leopard plant were used for measurements of the genital tract of the females and to count spermatozoa in the PUS. These measurements and counts, not included in the tables, were used for the diagnosis of this population and to assess the range of variability of the anatomical structures of the genital tract. All the populations mentioned above were also used for molecular analysis. A few specimens extracted from soil from a strawberry nursery in Cullowhee, NC, USA, were only used for molecular analysis.

Light microscopic study

Nematodes in the water suspension from the Petri dishes were picked with an eyebrow hair stuck on the end of a mounted needle. Specimens were placed in a drop of tap water on a glass slide, narcotised by gentle heat and then mounted in water agar on a slide for measurements and photographs using a modified Esser's (1986) method. Some specimens were also killed by gentle heat, fixed in a solution of 4% formaldehyde + 1% propionic acid and processed to pure glycerin using Seinhorst's (1966) or De Grisse's (1969) methods and mounted in permanent slides. Nematode specimens were examined, measured and photographed using compound microscopes (Nikon and Zeiss) equipped with differential interference contrast.

Specimens of the foliar nematode populations were preliminarily identified to species using morphological and morphometric evaluation before conducting molecular analyses.

DNA extraction, PCR, sequencing and phylogenetic analysis

For molecular analyses, nematode DNA from foliar nematode samples was extracted from single or several individuals using proteinase K and then used for PCR as described by Tanha Maafi *et al*. (2003). The forward D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and the reverse D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) primers (Subbotin *et al*., 2006) amplifying the D2-D3 expansion segments of 28S rRNA gene, the forward TW81 (5′-GTT TCC GTA GGT GAA CCT GC-3′) and the reverse AB28 (5′-ATA TGC TTA AGT TCA GCG GGT-3′) primers (Tanha Maafi *et al*., 2003) amplifying the ITS1-5.8S-ITS2 of rRNA gene, and the forward COI-F1 (5′-CCT ACT ATG ATT GGT GGT TTT GGT AAT TG-3′) and the reverse COI-R2 (5′-GTA GCA GCA GTA AA ATA AGC ACG-3′) primers (Kanzaki & Futai, 2002) amplifying the *COI* mtDNA gene fragment, were used in the present study. New sequences were deposited in GenBank under accession numbers: MT267787-MT267791 (*COI* gene), MT271867-MT271871 (D2-D3 of 28S rRNA gene) and MT271861-MT271866 (ITS rRNA gene) as indicated in Table 1, phylogenetic trees and networks.

The new sequences were aligned using ClustalX 1.83 (Thompson *et al*., 1997) with their corresponding gene sequences published and deposited in GenBank (Subbotin *et al*., 2006; Ye *et al*., 2007; Cuc & Pilon, 2007; Zhuo *et al*., 2010; Khan *et al*., 2012; De Jesus *et al*., 2016; Sánchez-Monge *et al*., 2017; Favoreto *et al*., 2018; Oliveira *et al*., 2019; Wang *et al*., 2019; Xu *et al*., 2020, and others). The alignments for D2-D3 of 28S rRNA and *COI* gene were generated using default parameters, whereas the ITS rRNA gene alignment was made with following parameters: gap opening – 5, gap extension – 3. Phylogenetic and sequence analysis have been made as described by Oliveira *et al*. (2019). The alignments for ITS rRNA and *COI* gene sequences were also used to construct phylogenetic network estimation using statistical parsimony (SP) as implemented in POPART software (http://popart.otago.ac.nz) (Bandelt *et al*., 1999).

Species delimiting

Species delimiting within the *A. besseyi* species complex studied was undertaken using an integrated approach that considered morphological and morphometric evaluation combined with molecular-based phylogenetic inference (tree-based methods) and sequence analyses (genetic distance methods) (Sites & Marshall, 2004).

Parasitic habits

An experiment was conducted in a growth chamber impervious to phytoparasitic arthropod infestations to determine the ability of a population of *A. besseyi sensu*

lato from leopard plant to parasitise soybean 'Patriot'. Although offering only limited space for the pilot study, a growth chamber was necessary to avoid potential complications caused by microarthropods, which can produce similar symptoms to the nematode. Four soybean seeds were sown in a 1.7 l plastic pot containing autoclaved sand soil. Then, the soil surface of the potted seeds was covered with 8 g of partially necrotic leopard leaves that were infested with about 200 specimens of the nematode. An equal number of seeds was sown in a pot containing non-inoculated soil as control. The two inoculated and non-inoculated pots were placed in a temperature-controlled growth room at 24 ± 2°C and covered with transparent plastic boxes for 1 week to prevent evaporation and facilitate seed germination. After the removal of the plastic boxes, the emerged seedlings were monitored for nematode symptoms for 6 weeks. Procedures used for nematode extraction, plant care, detection of plant symptoms induced by the nematode and assessment of final nematode populations were described by Oliveira *et al*. (2019).

Results and discussion

Morphological and morphometric characterisation of the *Aphelenchoides* species and populations selected in this study is given below.

Aphelenchoides besseyi Christie, 1942
(Figs 2, 3 in Oliveira *et al*. (2019); Figs 1, 2 in the present article)

The strawberry population that occurs in Florida was well characterised by Oliveira *et al*. (2019). The examination of the morphology of the additional specimens of this population did not indicate major differences in their characters compared to those reported in the previous study. For the convenience of the reader, we are reporting our measurements, as well as those in Oliveira *et al*. (2019), the original description of the strawberry population by Christie (1942) and the subsequent, more complete, description by Allen (1952).

MEASUREMENTS

See Tables 2, 3.

DESCRIPTION (from Christie, 1942 and Allen, 1952)

The characters provided by Christie (1942) for the strawberry population include: "Postvulvar uterine sac short, narrow, inconspicuous, usually extending less than one-third distance from vulva to anus, rarely containing spermatozoa; ovary wide, showing several developing ova in a single cross section through its middle region; excretory pore slightly anterior to nerve ring;... stylet with moderately well developed basal swellings."

The redescription by Allen (1952) includes "Female (Neotype): Length 0.7 mm; a = 50; b = 10; c = 10; Vulva 70%; ovary 33%; posterior uterine branch 6% (3 times body width). Body slender. Cuticle marked by fine striae. Lateral field occupying one-fourth of body diameter, marked by four incisures. Lip region expanded, wider than neck at base of lips. Lips without annulation. Six-radial head sclerotization delicate. Cheilorhabdions near oral aperture moderately sclerotized, and appearing as dark cuticularized pieces. Spear 10 μm long with moderately well-developed knobs. Median esophageal bulb well developed. Nerve ring one body width behind median bulb. Excretory pore located anterior to nerve ring. Esophageal gland extending 5 body widths behind median bulb, joining esophagus immediately behind median bulb. Intestine joining the esophagus as a slender tube immediately behind median bulb. Ovary relatively short. Oocytes not arranged in tandem, several in a cross section. Posterior uterine branch short, narrow, usually not containing spermatozoa. Tail tapering conoid. Terminus armed with four mucronate points. Mucrons usually divergent with star-shaped appearance. Male: Male tail curvature about 180 degrees when relaxed by gentle heat. Three pairs of ventro-submedian papillae, the anterior pair being adanal. Spicules ventrally curved. The ventral piece with a moderate ventral process at distal end. Terminus armed with four variable mucronate points."

REMARKS

Female morphology and morphometrics of the Florida population in this study and in that of Oliveira *et al*. (2019) agree with those reported by Christie (1942) and Allen (1952), except for the smaller body width than reported by Christie (1942) (13.3-17.8 *vs* 17-22 μm) and longer stylet than that of the neotype selected by Allen (1952) (10.9-12.5 *vs* 10 μm). The position of valvular apparatus in the metacorpus was variable and centrally located or in somewhat lower or upper portion of this organ. No information on the feature of the spermatheca

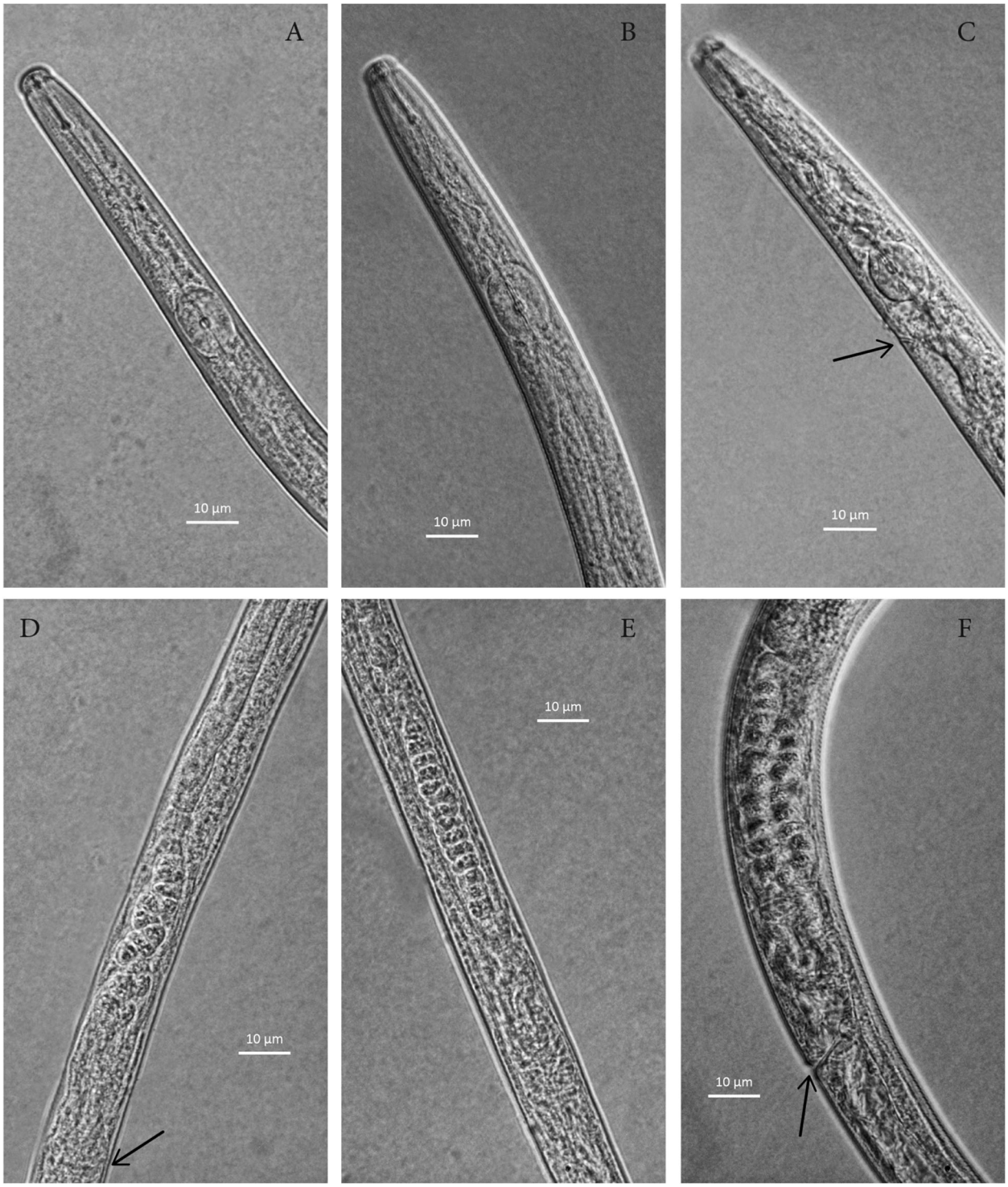


Fig. 1. Photomicrographs of *Aphelenchoides besseyi* female from *Fragaria* × *ananassa* in Florida. A-C: Anterior body region. Note different arrangement of valve in metacorpus and excretory pore (arrowed) located anterior to anterior margin of nerve ring; D-F: Spermathecae of different sizes and shapes showing spermatozoa arranged in single or multiple rows (arrow = vulva).

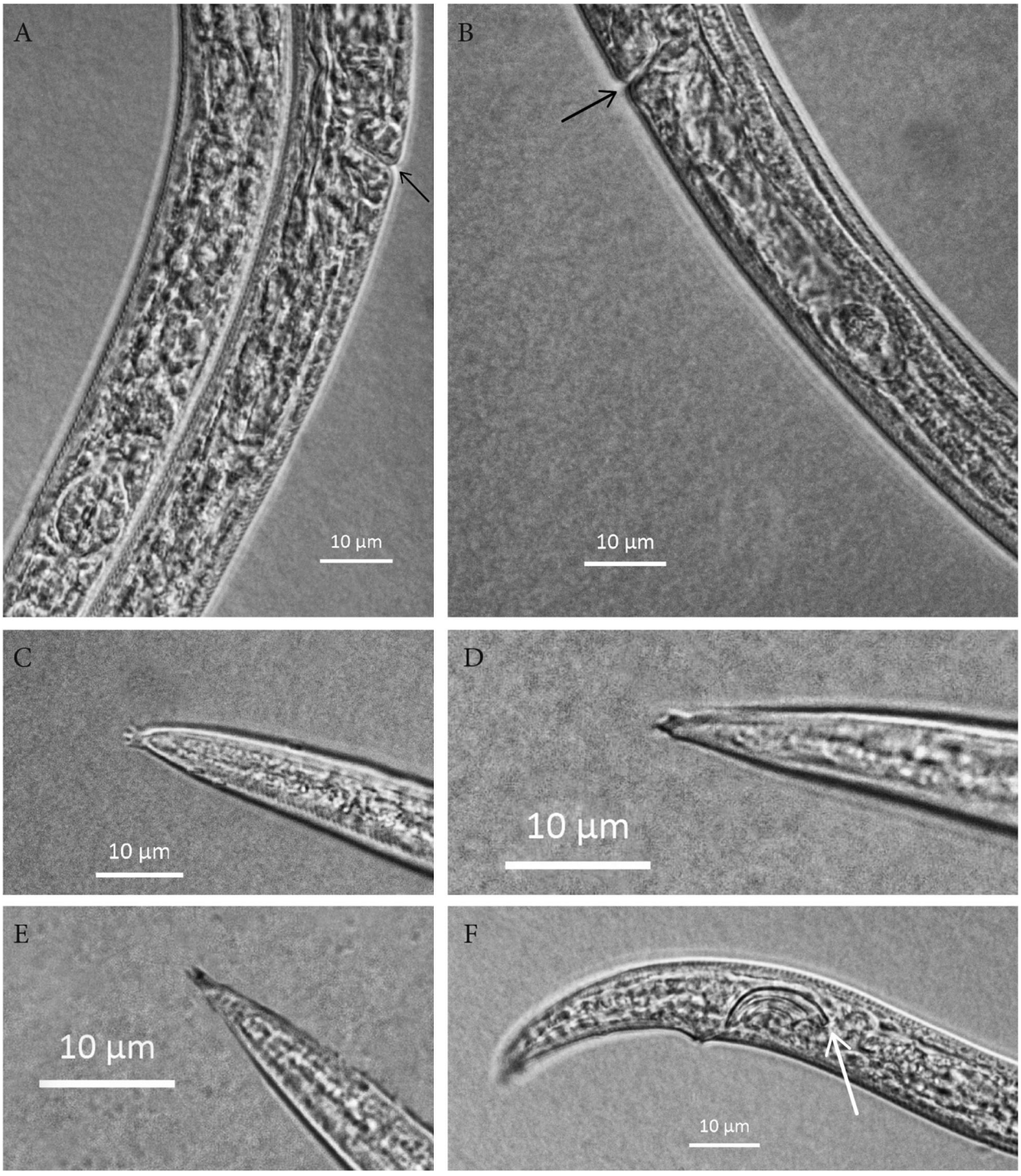


Fig. 2. Photomicrographs of *Aphelenchoides besseyi* female and male from *Fragaria* × *ananassa* in Florida. A, B: Posterior body sections of female showing post-vulval uterine branch (PUS) posterior to vulva (arrowed). Note an inconspicuous PUS devoid of spermatozoa in (A) and conspicuous PUS with a spermatozoon at distal end in (B); C-E: Female tails showing different shapes of terminal stellate mucro; F: Male tail showing spicules with conspicuous condylus (arrowed).

Table 2. Morphometrics of live females of a Florida population of *Aphelenchoides besseyi* from strawberry and from *Monilinia fructicola* cultures compared to those in the original description by Christie (1942) and a redescription by Allen (1952).

Character	Strawberry (N17-00341)		*Monilinia fructicola* (N18-01001-2)	Strawberry	
				Christie (1942)	Allen (1952)
n	15*	10**	10*	10	10
L	657 ± 75.9 (517-811)	620 ± 43.2 (560-694)	710 ± 23.6 (669-747)	– (660-750)	– (620-880)
a	41.7 ± 3.8 (34.0-49.7)	43.4 ± 3.4 (39.3-49.9)	50.3 ± 2.4 (45.3-53.3)	– (32-42)	– (38-58)
b	9.8 ± 0.6 (8.6-11.2)	9.3 ± 0.6 (8.3-10.3)	10.3 ± 0.2 (10.0-10.6)	– (10.2-11.4)	– (9-12)
b′	4.4 ± 0.2 (4.0-4.6)	4.1 ± 0.7 (3.3-6.2)	4.3 ± 0.1 (4.1-4.5)	–	–
c	16.6 ± 1.2 (14.8-19.1)	16.1 ± 0.9 (14.8-17.4)	17.6 ± 0.8 (16.1-18.9)	– (17-21)	– (15-20)
c′	3.9 ± 0.3 (3.6-4.7)	4.2 ± 0.2 (4.0-4.6)	4.0 ± 0.2 (3.8-4.4)	–	–
V	70.5 ± 0.5 (69.7-71.6)	69.6 ± 1 (68.1-71.5)	69.8 ± 0.6 (68.8-70.8)	– (68-70)	– (66-72)
Max. body diam.	15.7 ± 1.3 (13.3-17.8)	14.2 ± 0.5 (13.5-14.9)	14.1 ± 0.6 (13.4-15.2)	– (17-22)	–
Body diam. at anus	9.9 ± 0.7 (8.9-11.4)	9.0 ± 0.5 (8.2-10.0)	9.8 ± 0.5 (9.0-11.0)	–	–
G (Genital tract length/L (%))	26.8 ± 2.5 (23.2-31.1)	27.5 ± 1.8 (24.6-30.9)	25.9 ± 1.1 (24.3-27.6)	–	–
Anterior genital tract length	181 ± 19.2 (155-217)	171 ± 14.6 (145-196)	184 ± 9.7 (163-196)	–	–
Lip region diam.	6.9 ± 0.3 (6.0-7.5)	6.9 ± 0.05 (6.9-7.0)	6.9 ± 0.1 (6.7-7.0)	–	–
Lip region height	3.1 ± 0.2 (2.8-3.4)	3.0	3.0	–	–
Stylet length	12.0 ± 0.2 (11.5-12.5)	11.6 ± 0.3 (11.2-12.1)	11.0 ± 0.1 (10.9-11.2)	–	10
Stylet cone length	5.8 ± 0.3 (5.7-5.9)	5.3 ± 0.3 (5.0-5.9)	5.0 ± 0.1 (5.0-5.2)	–	–
Stylet knob height	1.7 ± 0.1 (1.5-1.9)	1.3 ± 0.1 (1.2-1.5)	1.6 ± 0.1 (1.5-1.7)	–	–
Stylet knob width	2.0 ± 0.1 (1.9-2.2)	1.9 ± 0.9 (1.8-2.1)	1.9 ± 0.1 (1.8-2.0)	–	–
Metacorpus length	14.3 ± 0.6 (13.3-15.5)	14.3 ± 0.6 (13.9-15.8)	14.5 ± 0.5 (14.0-15.2)	–	–
Metacorpus width	10.0 ± 0.5 (9.2-11.0)	9.4 ± 0.4 (9.0-10.0)	9.9 ± 0.4 (9.0-10.5)	–	–
Metacorpus valve length	3.0 ± 0.1 (2.9-3.3)	2.8 ± 0.1 (2.5-2.9)	3.3 ± 0.3 (3.0-3.8)	–	–
Metacorpus valve width	2.0 ± 0.2 (1.9-2.5)	2.0 ± 0.1 (1.8-2.3)	2.6 ± 0.1 (2.5-2.8)	–	–
Pharynx length	67 ± 4.8 (59-73)	66 ± 3.0 (61-70)	68 ± 1.4 (66-70)	– (64-68)	–
Pharyngeal overlap length	86 ± 10.0 (69-104)	86 ± 7.3 (78-105)	95 ± 8.7 (85-105)	–	–

Table 2. (Continued.)

Character	Strawberry (N17-00341)		*Monilinia fructicola* (N18-01001-2)	Strawberry	
				Christie (1942)	Allen (1952)
Ant. end to pharyngeal gland lobe	152 ± 13.9 (129-176)	152 ± 8.5 (142-172)	163 ± 7.4 (152-172)	–	–
Ant. end to excretory pore	80 ± 6.8 (68-93)	75 ± 5.9 (69-87)	79 ± 3.7 (75-86)	–	–
Post-uterine sac (PUS) length (L)	45 ± 6.6 (33-56)	46 ± 3.8 (40-53)	46 ± 5.7 (37-56)	–	–
Vulva to anus (VA)	152 ± 16.9 (124-182)	153 ± 9.5 (141-167)	174 ± 5.7 (161-182)	–	–
Ant. end to vulva	464 ± 54.4 (360-574)	459 ± 45.8 (381-538)	496 ± 19.0 (468-530)	–	–
Post. end to vulva	190 ± 21.7 (156-237)	190 ± 12.6 (173-207)	214 ± 6.1 (201-223)	–	–
Tail length	39 ± 3.9 (34-46)	38 ± 2.8 (35-43)	40 ± 1.8 (38-43)	– (36-42)	–
Spermatheca length	38 ± 9.5 (34-46)	52 ± 9.0 (41-66)	58 ± 5.4 (49-69)	–	–
Spermatheca width	8.1 ± 0.6 (7.0-9.5)	9.7 ± 1 (8.0-11.3)	8.2 ± 1.1 (6.0-10)	–	–
PUS/L	6.7 ± 0.8 (5.0-7.6)	7.5 ± 0.8 (6.6-9.4)	6.4 ± 0.9 (5.0-7.8)	–	–
PUS/VA %	29.3 ± 3.6 (21.9-33.7)	29.8 ± 2.0 (26-33.9)	26.4 ± 3.7 (20.7-31.8)	–	–

* Populations measured by Oliveira *et al*. (2019).
** The same population re-measured in this study.

Table 3. Morphometrics of live males of a Florida population of *Aphelenchoides besseyi* from strawberry and from *Monilinia fructicola* cultures compared to those in the original description by Christie (1942) and a redescription by Allen (1952).

Character	Strawberry (N17-00341)		*Monilinia fructicola* (N18-01001-2)	Strawberry	
				Christie (1942)	Allen (1952)
n	1*	2**	10*	10	–
L	473	525 ± 36.9 (499-551)	572 ± 19.4 (538-592)	– (540-620)	– (440-720)
a	31.8	35.2 ± 0.5 (34.8-35.6)	36.1 ± 1.6 (33.2-38.7)	– (36-39)	– (36-47)
b	7.4	8.7 ± 0.6 (8.3-9.1)	9.0 ± 0.3 (8.5-9.5)	(8.6-8.8)	(9-11)
b′	3.2	4.2 ± 0.6 (3.8-4.7)	4.3 ± 0.3 (3.9-4.8)	–	–
c	14.3	17.6 ± 0.6 (17.2-18.0)	17.7 ± 0.8 (16.0-19.1)	– (15-17)	– (14-19)
c′	3.0	2.7	2.7 ± 0.1 (2.5-2.8)	–	–
Max. body diam.	14.9	14.9 ± 1.3 (14.0-15.8)	15.9 ± 0.4 (14.9-16.3)	– (14-17)	–
Body diam. at cloacal opening	11.0	10.7 ± 0.3 (10.5-11.0)	12.0 ± 0.3 (11.5-12.5)	–	–

Table 3. (Continued.)

Character	Strawberry (N17-00341)		*Monilinia fructicola* (N18-01001-2)	Strawberry	
				Christie (1942)	Allen (1952)
Testis/L (%)	39.0	52.5 ± 16.2 (41.0-64.0)	44.2 ± 4.2 (38.0-51.4)	–	– (50-65)
Lip region diam.	7.0	6.5	6.6 ± 0.1 (6.4-6.8)	–	–
Lip region height	3.0	2.8 ± 0.1 (2.7-2.9)	3.0	–	–
Stylet length	11.4	12.0	10.7 ± 0.2 (10.4-11.0)	–	–
Stylet cone length	5.2	5.6	5.0 ± 0.1 (4.8-5.0)	–	–
Stylet knob height	1.2	1.7	1.5 ± 0.1 (1.4-1.6)	–	–
Stylet knob width	2.0	2.0	1.9 (1.8-1.9)	–	–
Metacorpus length	13.4	13.2 ± 0.5 (13-13.5)	14.3 ± 0.4 (14.0-15.0)	–	–
Metacorpus width	9.0	9.5 ± 0.7 (9-10)	10.0 ± 0.3 (9.5-10.6)	–	–
Metacorpus valve length	2.9	3.3 ± 0.3 (3.1-3.5)	3.2 ± 0.2 (3.0-3.5)	–	–
Metacorpus valve width	2.0	2.1	2.4 ± 0.1 (2.3-2.5)	–	–
Pharynx length	63	67 ± 4.8 (59-73)	63 ± 1.5 (61-67)	– (63-66)	–
Pharyngeal overlap length	82	60	69 ± 8.1 (60-84)	–	–
Ant. end to pharyngeal gland lobe	146	123 ± 10.5 (115-130)	131 ± 9.5 (121-150)	–	–
Ant. end to excretory pore	62	68	76 ± 3.0 (70-81)	–	–
Tail length	33	30 ± 1.0 (29-30)	32 ± 1.1 (30-34)	– (36-37)	–
Spicule dorsal limb length	18.8	18.1 ± 0.9 (17.5-18.8)	18.3 ± 0.7 (17.0-19.3)	–	–
Spike length	–	2	–	–	–
Testis length	186	279 ± 104.9 (205-353)	253 ± 27.8 (214-300)	–	–

* Measurements taken by Oliveira *et al*. (2019).
** Measurements taken in this study.

in the females was provided by Christie (1942) and Allen (1952). The specimens we examined showed a defined spermatheca elongated, oval or rectangular and packed with rounded spermatozoa, which usually were not found in the uterus. The length (46 μm) of the PUS illustrated by Allen (1952) is in the range of that of our population 32.6-56.4 μm. The length of PUS was not always less than one-third of the distance between vulva and anus (VA) as reported by Christie (1942). The ratio in percent of length of PUS/VA ranged 21.9-33.9, indicating that in this species the length of PUS is often (58% of the examined specimens) greater than one-third of VA.

The PUS did not contain spermatozoa in many females. Oliveira *et al*. (2019) found spermatozoa in the PUS in

about 5% of females. In our specimens, we found 1-3 spermatozoa in the PUS of about 15% of females with no evidence of a PUS packed with spermatozoa. The specimens of our population have a tail with a stellate mucron. However, the shape of the mucron was variable: stellate with 3-4 processes or brush-like or single with a short basal appendix.

The male morphology and morphometrics of the Florida population in this study and Oliveira *et al*. (2019) agree with those reported by Christie (1942) and Allen (1952). The length of the spicules in our population ranged from 17 to 19.3 μm and did not differ from the length (19 μm) illustrated by Allen (1952). The spicules in our specimens and in those studied by Oliveira *et al*. (2019) have a clear condylus and rostrum, although not very pronounced, as also seen in illustrations of the male by Allen (1952).

HOST AND LOCALITIES

In the original description, Christie (1942) indicated cultivated strawberry (*Fragaria* hybrids) in Southeastern USA as host and specified that specimens from strawberry in Willard, NC, USA, were used for the description. In the redescription and designation of the neotype by Allen (1952) and in the present study, specimens were from strawberry (*Fragaria* × *ananassa*) collected in Plant City, FL, USA. However, the Florida population characterised and used in this study originated from plants imported from Cashiers, NC, USA. This population parasitised gerbera daisy (*Gerbera jamesonii* Bolus *ex* Hook.f.) under glasshouse conditions in the previous study conducted by Oliveira *et al*. (2019).

Aphelenchoides oryzae Yokoo, 1948
(Fig. 1 in Yokoo (1948); Fig. 1 in Fortuner (1970); Fig. 3 in this study)

Yokoo (1948) used two rice populations from Kyushu and Hokkaido, Japan, for the original description of this species and provided a limited number of measurements. However, *A. oryzae* was later synonymised with *A. besseyi* by Allen (1952). Fortuner (1970) accepted the synonymy and provided a detailed description of this nematode using rice populations from Senegal. This new description has been incorporated with some modifications in other publications (Franklin & Siddiqi, 1972; Hunt, 1993). In our study, we do not accept this synonymisation and consider *A. oryzae* as a valid species. The descriptions by Yokoo (1948) and that by Fortuner (1970) modified by Franklin & Siddiqi (1972) as *A. besseyi s.l.*, are reported below.

MEASUREMENTS

See Table 4.

DESCRIPTION (from Yokoo, 1948 and Fortuner, 1970, as modified by Franklin & Siddiqi, 1972)

Selected characters for this species reported by Yokoo (1948) and derived by a figurative translation of the Japanese text of this description include: "Head distinct from the body and setoff. Stylet with defined knobs 12 μm long, almost one-fifth of the distance between the anterior body end and the metacorpus, which is almost elliptical in shape. Nerve ring posterior to the metacorpus. Excretory pore not well distinct. Anus located at 94 percent of body length and covered by a short cuticular overlap. Tail conical ending in a terminus with a triply branched mucro. Female: Body longer and thinner than that of the male and suddenly narrowing behind the vulva. Vulva located at 70% of body length. Genital tract consisting of single ovary, uterus and a post uterine sac, which is one-fourth of the distance between the vulva and the tail terminus in length. Male: Body shorter and larger than that of female. The tail curves like a sickle. It lacks bursa and shows three pairs of copulatory papillae, one pair adanal, a second pair in the middle and a third pair at the end of the tail. The spicules are semi-circular and lack gubernaculum."

The redescription by Fortuner (1970) and modified by Franklin & Siddiqi (1972) includes:

> "Female: Body slender, straight to slightly arcuate ventrally when relaxed; annuli fine, indistinct, about 0.9 μm wide near mid-body. Lip region rounded, unstriated, slightly offset and wider than body at lip base, about half as wide as mid body; labial framework hexaradiate, lightly sclerotized. Lateral fields about one-fourth as wide as body, with 4 incisures. Anterior part of spear sharply pointed, about 45% of total spear length, posterior part with light basal swellings which are 1.75 μm across. Median oesophageal bulb oval, with a distinct valvular apparatus slightly behind its centre. Oesophageal glands extending dorsally and subdorsally for 4 to 8 body-widths over intestine. Nerve ring about one body-width behind median oesophageal bulb. Excretory pore usually near anterior edge of nerve ring. Hemizonid 11-15 μm behind

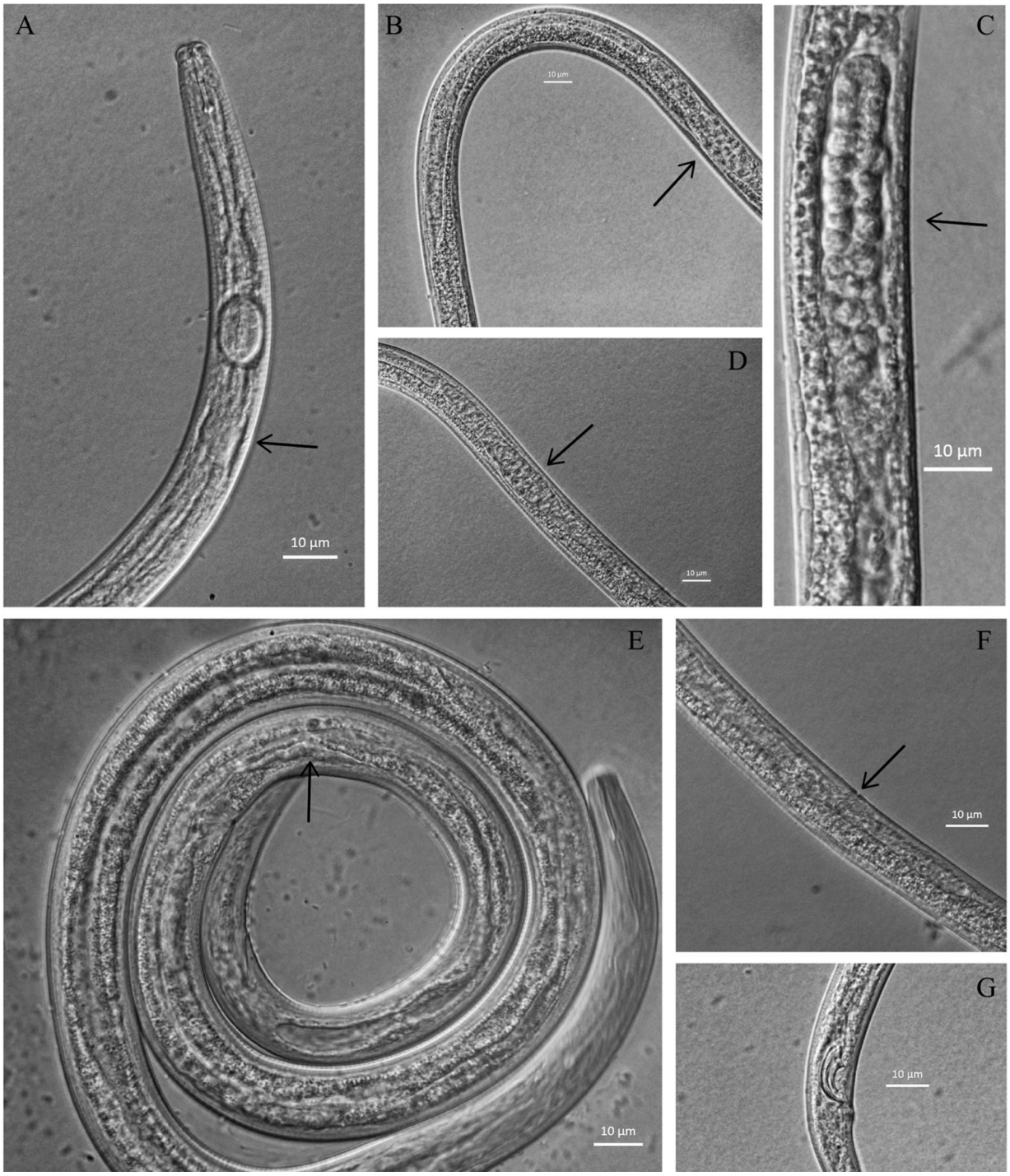


Fig. 3. Photomicrographs of *Aphelenchoides oryzae* female and male from *Oryza sativa* in Louisiana. A: Female anterior body showing excretory pore (arrowed) located at level of nerve ring; B-D: Mid-section of female body showing well defined ovoid spermathecae (arrowed) filled with spermatozoa packed in multiple rows; E: Coiled female showing a thin and short post-vulval uterine sac (PUS) devoid of spermatozoa; F: Mid-section of female body showing PUS posterior to vulva (arrowed) containing spherical bodies resembling sperm; G: Male posterior body showing spicules with small condylus and pointed rostrum.

excretory pore. Vulva transverse, with slightly raised lips. Spermatheca elongate oval (up to 8 times as long as wide when fully distended) usually packed with sperms. Ovary relatively short and not extending to oesophageal glands, with oocytes in 2-4 rows. Post vulvar uterine sac narrow, inconspicuous, not containing sperms, 2.5-3.5 times anal body width long but less than one-third distance from vulva to anus. Tail conoid, 3.5 to 5 anal body widths long; terminus bearing a mucro of diverse shape with 3-4 pointed processes. Male: About as numerous as females. Posterior end of body curved to about 180 degrees in relaxed specimens. Lip region, spear and oesophagus as described for female; tail conoid, with terminal mucro with 2-4 pointed processes. First pair of ventrosubmedian papillae adanal, second slightly behind middle of tail and third subterminal. Spicules typical of the genus except that the proximal end lacks a dorsal process (apex or condylus) and has only a moderately developed ventral one (rostrum). Testis single outstretched."

Remarks

The measurements of the rice populations from Louisiana fit those reported by Yokoo (1948), Fortuner (1970) and Franklin & Siddiqi (1972). However, some differences were observed in several characters. The body length in the females of the Louisiana population was variable. Some specimens extracted from seeds stored in a refrigerator for 28 months showed a longer body than the specimens extracted from seeds stored for less than 1 year after harvest (717-1000 *vs* 586-804 μm). The stylet of the Louisiana population did not differ from that reported for the populations from Japan and Senegal described by Yokoo (1948) and Fortuner (1970), respectively. The stylet cone of our population was about 45-48% of the stylet length rather than 45% as reported by Franklin & Siddiqi (1972). The stylet knob widths were also slightly greater (2 rather than 1.75 μm). No differences were observed in the length of the pharyngeal glands, the position of the excretory pore at nerve ring level, and the configuration of the female reproductive system. The spermatheca was variable in size and oval or elongate in shape. It was filled with round (subspherical in three-dimensional view) spermatozoa usually 4-5 μm in diam. with the prominent nucleus surrounded by a granular cytoplasm and small black dots as reported by Fortuner (1970). There is no clear information on the position of the spermatozoa in the female genital tract in the descriptions of this species by Yokoo (1948) or Fortuner (1970). The spermatozoa are reported to be confined in the long spermatheca in the description of Fortuner and are not present in the uterus or in the PUS. However, some (2-4) cells with prominent nuclei resembling spermatozoa were illustrated in the PUS by Fortuner (1970). We confirmed this observation and found 25% of the examined specimens from Louisiana with 1-4 spermatozoa in the PUS. In about 5% of the specimens the spermatozoa were also scattered in the uterus. The length values of the PUS in the Louisiana specimens agreed with those reported by Fortuner (1970) (30.6-49.5 *vs* 24-47 μm) and were consistently less than one-third of VA in all the specimens we examined as emphasised in the description of this species by Fortuner (1970) and Yokoo (1948) (one-fourth of the distance between the vulva and the tail terminus). Males of the Louisiana population showed spicules with an evident condylus, which is inconspicuous or absent in the rice population from Senegal used by Fortuner (1970).

We are aware that the morphological characters in both sexes differentiating *A. oryzae* from *A. besseyi* are insufficient for morphological separation of the two species. The major difference between them is represented by the length of the PUS, which is less than one-third of VA in *A. oryzae*, but often greater in *A. besse*yi (the ranges of PUS/VA% are 16.8 to 26.1 *vs* 20.7 to 33.9). Since these characters may overlap in some specimens, their morphological separation is unreliable without the corroboration of the molecular analysis and these species are therefore considered cryptic.

Hosts and localities

Yokoo indicated rice grown in Kyushu and Hokkaido, Japan, as the host of this species. However, in addition to rice, genetically identical *A. oryzae* populations have been reported from foxtail millet (*Setaria italica*), tuberose (*Polianthes tuberosa*), Guinea yam (*Dioscorea cayenensis*), and other plants from other countries such as Brazil, China, Costa Rica, Iran, Italy, Russia, Spain, Turkey, USA and Viet Nam, amongst others.

Table 4. Morphometrics of live females and males of a Louisiana population of *Aphelenchoides oryzae* from rice measured twice at an interval of 28 months and compared to those in the original description by Yokoo (1948) and a redescription by Fortuner (1970).

Character	Rice, Louisiana				Rice			
	(N17-00400)		(N17-00400)*		Yokoo (1948)		Fortuner (1970)	
	Female	Male	Female	Male	Female	Male	Female	Male
n	10	3	10	6	–	–	20	9
L	727 ± 66.8 (586-804)	551 ± 40.3 (526-598)	841 ± 96.1 (717-1000)	587 ± 67.5 (498-700)	650 (504-732)	520 (458-600)	680 (570-840)	570 (530-610)
a	45.3 ± 3.7 (39.7-51.8)	37.9 ± 2.6 (35.2-40.3)	51.2 ± 4.0 (45.5-58.9)	39.1 ± 2.8 (35.5-42.9)	43.5 (38.1-48.1)	38 (33.7-44)	47.7 (39.3-53.5)	44.4 (40.7-46.9)
b	10.9 ± 0.5 (9.4-12.1)	9.0 ± 0.3 (8.6-9.2)	11.5 ± 1.2 (10.1-13.8)	8.9 ± 1.0 (7.6-10.4)	10.6 (9.1-12.2)	8.9 (8.3-10.5)	11.4 (9.2-13.1)	9.5 (8.9-10.7)
b′	4.8 ± 0.5 (3.9-5.6)	4.0 ± 0.1 (3.9-4.1)	4.9 ± 0.5 (4.2-5.7)	3.8 ± 0.2 (3.5-4.1)	–	–	4.8 (4.0-5.7)	4.1 (3.6-4.9)
c	17.5 ± 1 (15.6-19.6)	15.2 ± 0.5 (14.8-15.8)	19.0 ± 1.5 (16.9-22.0)	16.8 ± 1.3 (14.8-18.4)	16.3 (13-19.6)	18 (13.3-22)	17.7 (13.8-20.4)	17.9 (16-20)
c′	4 ± 0.3 (3.6-4.5)	2.9 ± 0.1 (2.8-3.1)	4.2 ± 0.2 (3.8-4.4)	2.8 ± 0.3 (2.4-3.2)	–	–	–	–
V	71 ± 0.7 (69.4-71.8)	–	70.6 ± 0.5 (69.6-71.4)	–	71.6 (60.3-83)	–	71.2 (68.7-73.6)	–
Max. body diam.	16.0 ± 0.8 (14.2-17.3)	14.5 ± 0.6 (13.8-14.9)	16.5 ± 1.4 (13.2-18.3)	14.8 ± 0.9 (14.0-16.3)	15 (13-18)	14 (12-16)	–	–
Body diam. at anus or cloacal opening	10.1 ± 0.6 (9.2-11.3)	12 ± 0.3 (11.8-12.3)	10.4 ± 0.8 (9.4-11.4)	11.8 ± 0.7 (11.0-12.9)	–	–	–	–
G or T (Genital tract length/ L (%))	27.1 ± 2.9 (22.8-33.4)	37.5 ± 3.9 (33.2-40.9)	29.4 ± 2.5 (25.1-32.6)	37 ± 3.5 (32.1-41.8)	–	–	27.9 (19.9-39.1)	40.5 (28.2-52.3)
Anterior genital tract length	197 ± 19.2 (169-219)	–	245 ± 48.8 (175-299)	–	–	–	–	–
Lip region diam.	7.0 ± 0.1 (7.0-7.4)	7.0 ± 0.1 (6.9-7.1)	7.0 ± 0.03 (7.0-7.1)	7.0 ± 0.05 (6.9-7.1)	–	–	–	–
Lip region height	3.0 ± 00.7 (3.0-3.2)	3.1 ± 0.1 (3.0-3.2)	3.0 ± 0.07 (3.0-3.2)	3.0	–	–	–	–
Stylet length	12.4 ± 0.2 (12-12.8)	12.2 ± 0.3 (12.0-12.5)	12.0 ± 0.3 (11.4-12.5)	12.1 ± 0.5 (11.2-12.6)	12	–	11.9 (10.0-12.5)	11.4 (10.0-12.5)
Stylet cone length	5.7 ± 0.2 (5.4-5.9)	5.7 ± 0.2 (5.6-5.9)	5.5 ± 0.2 (5.2-5.8)	5.5 ± 0.3 (5.1-5.8)	–	–	–	–
Stylet knob height	1.8 ± 00.6 (1.7-2.0)	1.7 ± 0.1 (1.6-1.8)	1.4 ± 0.1 (1.2-1.6)	1.3 ± 0.2 (1.1-1.6)	–	–	–	–
Stylet knob width	2.2 ± 0.1 (2.0-2.4)	2.1 ± 0.1 (2.0-2.2)	2.2 ± 0.2 (2.0-2.7)	2.1 ± 0.1 (2.0-2.2)	–	–	2	–
Metacorpus length	14.4 ± 0.4 (13.8-15.0)	14.1 ± 0.8 (13.5-15.0)	14.4 ± 0.4 (13.9-15.0)	14.2 ± 0.6 (13.4-14.9)	–	–	–	–
Metacorpus width	9.8 ± 0.4 (9.0-10.5)	9.9 ± 0.1 (9.9-10.0)	10 ± 0.3 (9.2-10.5)	9.5 ± 0.6 (9.0-10.3)	–	–	–	–
Metacorpus valve length	3.1 ± 0.1 (3.0-3.2)	3.0 ± 0.1 (2.9-3.1)	3.2 ± 0.2 (2.8-3.5)	3.0 ± 0.1 (2.9-3.1)	–	–	–	–
Metacorpus valve width	2.0 ± 0.1 (1.9-2.2)	2.0 ± 0.1 (1.9-2.1)	2.3 ± 0.1 (2.1-2.5)	2.2 ± 0.05 (2.1-2.3)	–	–	–	–
Pharynx length	66 ± 3.3 (62-73)	61 ± 3.4 (57-64)	73 ± 3.0 (68-79)	66 ± 1.3 (64-67)	–	–	–	–

Table 4. (Continued.)

Character	Rice, Louisiana				Rice			
	(N17-00400)		(N17-00400)*		Yokoo (1948)		Fortuner (1970)	
	Female	Male	Female	Male	Female	Male	Female	Male
Pharyngeal overlap length	83 ± 6.8 (76-95)	75 ± 7.1 (71-84)	98 ± 7.6 (85-107)	86 ± 9.7 (74-103)	–	–	–	–
Ant. end to pharyngeal gland lobe	150 ± 8.5 (141-164)	136 ± 10.2 (129-148)	169 ± 6.9 (159-179)	152 ± 10.7 (139-170)	–	–	–	–
Ant. end to excretory pore	80 ± 7.2 (72-86)	75 ± 4.8 (70-79)	86 ± 3.2 (81-90)	77 ± 6.3 (67-82)	–	–	(58-83)	–
Post-vulval uterine sac (PUS)	39 ± 3.6 (31-43)	–	42 ± 4.6 (37-49)	–	–	–	38.7 ± 10.6 (24-47)	–
Vulva to anus (VA)	170 ± 20.8 (131-202)	–	203 ± 28.6 (168-257)	–	–	–	–	–
Ant. end to vulva	516 ± 45 (419-567)	–	593 ± 65.4 (506-697)	–	–	–	–	–
Post. end to vulva	211 ± 22.7 (167-246)	–	247 ± 30.9 (207-303)	–	–	–	–	–
Tail length	41 ± 3.2 (35-44)	36 ± 1.4 (35-38)	44 ± 3.0 (38-48)	35 ± 2.7 (31-39)	–	–	51.4	35.3 ± 4.1 (24-40)
Spermatheca length	46 ± 6.9 (37-56)	–	61 ± 11.7 (43-79)	–	–	–	61.4	–
Spermatheca width	9.6 ± 1.3 (5.9-10.5)	–	12.9 ± 1.7 (9.0-13.8)	–	–	–	–	–
Spicule dorsal limb length	–	16.0 ± 1.0 (15.0-17.0)	–	18. 5 ± 0.8 (17.5-19.5)	–	18	–	19.2 (18-21)
PUS/VA %	23.9 ± 1.5 (21.5-26.1)	–	21.0 ± 2.1 (16.8-24.4)	–	–	–	–	–
Lateral field width	4 ± 0.2 (3.9-4.5)	–	–	4	–	–	–	–
Number of tail tip spikes	2-3	2	–	2-3	–	–	3.4	2-4
Testis length	–	208 ± 3.5 (175-245)	–	218 ± 3.8 (191-293)	–	–	–	–
PUS/L	5.9 ± 0.3 (4.9-5.8)	–	5.0 ± 0.4 (4.3-5.5)	–	–	–	4.9 (4.1-6.2)	–

*Population from the same rice seed stock remeasured after 28 months from the first measurements.

Aphelenchoides pseudobesseyi* **sp. n.**
(Figs 4-7)

The results of morphological analyses of foliar nematode populations from the ornamental plants, coneflower, leopard plant and wood fern, showed some morphological differences in the genital tract of the females compared to *A. besseyi* and *A. oryzae*. These populations are identified here as a new species called *A. pseudobesseyi* sp. n. This new species, however, shares the characters of *A. besseyi* from strawberry reported by Christie (1942), Allen (1952) and Oliveira *et al*. (2019), and *A. oryzae* as described by Yokoo (1948), Fortuner (1970) and Franklin & Siddiqi (1972). The descriptions provided by these authors for *A. besseyi* and *A. oryzae* are used and modi-

* Specific epithet derived from the Greek term $\varepsilon\upsilon\delta\eta\sigma$ = false, and the root-*besseyi*.

fied appropriately for the description of *A. pseudobesseyi* sp. n.

Measurements

See Table 5.

Description

Female

Body slender, straight to slightly arcuate ventrally, annuli fine and indistinct under light microscopy. Lip region rounded, slightly offset, about half as wide as mid-body diam., labial framework lightly sclerotised. Lateral fields with four incisures. Total stylet length 11.1-12.8 μm across three populations. Anterior part of spear comprising 41-48% of total spear length. Stylet knobs 1.9-2.5 μm across. Pharyngeal glands extending dorsally and subdorsally over intestine. Nerve ring located about one-body diam. posterior to metacorpus. Excretory pore usually near anterior edge of nerve ring or anterior to it, but close to posterior edge of metacorpus in some specimens. Hemizonid not observed. Ovary not extending to pharyngeal glands, oocytes in two to four rows. Mature oocytes rectangular, 17 $\times$ 14 μm in size, with prominent nucleus. Spermatheca elongate, oval, 40-100 μm long, packed with spermatozoa 5-7 μm in diam. arranged in single or multiple rows. Uterus containing spermatozoa in 41% of specimens examined. Vulva transverse, with slightly raised lips. PUS conspicuous and large, 8-14 μm wide, empty or containing sperm in almost half (48-50%) of specimens (59) examined across two populations. Up to 16 spermatozoa observed in PUS. PUS length more than one-third of VA distance observed in 40-70% of examined specimens (69) across examined populations, with lowest percentage recorded for the wood fern population and the highest for that from leopard plant. PUS packed with four or 16 spermatozoa in 13% of examined specimens. Tail conoid, ending in a terminus bearing a cuspidate or brush-like mucron with two or three pointed processes.

Male

About as numerous as female. Posterior end of body curved by about 180 degrees. Lip region, spear and pharynx as described for female. Tail conoid, with terminal mucron with 2-4 pointed processes. First pair of ventrosubmedian papillae adcloacal, second slightly posterior to middle of tail and third subterminal. Spicules typical of genus with a rounded or rectangular condylus and a moderately developed and pointed rostrum. Testis single, outstretched.

Type host and locality

Leaves of *Farfugium japonicum* collected in November 2017 in St Augustine, FL, USA (latitude 29°90′50.8″N, longitude 81°41′34.2″W).

Other hosts and localities

Other populations from *Echinacea* sp. and *D. erythrosora* came from seeds and leaves of plants located in Florida, USA (Table 1). This species has also been found on bird's nest fern in Taiwan (Hsieh *et al*., 2012), beans in Costa Rica (Sánchez-Monge *et al*., 2017), soybean (Meyer *et al*., 2017), cotton (Favoreto *et al*., 2018) in Brazil, and strawberry in Taiwan (Lin & Yang, unpubl.).

Type material

The type material was obtained from live specimens that were fixed after morphological examination. The holotype female, ten paratype females and six paratype males, mounted on glass slides, are deposited in the nematode collection of the National Museum of Natural Sciences, Madrid, Spain. An additional four paratype females and two paratype males were sent to each of the United States Department of Agriculture Nematode Collection, Beltsville, MD, USA; University of California Riverside Nematode Collection, Riverside CA, USA; WaNeCo, Plant Protection Service, Wageningen, The Netherlands; and the Nematode Collection of FERA, Sand Hutton YO41 1LZ, UK. This work has been registered in Zoobank with the following number: Urn:lsid:zoobank.org:pub:0CF127C4-6F23-4E75-A234-12A4626A72DD.

Diagnosis and relationships

Aphelenchoides pseudobesseyi sp. n. is characterised by females having a conspicuous and large PUS, 8-14 μm wide and more than one-third of VA long in 40-70% of the examined specimens, containing some spermatozoa in almost half (48-50%) of the specimens (59) examined across two populations, and filled with spermatozoa in 13%. Males of this new species have a rounded or rectangular condylus.

The characters of the females separate this new species from those of *A. besseyi*, which have PUS thinner (6.5 μm

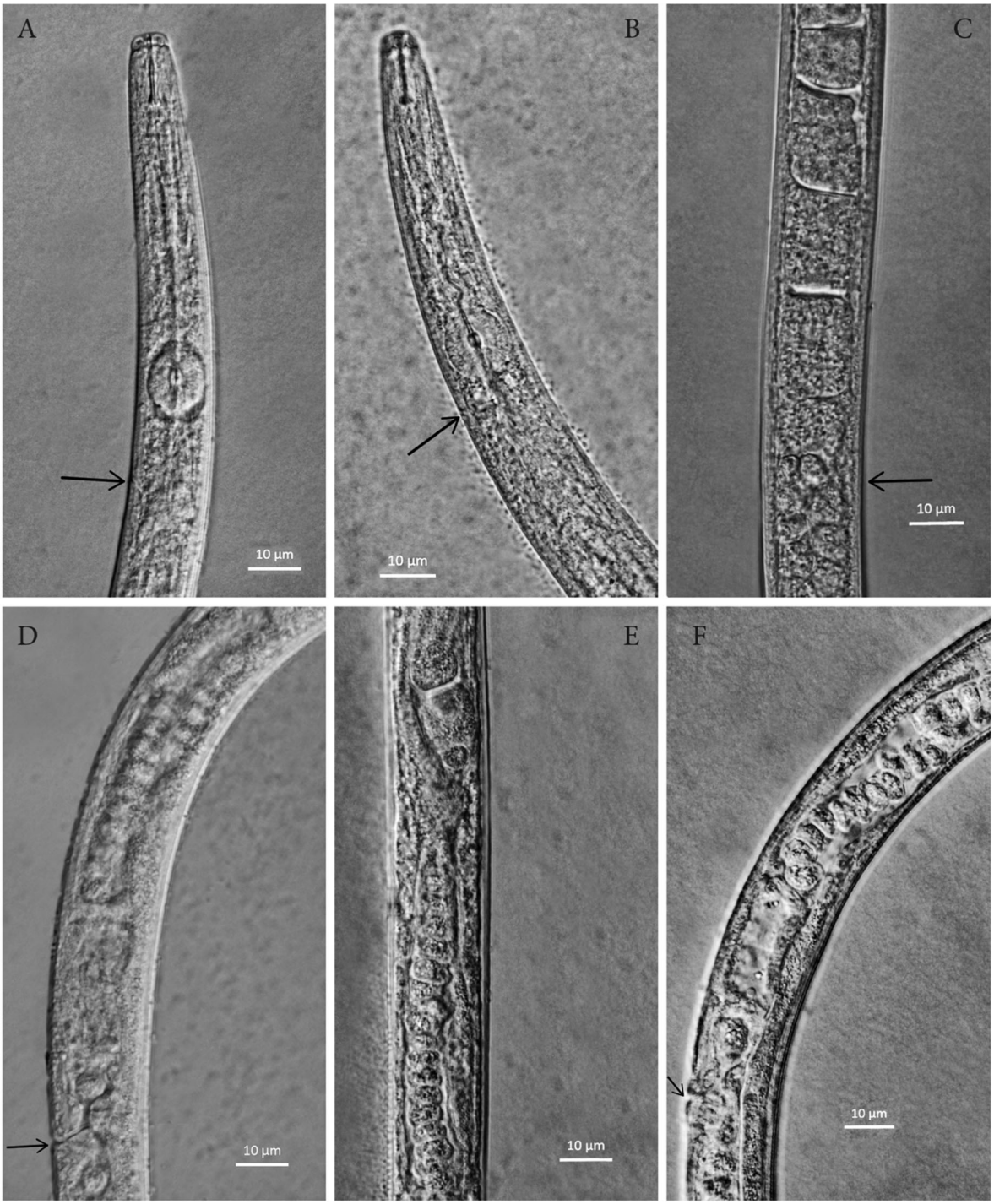


Fig. 4. Photomicrographs of *Aphelenchoides pseudobesseyi* sp. n. female from *Farfugium japonicum* in Florida. A, B: Anterior body showing excretory pore (arrowed) located at anterior margin of nerve ring or above it, slightly posterior to the posterior margin of the metacorpus; C: Mid-section of body showing rectangular mature oocytes with prominent nucleus (arrowed); D-F: Mid-section of body showing spermathecae packed with dotted spermatozoa spherical in shape or compressed in rectangular cases. Note spermatozoa in uterus and in proximity to vagina (arrow = vulva).

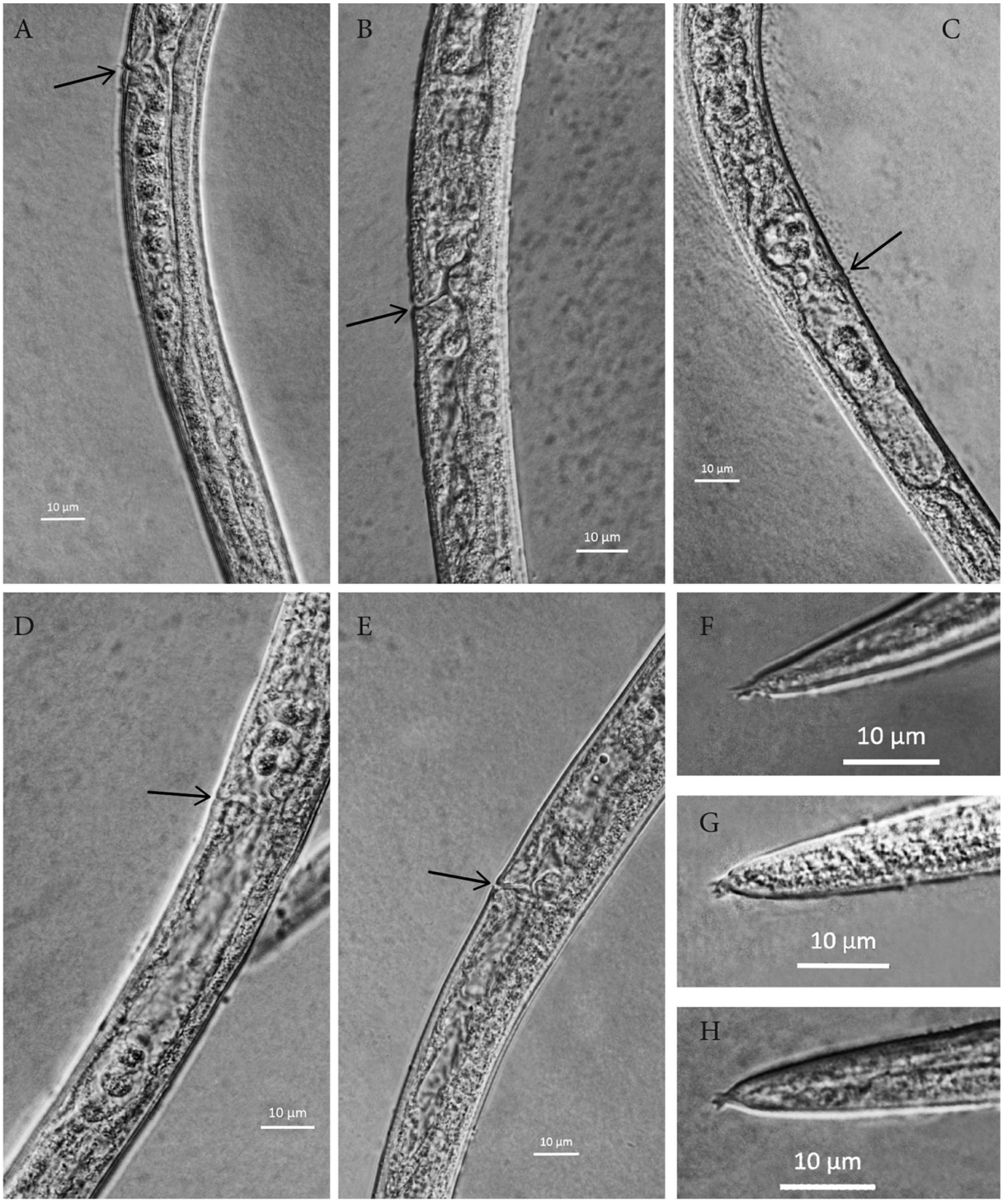


Fig. 5. Photomicrographs of *Aphelenchoides pseudobesseyi* sp. n. female from *Farfugium japonicum* in Florida. A-D: Posterior body showing post-vulval uterine sac (PUS) full or partially filled with dotted spermatozoa located in its proximal, central or distal portions. Note vulva (arrowed) and wide PUS in (C) and (D); E: Long PUS devoid of sperm; F-H: Tail showing different shapes of terminal stellate mucro.

Table 5. Morphometrics of mounted holotype, live type material before mounting (N17-01199) and additional live type material (N18-00688) of *Aphelenchoides pseudobesseyi* sp. n. from the ornamental leopard plant, *Farfugium japonicum* – the type host. Measurements of live females and males of *A. pseudobesseyi* n. sp. from Florida populations *ex.* coneflower (*Echinacea* sp.) and wood fern (*Dryopteris erythrosora*) are also provided.

Character	*Farfugium japonicum*					*Echinacea* sp.				*Dryopteris erythrosora*	
	(N17-01199)			(N18-00688)*		(N17-00754)		(N17-00754)**		(N18-00052)	
	Female		Male	Female	Male	Female	Male	Female	Male	Female	Male
	Holotype	Paratypes	Paratypes	Paratypes	Paratypes						
n	–	10	7	10	10	10	5	10	2	10	3
L	650	692 ± 51.3 (611-757)	559 ± 75.1 (443-661)	744 ± 73.8 (604-846)	579 ± 47.0 (503-630)	780 ± 64.0 (652-873)	634 ± 59.3 (572-709)	722 ± 37.3 (686-786)	579 ± 13.1 (569-586)	629 ± 57.5 (540-702)	509 ± 34.5 (478-546)
a	44.8	38.2 ± 1.9 (34.8-41.4)	35.4 ± 3.9 (28.7-41.0)	37.1 ± 3.5 (31.4-41.4)	34.8 ± 4.2 (30-40.7)	40.2 ± 4.9 (35.7-47.4)	36.1 ± 1.6 (33.6-37.8)	37.7 ± 3.3 (34-45.3)	37.2 ± 4.6 (33.9-40.5)	43.3 ± 3.7 (38-49)	38.3 ± 3.4 (36-42.3)
b	10.0	10.0 ± 0.5 (9.4-11.2)	8.6 ± 0.9 (7.1-9.5)	10 ± 0.8 (8.6-11.4)	8.7 ± 0.3 (8.3-9.4)	11.8 ± 2.8 (10-19.6)	9.5 ± 0.5 (8.9-10.0)	11.0 ± 1.2 (10.3-12.1)	8.7 ± 0.6 (8.3-9.2)	9.5 ± 0.6 (8.7-10.6)	8.1 ± 0.5 (7.5-8.6)
b′	4.6	4.5 ± 0.4 (4.1-5.3)	4.0 ± 0.5 (3.2-4.6)	4.6 ± 0.4 (4.3-5.5)	4.0 ± 0.1 (3.9-4.2)	5.1 ± 0.2 (4.7-5.4)	4.3 ± 0.2 (4.0-4.5)	4.9 ± 0.2 (4.5-5.2)	3.9 ± 0.4 (3.6-4.2)	4.6 ± 0.2 (4.3-5.0)	4.2 ± 0.2 (3.9-4.3)
c	16.3	16.7 ± 1.0 (15.0-18.4)	16.2 ± 2.1 (13.6-20.2)	17.3 ± 0.8 (16-18.4)	16.5 ± 1.0 (14.7-17.8)	17.9 ± 0.8 (16.9-19.6)	19.3 ± 1.6 (17.5-21.1)	17.2 ± 0.8 (16.1-18.8)	17.7 ± 1.5 (16.8-18.6)	16.2 ± 0.9 (14.7-17.7)	16.2 ± 1.4 (14.6-17.2)
c′	4.5	3.8 ± 0.3 (3.4-4.3)	2.8 ± 0.3 (2.5-3.3)	4.0 ± 0.3 (3.5-4.5)	2.7 ± 0.2 (2.4-3.0)	3.9 ± 0.2 (3.6-4.4)	2.6 ± 0.1 (2.5-2.8)	4.0 ± 0.2 (3.5-4.3)	2.8 ± 0 (2.8-2.8)	4.0 ± 0.3 (3.4-4.4)	2.7 ± 0.1 (2.6-2.9)
V	70.7	70.2 ± 1 (68.2-72.2)	–	70.2 ± 1.1 (68.3-71.7)	–	69.8 ± 1 (67.6-70.8)	–	69.6 ± 0.8 (68.7-71.2)	–	69.4 ± 0.9 (68.1-70.7)	–
Max. body diam.	14.5	18.0 ± 1.2 (16.3-20.0)	15.7 ± 0.6 (14.5-16.3)	20 ± 0.9 (19.2-21.9)	16.6 ± 0.9 (15.3-17.8)	19.5 ± 2.3 (16.6-22.7)	17.5 ± 1.2 (15.8-18.8)	19.3 ± 1 (17.8-20.5)	15.6 ± 1.6 (14.5-16.8)	14.4 ± 0.3 (14-15)	13.3 ± 0.6 (12.9-14)
Body diam. at anus or cloacal opening	9.0	10.7 ± 0.5 (9.5-11.3)	12.0 ± 0.5 (11.5-13.2)	10.6 ± 0.3 (10-11)	12.5 ± 0.3 (12.3-13.4)	10.9 ± 0.8 (9.0-11.8)	12.6 ± 0.9 (11.4-13.8)	10.4 ± 0.8 (10.5-10.9)	11.6 ± 0.4 (11.4-11.8)	9.6 ± 0.6 (8.9-10.8)	11.1 ± 0.2 (11-11.3)
G or T (Genital tract length/L (%))	43.5	34.9 ± 3.9 (29.2-39.9)	45.9 ± 8.6 (35.2-59.2)	35.7 ± 4.1 (28.9-40.4)	45.0 ± 10 (33.0-57.6)	35.7 ± 5.9 (27.0-44.0)	56.7 ± 3.7 (53.4-62.7)	34.9 ± 4.6 (29.6-45.2)	52.1 ± 1.5 (51.0-53.2)	28.9 ± 3.2 (23.6-34.6)	36.4 ± 1.4 (35.2-38)
Anterior genital tract length	283	242 ± 32.6 (185-287)	–	262 ± 49.7 (185-341)	–	279 ± 54.3 (219-363)	–	262 ± 49.3 (214-344)	–	181 ± 17.5 (160-206)	–
Lip region diam.	6.7	7.1 ± 0.3 (7.0-7.5)	7.0 ± 0.2 (6.8-7.2)	7.0 ± 0.1 (6.9-7.2)	7.0 ± 0.1 (6.7-7.1)	6.9 ± 0.1 (6.7-7.1)	6.9 ± 0.1 (6.7-7.0)	6.9 ± 0.2 (6.5-7.0)	6.9 ± 0.07 (6.8-7.0)	7.0± 0.1 (6.9-7.1)	6.8 ± 0.2 (6.7-7.0)
Lip region height	3.0	3.0 ± 0.1 (2.9-3.1)	3.0	3.0 ± 0.1 (2.9-3.1)	3.0 ± 0.1 (2.9-3.1)	3.0 ± 0.06 (2.9-3.1)	2.9 ± 0.04 (2.9-3.0)	3.0 ± 0.04 (2.9-3.0)	2.6 ± 0.1 (2.5-2.7)	3.0 ± 0.1 (2.9-3.1)	3.0

Table 5. (Continued.)

Character	*Farfugium japonicum*					*Echinacea* sp.				*Dryopteris erythrosora*	
	(N17-01199)			(N18-00688)*		(N17-00754)		(N17-00754)**		(N18-00052)	
	Female		Male	Female	Male	Female	Male	Female	Male	Female	Male
	Holotype	Paratypes	Paratypes	Paratypes	Paratypes						
Stylet length	12.0	12.4 ± 0.2 (12.0-12.8)	12.0 ± 0.1 (11.9-12.2)	12.2 ± 0.2 (12.0-12.5)	12.2 ± 0.2 (12.0-12.5)	11.8 ± 0.2 (11.2-12.0)	12.0 ± 0.2 (11.9-12.3)	11.5 ± 0.3 (11.1-12.0)	11.7 ± 0.3 (11.5-12.0)	11.7 ± 0.2 (11.2-12.1)	11.7 ± 0.1 (11.5-11.8)
Stylet cone length	5.5	5.5 ± 0.2 (5.3-5.7)	5.6 ± 0.2 (5.3-5.9)	5.4 ± 0.2 (5.2-5.9)	5.7 ± 0.1 (5.5-5.9)	5.5 ± 0.2 (5.0-5.8)	5.8 ± 0.1 (5.7-5.9)	5.3 ± 0.4 (5.0-5.9)	5.2 ± 0.1 (5.1-5.3)	5.6 ± 0.3 (5.2-5.8)	5.2 ± 0.2 (5.1-5.5)
Stylet knob height	1.3	1.7 ± 0.1 (1.5-1.9)	1.7 ± 0.1 (1.6-1.9)	1.7 ± 0.2 (1.4-2.0)	1.6 ± 0.2 (1.2-1.9)	1.8 ± 0.05 (1.7.-2.0)	1.7 ± 0.1 (1.6-1.8)	1.4 ± 0.1 (1.2-1.6)	1.4 ± 0.3 (1.2-1.7)	1.7 ± 0.1 (1.6-1.8)	1.7 ± 0.2 (1.5-1.8)
Stylet knob width	2.0	2.2 ± 0.2 (2.0-2.6)	2.3 ± 0.2 (2.1-2.4)	2.3 ± 0.1 (2.1-2.5)	2.1 ± 0.1 (2.0-2.2)	2.4 ± 0.2 (2.2-2.6)	2.6 ± 0.1 (2.5-2.7)	2.0 ± 0.05 (2.0-2.1)	2.0 ± 0.1 (1.9-2.1)	2.3 ± 0.3 (2.1-2.7)	2.1 ± 0.1 (2.0-2.2)
Metacorpus length	13.0	15.5 ± 0.6 (15.0-16.8)	14.2 ± 0.6 (13.3-15.3)	14.6 ± 0.5 (14.0-15.3)	13.6 ± 0.6 (13.0-14.8)	13.9 ± 0.6 (13.0-15.0)	14.5 ± 0.4 (14.3-15.3)	14.2 ± 0.4 (13.9-15.3)	13.5	14.1 ± 0.7 (13.0-14.8)	13.7 ± 0.3 (13.4-14.0)
Metacorpus width	9.0	10.7 ± 0.7 (10.0-12.2)	10.4 ± 0.7 (9.9-11.8)	11.2 ± 0.6 (10.4-12.3)	10.2 ± 0.4 (9.8-11.0)	10.7 ± 1.2 (9.0-12.3)	10.6 ± 0.9 (9.2-11.8)	10.2 ± 0.7 (9.2-11.5)	9.5	9.6 ± 0.9 (9.0-11.8)	9.0
Metacorpus valve length	3.0	3.3 ± 0.3 (3.0-3.8)	3.4 ± 0.3 (3.1-3.9)	3.3 ± 0.2 (3.0-3.7)	3.4 ± 0.3 (3.1-3.9)	3.3 ± 0.3 (3.0-3.9)	3.1 ± 0.1 (3.0-3.3)	3.2 ± 0.1 (3.0-3.3)	3.0 ± 0.1 (2.9-3.1)	3.2 ± 0.3 (3.0-3.8)	3.4 ± 0.5 (3.2-3.8)
Metacorpus valve width	2.3	2.4 ± 0.3 (2.1-2.8)	2.3 ± 0.2 (2.1-2.5)	2.4 ± 0.2 (2.2-2.8)	2.5 ± 0.2 (2.2-2.7)	2.6 ± 0.3 (2.1-3.0)	2.4 ± 0.1 (2.3-2.6)	2.5 ± 0.3 (2.1-3.0)	2.2 ± 0.1 (2.1-2.3)	2.4 ± 0.3 (2.1-2.9)	2.3 ± 0.2 (2.1-2.5)
Pharynx length	65	69 ± 4.1 (64-77)	64 ± 3.7 (60-71)	74 ± 4.6 (68-84)	66 ± 5.4 (59-74)	71 ± 4.4 (64-78)	61 ± 3.4 (57-64)	65 ± 3.2 (59-69)	66 ± 6.3 (61-70)	66 ± 3.6 (61-72)	62 ± 1.3 (61-63)
Pharyngeal overlap length	82	81 ± 12.4 (62-95)	74 ± 9.0 (66-91)	84 ± 8.5 (70-99)	77 ± 4.8 (69-84)	80 ± 10.0 (63-90)	78 ± 5.6 (70-85)	82 ± 5.0 (73-92)	79 ± 8.3 (73-85)	69 ± 7.3 (61-80)	59 ± 4.2 (55-63)
Ant. end to pharyngeal gland lobe	141	150 ± 15.4 (127-170)	138 ± 12.3 (128-162)	158 ± 12.9 (138-183)	149 ± 10.2 (128-159)	151 ± 13.8 (128-165)	147 ± 8.8 (136-156)	147 ± 7.0 (138-156)	149 ± 20.2 (135-163)	136 ± 8.8 (123-148)	121 ± 5.1 (116-126)
Ant. end to excretory pore	74	76 ± 4.8 (69-82)	71 ± 5.9 (61-79)	76 ± 5.7 (66-86)	68 ± 4.9 (58-73)	85 ± 6.1 (72-92)	78 ± 9.3 (67-88)	75 ± 4.7 (70-82)	79 ± 8.3 (73-85)	74 ± 4.7 (67-81)	68 ± 8.1 (60-76)
Post-vulval uterine sac (PUS) length	46	56 ± 6.1 (45-65)	–	60 ± 7.5 (49-71)	–	55 ± 5.9 (45-65)	–	52 ± 4.5 (42-60)	–	42 ± 4.5 (36-49)	–
Vulva to anus (VA)	150	164 ± 11.8 (150-183)	–	177 ± 16.9 (150-206)	–	192 ± 17.7 (154-220)	–	177 ± 8.3 (170-197)	–	153 ± 16.6 (124-173)	–
Ant. end to vulva	460	486 ± 39.9 (417-535)	–	523 ± 59.2 (416-594)	–	545 ± 45.6 (461-619)	–	503 ± 28.6 (471-543)	–	437 ± 40.2 (382-490)	–
Post. end to vulva	190	206 ± 13.2 (192-225)	–	221 ± 22.6 (188-252)	–	233 ± 21.5 (190-262)	–	218 ± 11.5 (203-243)	–	192 ± 18.6 (158-216)	–

Table 5. (Continued.)

Character	*Farfugium japonicum*					*Echinacea* sp.				*Dryopteris erythrosora*	
	(N17-01199)			(N18-00688)*		(N17-00754)		(N17-00754)**		(N18-00052)	
	Female		Male	Female	Male	Female	Male	Female	Male	Female	Male
	Holotype	Paratypes	Paratypes	Paratypes	Paratypes						
Tail length	40	41 ± 2.7 (37-46)	34 ± 2.9 (31-40)	43 ± 3.9 (37-47)	35 ± 2.2 (31-39)	43 ± 3.8 (36-48)	33 ± 1.0 (32-35)	42 ± 2.5 (39-46)	33 ± 1.5 (32-34)	39 ± 2.7 (34-42)	31 ± 1.3 (30-32)
Spermatheca length	50	68 ± 20.8 (40-103)	–	58 ± 16.4 (43-100)	–	64 ± 4.8 (54-69)	–	74 ± 20.9 (51-104)	–	65 ± 10.5 (52-89)	–
Spermatheca width	9.3	12.0 ± 2.9 (9.0-18.8)	–	12.3 ± 1.6 (10.0-14.0)	–	12.7 ± 1.7 (10.0-15.3)	–	12.5 ± 1.6 (10.0-15.0)	–	9.1 ± 0.9 (8.0-10.0)	–
Spicule dorsal limb length	–	–	19.5 ± 1.1 (18.0-20.7)	–	19.9 ± 0.5 (19.0-20.7)	–	18.9 ± 1.5 (17.0-20.7)	–	18.8	–	20.2 ± 0.4 (19.8-20.7)
Gubernaculum length	–	–	–	–	–	–	–	–	–	–	–
PUS/VA (%)	30.6	33.8 ± 3.2 (27.9-40.0)	–	34.1 ± 2.3 (31.6-39.0)	–	28.5 ± 3.1 (23.8-34.0)	–	29.5 ± 3.1 (21.5-32.9)	–	27.6 ± 4.2 (21.7-33.5)	–
Lateral field width	3.3	3.5 ± 0.4 (3.0-4.0)	3.4 ± 0.1 (3.3-3.5)	–	–	4.2 ± 0.3 (4.0-4.5)	4.5	–	–	2.8 ± 0.2 (2.5-3.0)	2.9 ± 0.0 (2.9-2.9)
Number of tail spikes	3	2-3	2-3	–	–	2-3	2	–	2-3	2-3	1-2
Testis length	–	–	257 ± 64.3 (210-394)	–	265 ± 51.2 (170-337)	–	359 ± 32.0 (324-398)	–	302 ± 0.7 (302-303)	–	–
PUS/L	7.0	8.1 ± 1.0 (6.6-9.7)	–	8.0 ± 0.7 (7.3-9.2)	–	6.9 ± 0.7 (5.9-8.3)	–	5.0 ± 0.4 (4.3-5.5)	–	6.6 ± 0.8 (5.5-7.9)	–

* Population from the same *Farfugium japonicum* plants resampled after 8 months. Specimens were measured alive and then fixed and are designated as paratypes as they came from the same host and locality as the mounted type material and formed part of the description.

** Population from the same *Echinacea* sp. seed stock remeasured after 12 months from the first measurements.

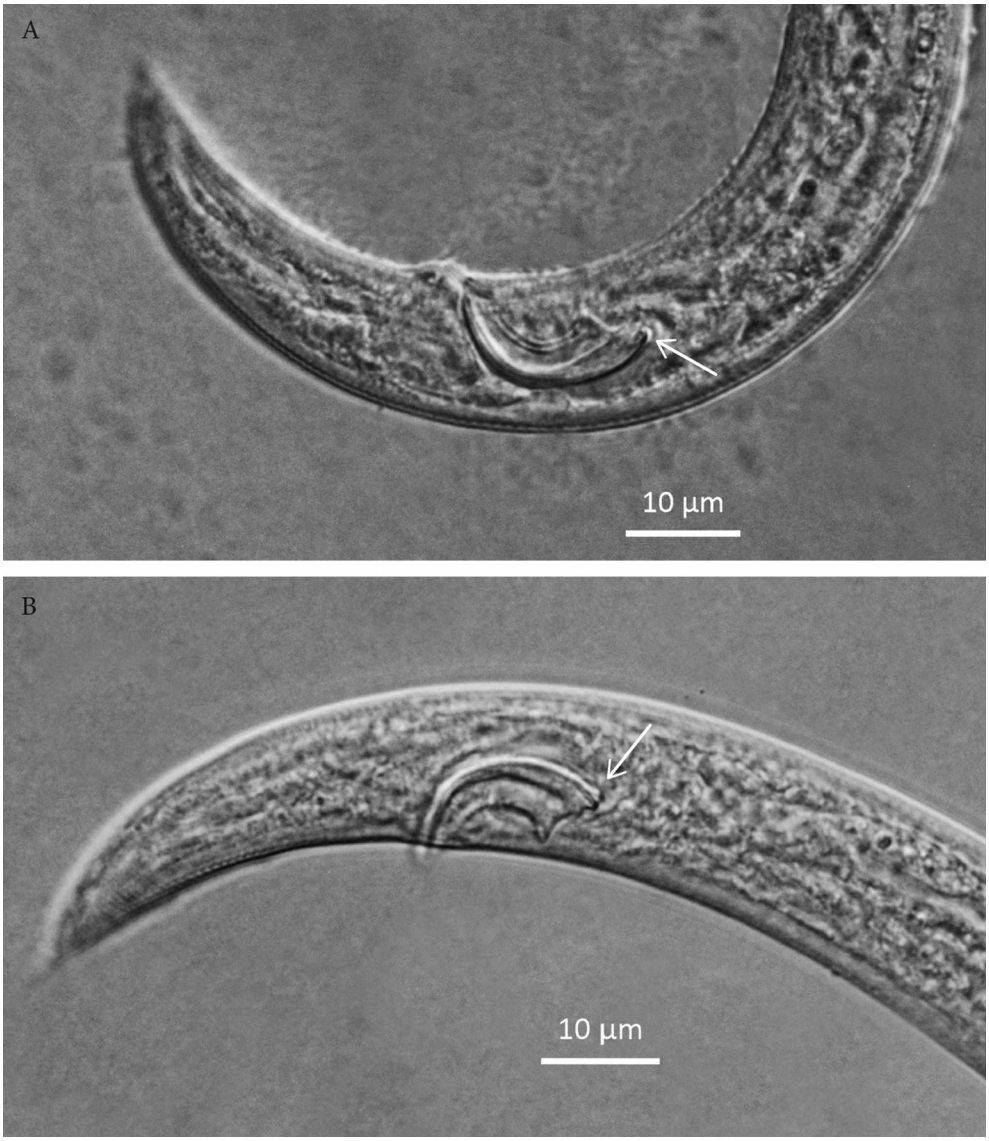


Fig. 6. Photomicrographs of *Aphelenchoides pseudobesseyi* sp. n. male from *Farfugium japonicum* in Florida. A, B: Body posterior portion showing spicules with spherical or rectangular condylus.

wide) and partially filled with spermatozoa. However, specimens of this new species with PUS devoid of spermatozoa occur in more than half (50-52%) of the specimens (59) examined across two populations, complicating the separation of these two species, which share the same male characteristics. The morphological differentiation of these two species is not sound without the support of molecular analysis and they should be considered cryptic.

A more reliable separation of *A. pseudobesseyi* sp. n. females from those of the described *A. oryzae* populations from Louisiana, Japan and Senegal is provided by the length of their PUS, which is greater than one-third of VA in 40-70% of the examined specimens, whereas it is consistently shorter than one-third of VA in those of *A. oryzae* (Yokoo, 1948; Fortuner, 1970). Furthermore, 13% of the specimens of *A. pseudobesseyi* sp. n. have the PUS full of spermatozoa rather than being partially filled with spermatozoa as in *A. oryzae*. However, specimens

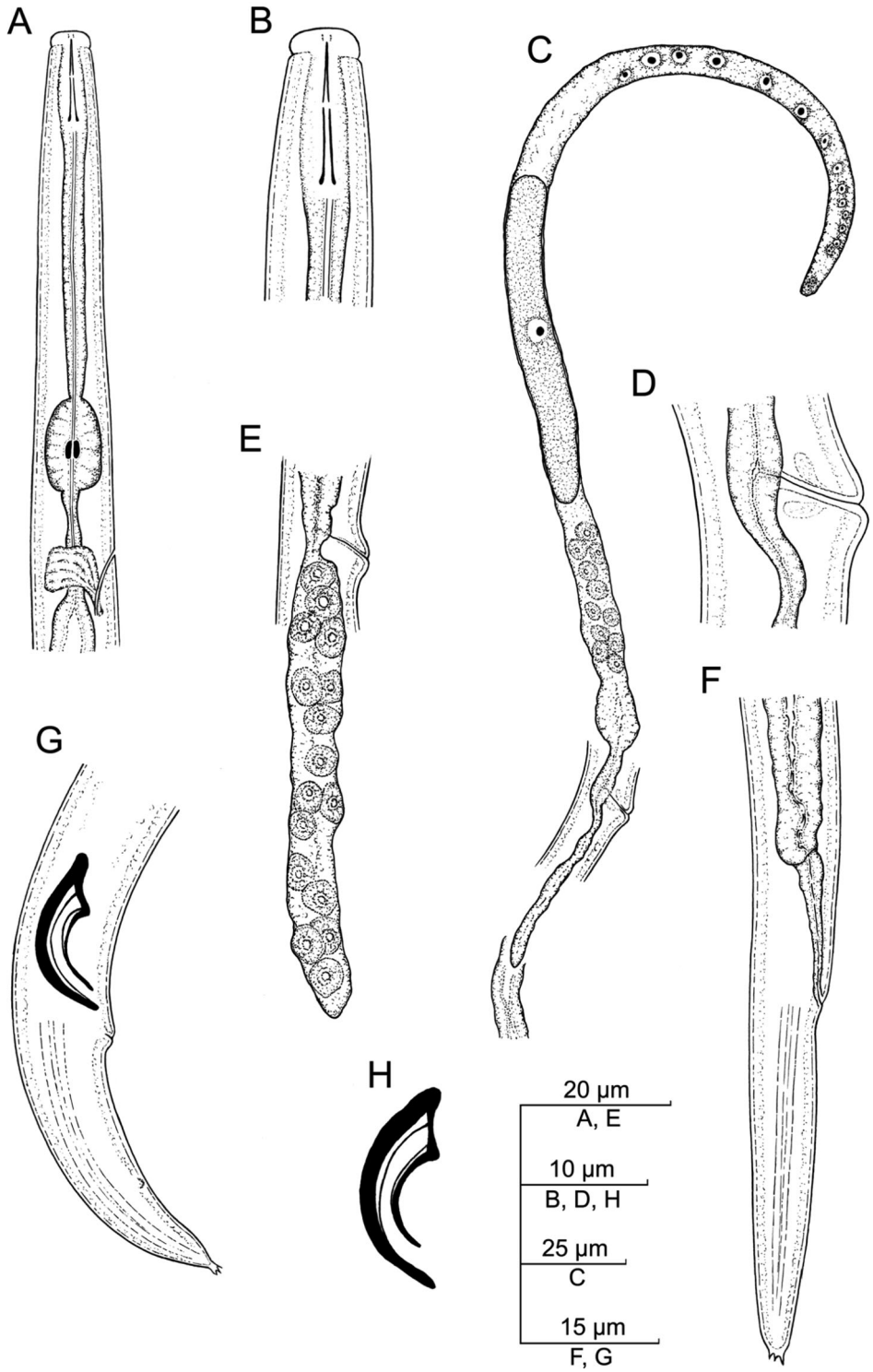


Fig. 7. Camera lucida drawings of *Aphelenchoides pseudobesseyi* sp. n. from *Farfugium japonicum* in Florida. A: Female pharyngeal region; B: Female anterior end; C: Female genital tract; D: Female vulval region; E: Post-vulval uterine sac filled with spermatozoa; F: Female posterior body; G: Male posterior body; H: Male spicules with moderately developed condylus and rostrum.

of the new species with a PUS shorter than one-third of VA and devoid of sperm may occur in at least 60% of the specimens of some populations, such as those from wood fern, thus complicating their separation from *A. oryzae*, especially when few specimens are available for identification. Males usually have spicules with a rounded or rectangular condylus that is inconspicuous in *A. oryzae* males. This character can facilitate their separation.

Aphelenchoides pseudobesseyi sp. n., together with *A. besseyi* and *A. oryzae*, are amphimictic species belonging to *Aphelenchoides* Group 3 as they have a stellate tail (Shahina, 1996). This new species differs from the remaining 15 amphimictic species in this Group by the following characters: from *A. aligarhiensis* Siddiqi, Husain & Khan, 1967 by greater values of ratios a and c′ (31.4-39 and 3.4-4.5 *vs* 23-35 and 3.2, respectively); from *A. appendurus* Singh, 1967 by shorter stylet (11.1-12.8 *vs* 16.5-17.0 μm); from *A. fujianensis* Zhou, Cui, Ye, Luo, Wang, Hu & Liao, 2010 by shorter tail (36-47 *vs* 46-58 μm); from *A. gorganensis* Miraeiz, Heydari & Bert, 2017 by greater values of ratio c′ (3.4-4.5 *vs* 2.6-3.4); from the Australian isolate of *A. hylurgi* Massey, 1974 (see Bird *et al*., 1989) by the longer tail (36-47 *vs* 26-38 μm); from *A. lichenicola* Siddiqi & Hawksworth, 1982 and *A. panaxifolia* Liu, Wu, Duan & Liu, 1999 by the longer stylet (11.1-12.8 *vs* 9.5-10 and 7.5-10 μm, respectively); from *A. medicagus* Wang, Bert, Gu, Couvreur & Li, 2019 by greater values of ratio c′ (3.4-4.5 *vs* 2.5-3.0); from *A. menthae* Lisetskaya, 1971 and *A. panadentus* Mobasseri, Pourjam & Pedram, 2018 by more incisures in the lateral field (4 *vs* 2 and 3, respectively); from *A. ritzemabosi* (Schwartz, 1911) Steiner & Buhrer, 1932 by a differently positioned excretory pore (anterior to or at level of nerve ring *vs* posterior) and PUS length/VA ratio (shorter than 50% of VA *vs* longer); from *A. siddiqii* Fortuner, 1970 by the longer tail (36-47 *vs* 26 μm); from *A stellatus* Fang, Gu, Wang & Li, 2014 by smaller values of ratio a and shorter stylet (31.4-39 *vs* 39.9-44.8 and 11.1-12.8 *vs* 12.3-17.5 μm, respectively); from *A. tabarastanensis* Golhasan, Fang, Li, Tanha Maafi & Heydari, 2019 in greater values of ratio c′ (3.4-4.5 *vs* 2.3-2.9); and from *A. wallacei* Singh, 1977 by the shorter stylet (11.1-12.8 *vs* 13.5-14.0 μm).

Molecular characterisation and phylogenetic relationships of *Aphelenchoides pseudobesseyi* sp. n. with other species

D2-D3 of 28S rRNA gene

The 28S rRNA gene alignment was 632 bp in length and contained 56 sequences of *Aphelenchoides* species including *A. ritzemabosi* and *A. gorganensis* used as outgroups. The BI revealed several clades within the *A. besseyi* species complex: *i*) *A. pseudobesseyi* sp. n. (15 sequences); *ii*) *A. besseyi* (two sequences); and *iii*) *Aphelenchoides* sp. from *Brachiaria* spp. (three sequences) and a group of *A. oryzae* sequences (41 sequences) (Fig. 8). Intraspecific sequence variation for *A. pseudobesseyi* sp. n. was 0-2.9% (0-18 bp) and for *A. oryzae*, 0-0.3% (0-3 bp). *Aphelenchoides pseudobesseyi* sp. n. sequences differed from those of *A. besseyi* by 4.7-7.4%, from those of *Aphelenchoides* sp. from *Brachiaria* spp. by 5.4-7.4%, and from those of *A. oryzae* by 4.1-9.3%.

COI gene

The *COI* gene alignment was 580 bp in length and contained 81 sequences of *Aphelenchoides* species and two sequences of *Robustodorus subtenuis* used as an outgroup. The BI revealed several clades within the *A. besseyi* species complex (Fig. 9). Phylogenetic relationships within some species are given in Figure 10. Intraspecific sequence variation for *A. pseudobesseyi* sp. n. was 0-0.3% (0-2 bp) and for *A. oryzae*, 0-6.0% (0-35 bp). *Aphelenchoides pseudobesseyi* sp. n. sequences differed from those of *A. besseyi* by 16%, and from those of *A. oryzae* and the mycetophagous *A. pseudogoodeyi*, by 14-17%.

ITS rRNA gene

The ITS rRNA gene alignment was 726 bp in length and contained 133 sequences of *Aphelenchoides*. Phylogenetic relationships within three species (*A. oryzae*, *A. besseyi* and *A. pseudobesseyi* sp. n.) are given in Figure 11. Intraspecific sequence variation for *A. pseudobesseyi* sp. n. was 2.2-3.0% (16-21 bp) and for *A. oryzae*, 0-1.4% (0-13 bp). *Aphelenchoides pseudobesseyi* sp. n. sequences differed from those of *A. besseyi* by 7.9-8.3%, and from those of *A oryzae* by 6.9-8.5%.

Parasitic habits

In the pilot study, the Florida population from leopard plant, described here as *A. pseudobesseyi* sp. n., infested the four soybean seedlings soon after seed germi-

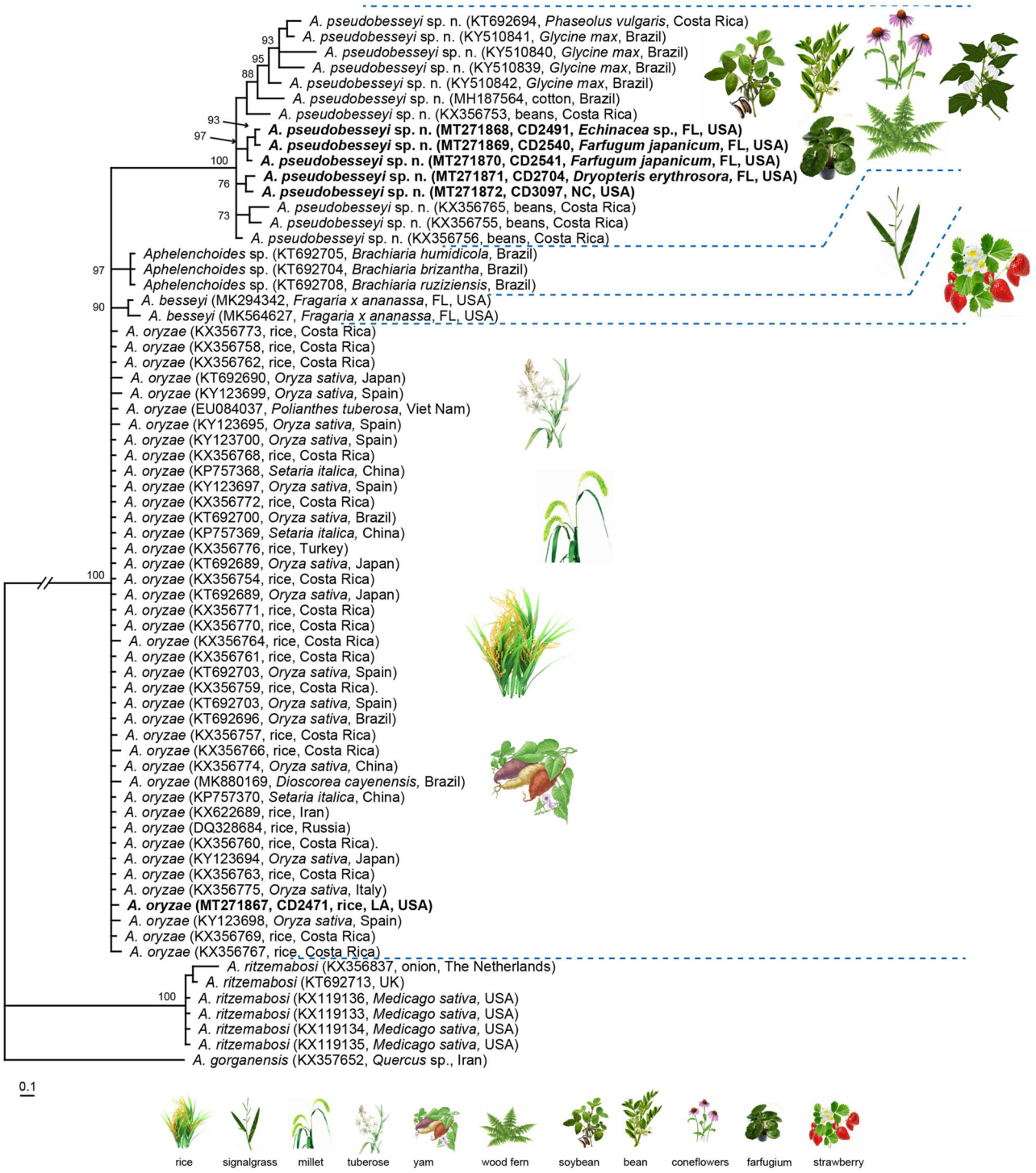


Fig. 8. Phylogenetic relationships of *Aphelenchoides pseudobesseyi* sp. n. with other related species as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequences under the GTR + I + G model with mapping of plant-hosts. Posterior probability more than 70% is given for appropriate clades. New sequences are indicated in bold. Species delimiting and new naming are given based on the present phylogenetic and sequence analysis.


A. oryzae (MF669527, rice, Taiwan)
A. oryzae (MN072933, rice, China)
A. oryzae (MF669525, rice, Taiwan)
A. oryzae (MF669523, rice, Taiwan)
A. oryzae (MN072934, rice, China)
A. oryzae (MN072932, rice, China)
A. oryzae (MN072930, rice, China)
A. oryzae (KX356862, rice, China)
A. oryzae (NC 025291, China)
A. oryzae (KJ739799, China)
A. oryzae (MN072931, rice, China)
A. oryzae (KT782810, Oryza sativa, Spain)
A. oryzae (KX356864, rice, Turkey)
A. oryzae (KX356863, rice, Italy)
A. oryzae (HQ540527, rice, Taiwan)
A. oryzae (MF669526, rice, Taiwan)
A. oryzae (MT267787, CD2471, rice, LA, USA)
A. oryzae (KX356855, rice, Costa Rica)
A. oryzae (KX356856, rice, Costa Rica)
A. oryzae (KX356854, rice, Costa Rica)
A. oryzae (KX356851, rice, Costa Rica)
A. oryzae (MN072937, rice, China)
A. oryzae (MN072936, rice, China)
A. oryzae (MN072935, rice, China)
A. oryzae (KX356845, Costa Rica)*
A. oryzae (KX356852, rice, Costa Rica)
A. oryzae (KX356844, rice, Costa Rica)
A. oryzae (KX356849, rice, Costa Rica)
A. oryzae (KX356847, rice, Costa Rica)
A. oryzae (KX356850, rice, Costa Rica)
A. oryzae (HQ540531, rice, Taiwan)
A. oryzae (KT782798, Oryza sativa, Japan)
A. oryzae (MF669518, rice, Taiwan)
A. oryzae (KX356848, rice, Costa Rica)
A. oryzae (KT782796, Oryza sativa, Japan)
A. oryzae (KT782807, Oryza sativa, Brazil)
A. oryzae (HQ540526, rice, Taiwan)
A. oryzae (KX356846, rice, Costa Rica)
A. oryzae (KX356853, rice, Costa Rica)
A. oryzae (GU367867, Oryza sativa, China)
A. oryzae (MN072938, rice, China)
A. besseyi (MK559497, Fragaria x ananassa, FL, USA)
A. besseyi (MK303401, Fragaria x ananassa, FL, USA)
Aphelenchoides sp. (KT782811, Brachiaria brizantha, Brazil)
A. pseudobesseyi sp. n. (MT267788CD3097, soil, NC, USA)
A. pseudobesseyi sp. n. (MT267789, CD2491, Echinacea sp., FL, USA)
A. pseudobesseyi sp. n. (MT267790, CD2704, Dryopteris erythrosora, FL, USA)
A. pseudobesseyi sp. n. (MF669521, bird's nest fern, Taiwan)
A. pseudobesseyi sp. n. (MF669522, bird's nest fern, Taiwan)
A. pseudobesseyi sp. n. (HQ540533, bird's nest fern, Taiwan)
A. pseudobesseyi sp. n. (MF669520, bird's nest fern, Taiwan)
A. pseudobesseyi sp. n. (MT267791, CD2540, Farfugum japanicum, FL, USA)
A. pseudobesseyi sp. n. (KT782801, Phaseolus vulgaris, Costa Rica)
A. pseudogoodeyi (KT782793, Panicum maximum, Brazil)
A. pseudogoodeyi (KT782800, Brachiaria brizantha, Brazil)
A. pseudogoodeyi (KT782809, Brachiaria decumbens, Brazil)
A. pseudogoodeyi (KT782797, Oryza sativa, Japan)
A. pseudogoodeyi (AY508072, FL, USA)
A. pseudogoodeyi (EU325686, Hosta sp. USA)
A. pseudogoodeyi (MK303402, Fragaria x ananassa, FL, USA)
A. pseudogoodeyi (HQ540532, strawberry, Taiwan)
A. pseudogoodeyi (HQ540528, bird's nest fern, Taiwan)
A. pseudogoodeyi (KT782802, Oryza sativa, Brazil)
A. pseudogoodeyi (GU367868, Zingiber sp., Togo)
A. pseudogoodeyi (KX356890, bark, Costa Rica)
A. pseudogoodeyi (KX356892, bark, Costa Rica)
A. pseudogoodeyi (KT782795, Oryza sativa, Japan)
A. pseudogoodeyi (KX356891, bark, Costa Rica)
A. pseudogoodeyi (KT782806, Oryza sativa, Brazil)
A. pseudogoodeyi (KT782791, Brachiaria decumbens, Brazil)
A. pseudogoodeyi (KT782794, Oryza sativa, Brazil)
A. pseudogoodeyi (KT782790, Brachiaria brizantha, Brazi)
A. fujianensis (FJ520226, Pinus massoniana, China)
A. ritzemabosi (KT782812, UK)
A. ritzemabosi (KX356906, The Netherlands)
A. ritzemabosi (GU367869, Dendranthema sp., China)
A. medicagus (MH853827, Medicago sativa, USA)
Aphelenchoides sp. (GU367866, wood packaging material, USA)
Aphelenchoides sp. (GU367863, wood packaging material, USA)
Aphelenchoides sp. (KX356887, bark, Costa Rica)
Aphelenchoides sp. (EU287593, Pinus radiata, Australia)
Robustodorus subtenuis (KX356860, onion, The Netherlands)
Robustodorus subtenuis (KX356859, crocus, The Netherlands)
0.1
rice
ringer
signalgrass
wood fern
bird's nest fern
hosta
bean
coneflowers
farfugium
strawberry

nation. The inoculum used caused severe stunting of the seedlings. Nematodes penetrated the cotyledons and migrated into the leaf primordia of the shoot apex inducing crinkling, distortion, necrosis and a spider-like appearance of the cotyledons and young leaves (Fig. 12). The number of nematodes found inside the leaf and stem tissue of the four seedlings, 40 days after inoculation, was 120. This final population level was smaller than the initial population (200 specimens) in the inoculum, suggesting a lack of nematode reproduction.

Problems of species delimiting and identification

Species delimiting in the *A. besseyi* species complex in this study are based on analysis of rRNA and mtDNA gene sequences and some morphological characters. The analysis of D2-D3 of 28S rRNA, ITS rRNA and *COI* gene sequences clearly separated *A. besseyi*, *A. oryzae* and *A. pseudobesseyi* sp. n. from each other. Other genes, 18S rRNA (Wu *et al*., 2016; Oliveira *et al*., 2019) and glycoside hydrolase GH45 and GH5 genes (Wu *et al*., 2016), also allowed us to distinguish these species. Moreover, the analysis also indicates that some other cryptic species could be delimited within the complex; however, more molecular data and information on morphology and morphometrics need to be obtained and studied.

Our research confirms the difficulty in the morphological separation of species previously considered as '*A. besseyi*' detected on rice, ornamental plants and strawberry in the Southeastern states of the USA. These populations differ morphologically in the structure of the genital tract of the females. Using these characters we have separated these populations into three cryptic species: *A. besseyi*, *A. oryzae* and *A. pseudobesseyi* sp. n. Their morphological identification may be possible but it is challenging and requires careful examination of many specimens for an accurate diagnosis. Furthermore, the few diagnostic characters that we found overlap to a significant degree and, in many cases, make identification unreliable without the support of the molecular analysis.

Fig. 9. Phylogenetic relationships of *Aphelenchoides pseudobesseyi* sp. n. with other related species as inferred from Bayesian analysis using the *COI* mtDNA gene sequences under the GTR + I + G model with mapping of plant-hosts. Posterior probability more than 70% is given for appropriate clades. New sequences are indicated in bold. Species delimiting and new naming are given based on the present phylogenetic and sequence analysis. * – the plant host is likely misnamed.

We would like to emphasise that, in the molecular study, only one population of *A. besseyi* was used for comparison with several populations of *A. oryzae* and *A. pseudobesseyi* sp. n. One of the reasons for the lack of additional populations is due to the annual fluctuation of this species in Florida strawberry fields, which were more infested in 2016 and 2017 than in 2018 through 2020. Christie (1959) noticed this nematode behaviour and stated that infestations of *A. besseyi* were widespread and more devastating in the 1930s than in the late 1950s when they became rare. He hypothesised that improvement in the phytosanitary conditions of the nurseries may have caused the decline of the nematode populations in the fields in those years.

Mapping of host plants on phylogenetic trees reveals some trends in the parasitism of species and population groups in the *A. besseyi* species complex. Our study showed that the rice white tip nematode, *A. oryzae*, parasitises only rice and several other monocots, there being no molecularly confirmed reports of this species on dicots. These findings agree with results of host tests suggesting that this species does not parasitise strawberry and, *vice versa*, populations of *A. besseyi* from strawberry do not parasitise rice (Riggs, 1991). On the contrary, *A. pseudobesseyi* sp. n. is found on dicots and ferns, and normally seems not to parasitise monocots, probably occurring on these plants only under experimental or certain other conditions. Marlatt (1966) found *A. besseyi s.l.* (most likely *A. pseudobesseyi* sp. n.) damaging leaves of rubber plant (*Ficus elastica*) growing in southern Florida. This nematode was also found without evidence of damage to inflorescences of the monocot smut-grass, *Sporobolus poiretii*, growing close to infected rubber plants (Marlatt, 1970). Yu & Tsay (2004) also reported experimental infection of strawberry and rice by '*A. besseyi s.l.*' (likely to be *A. pseudobesseyi* sp. n.) originating from bird's nest fern in Taiwan. The occurrence of a population of *A. pseudobesseyi* sp. n. in soil from a field previously cultivated with strawberry in North Carolina (NC) may confirm this finding, and may indicate that strawberry is a host of this new species also in the USA. However, strawberry plants transplanted in a large pot containing the NC soil in a glasshouse were not infested by the nematode.

In the small pilot study conducted in a growth chamber (done this way to avoid potential symptom complications caused by feeding activities of microarthropods), the *A. pseudobesseyi* sp. n. from leopard plants severely stunted soybean seedlings, another dicot, although it did not

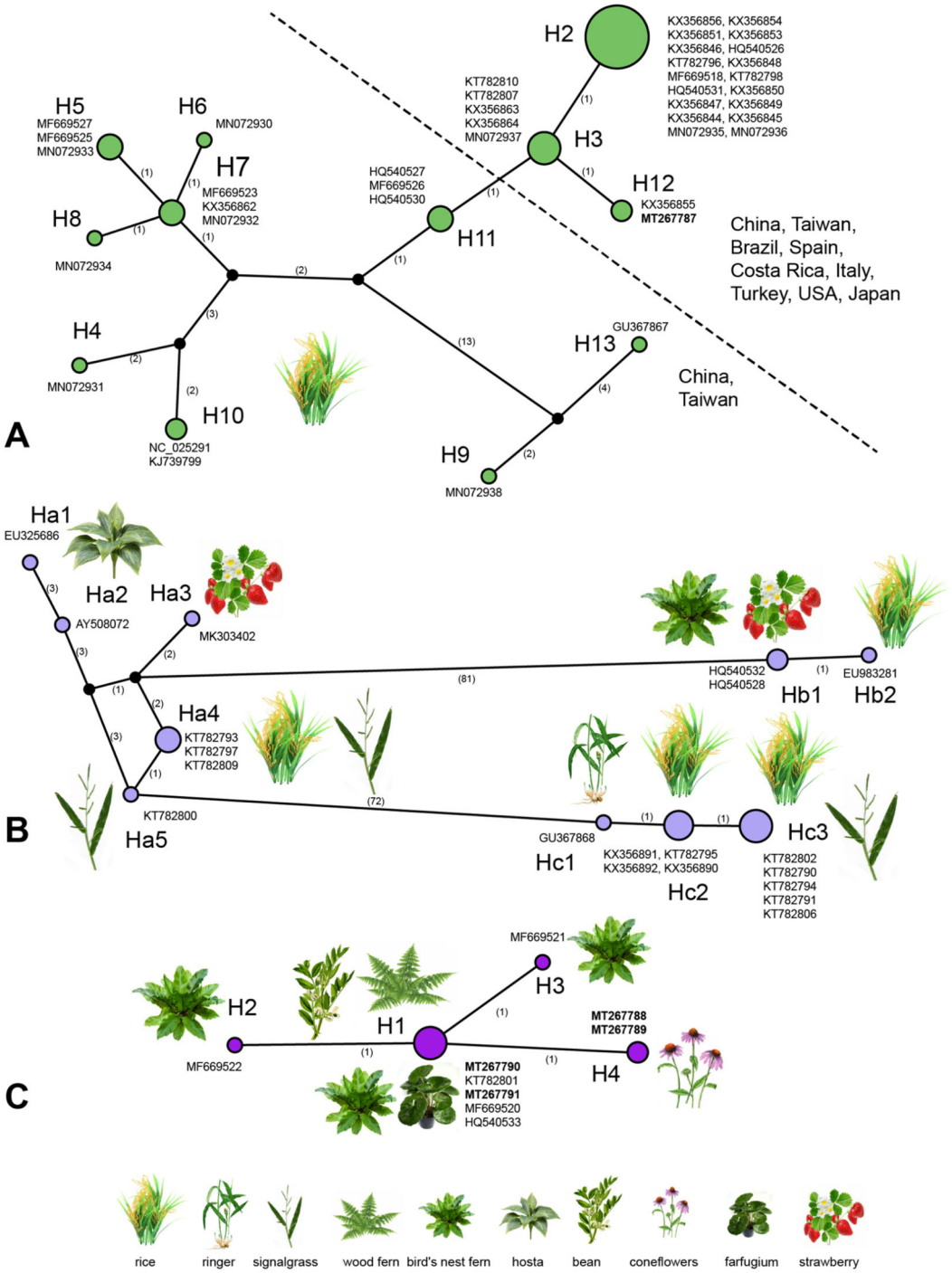
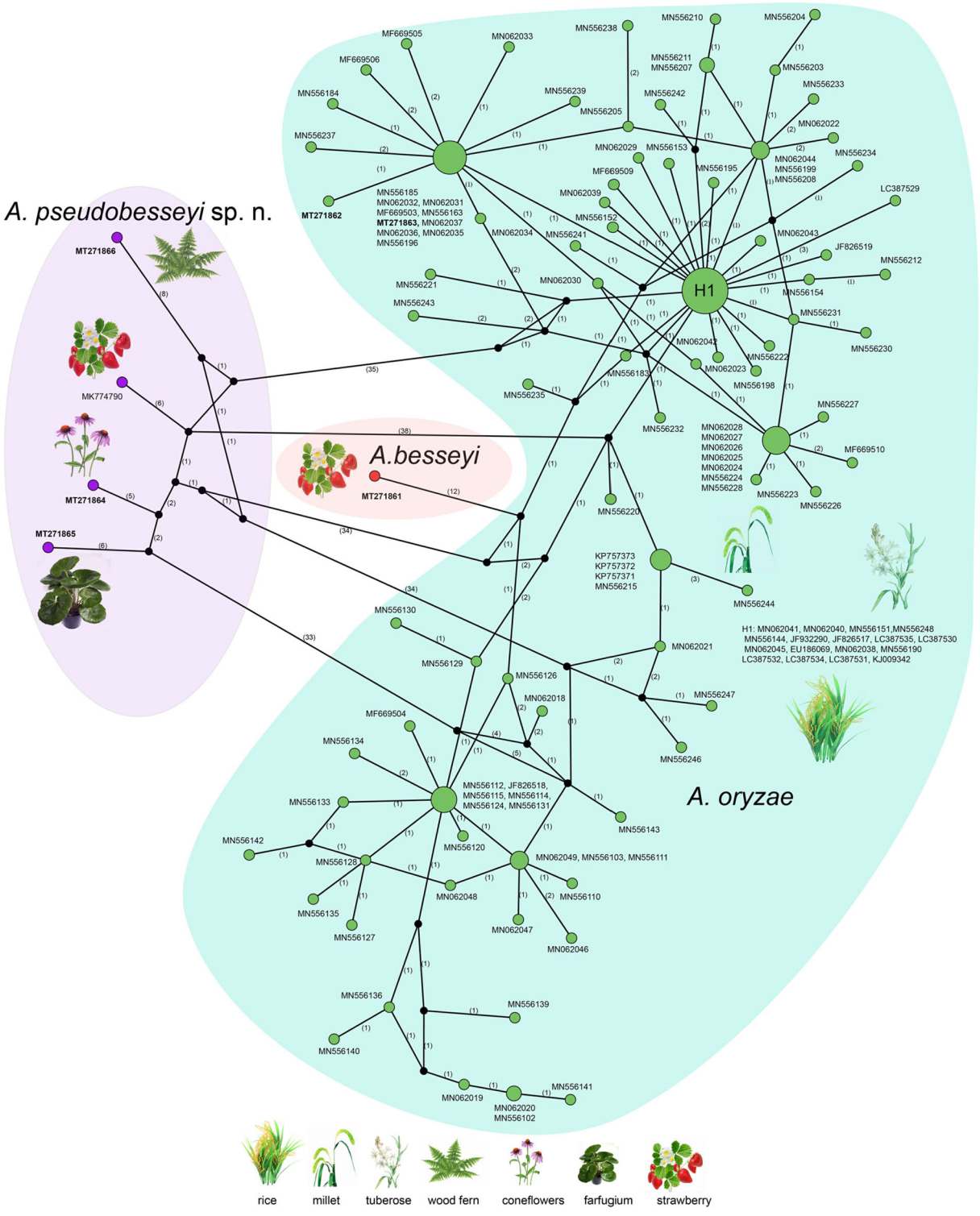


Fig. 10. Statistical parsimony networks showing the phylogenetic relationships between *COI* mtDNA gene haplotypes of *Aphelenchoides oryzae* (A), *A. pseudogoodeyi* (B) and *A. pseudobesseyi* sp. n. (C) with mapping of plant-hosts. Pies (circles) represent sequences of each species with the same haplotype and their size is proportional to the number of these sequences in the samples. Numbers of nucleotide differences between the sequences are indicated on lines connecting the pies. Small black circles represent missing haplotypes. New sequences are given in bold. *COI* haplotype codes for *A. oryzae* are given as proposed by Xu *et al.* (2020). Geographical division of haplotypes is also provided.


A. pseudobesseyi sp. n.
A.besseyi
A. oryzae
H1
MT271866
MK774790
MT271864
MT271865
MT271862
MT271861
MF669505
MF669506
MN062033
MN556238
MN556210
MN556204
MN556211
MN556207
MN556203
MN556233
MN556184
MN556239
MN556205
MN556242
MN062022
MN556237
MN062029
MN556153
MN556234
MN062044
MN556199
MN556208
MF669509
MN556195
LC387529
MN556185
MN062032, MN062031
MF669503, MN556163
MN062037
MN062036, MN062035
MN556196
MN062039
MN556152
MN062034
MN556241
MN062043
JF826519
MN556212
MN556221
MN062030
MN556154
MN556243
MN556231
MN556230
MN062042
MN556222
MN062023
MN556183
MN556198
MN556235
MN556227
MN556232
MN062028
MN062027
MN062026
MN062025
MN062024
MN556224
MN556228
MF669510
MN556223
MN556226
MN556220
KP757373
KP757372
KP757371
MN556215
MN556244
MN556130
MN062021
H1: MN062041, MN062040, MN556151,MN556248
MN556144, JF932290, JF826517, LC387535, LC387530
MN062045, EU186069, MN062038, MN556190
LC387532, LC387534, LC387531, KJ009342
MN556129
MN556126
MN062018
MN556247
MF669504
MN556246
MN556134
MN556112, JF826518,
MN556115, MN556114,
MN556124, MN556131
MN556133
MN556143
MN556142
MN556120
MN556128
MN062049, MN556103, MN556111
MN062048
MN556110
MN556135
MN556127
MN062047
MN062046
MN556136
MN556139
MN556140
MN062019
MN062020
MN556102
MN556141
rice
millet
tuberose
wood fern
coneflowers
farfugium
strawberry

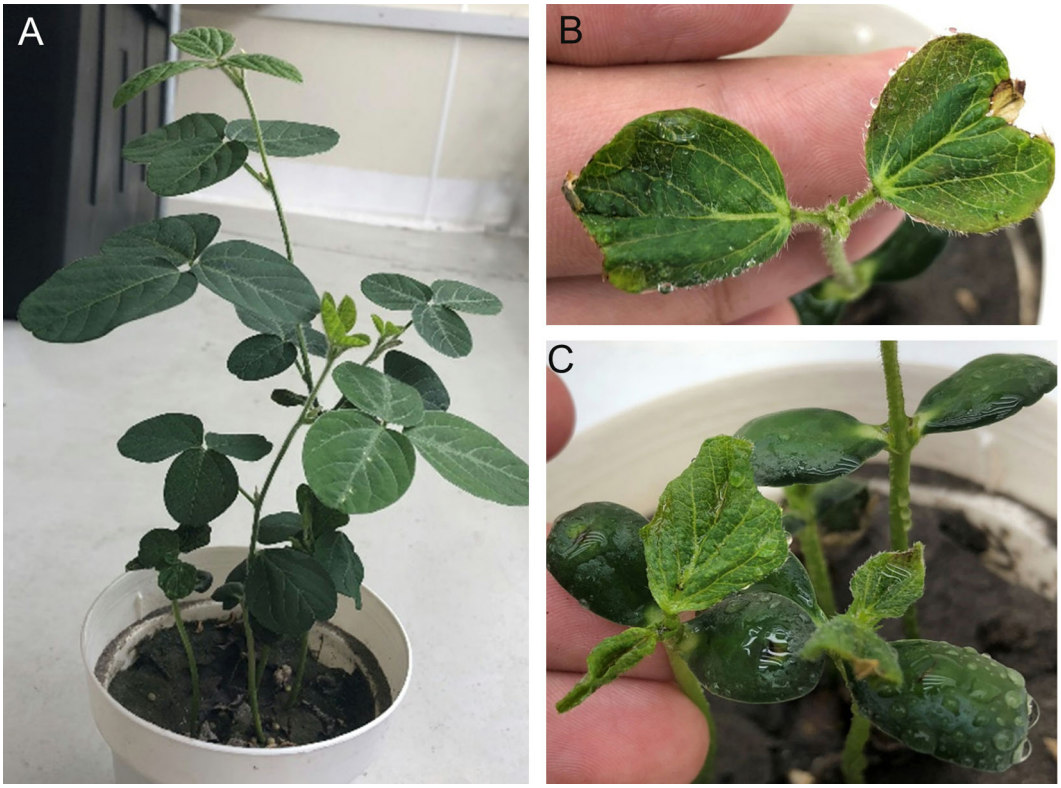


Fig. 12. Symptoms induced by a population of *Aphelenchoides pseudobesseyi* sp. n. from leopard plant on soybean 'Patriot' seedlings, 17 days after inoculation with 200 nematodes. A: Seedlings showing healthy leaves; B: Seedling with two cotyledons stunted, distorted, deformed, and necrotic; C: Seedlings with the same symptoms on young leaves.

reproduce on this plant species. More host tests are needed to confirm the ability of this population to persist on soybean. Some discordance between the lists of plant hosts published by other authors for the species could be explained by the fact that, in infection experiments, nematodes can invade, and even multiply and induce symptoms in non-favourable hosts for several generations until they lose their infectivity. In contrast, their parasitic ability persists on natural hosts, which remain inhabited by the nematode under field conditions for a very long time.

Several molecular diagnostics tools have been developed for identification of *A. besseyi s.l.* Conventional and real-time PCR methods have been designed by several authors (Cui *et al*., 2010; Rybarczyk-Mydłowska *et al*., 2012; Devran *et al*., 2017; Buonicontro *et al*., 2018; Çelik & Devran, 2019; Çelik *et al*., 2020). Bai *et al*. (2017) and Yang & Yu (2019) also proposed loop-mediated isothermal amplification assay (LAMP) for *A. besseyi* diagnostics. Detection of all these methods, except for that developed by Cui *et al*. (2010) and Yang & Yu (2019), are based on differences in 18S rRNA gene sequences between species. However, *in silico* analysis and published results (Yang & Yu, 2019) showed that most of these methods cannot separate the rice white tip nematode *A. oryzae* from other species in this complex. To the best of our knowledge, only a recently published LAMP assay

Fig. 11. Statistical parsimony network showing the phylogenetic relationships between ITS rRNA gene haplotypes of *Aphelenchoides oryzae*, *A. besseyi* and *A. pseudobesseyi* sp. n. with mapping of plant-hosts. The sequences of each species are marked by different colours. Pies (circles) represent sequences of each species with the same haplotype and their size is proportional to the number of these sequences in the samples. Numbers of nucleotide differences between the sequences are indicated on lines connecting the pies. Small black circles represent missing haplotypes. New sequences are given in bold. Species delimiting and new naming are given based on the present phylogenetic and sequence analysis.

developed by Yang & Yu (2019), in which *COI* mtDNA is used to differentiate species and populations, allows specific detection of *A. oryzae*.

Generating *COI* gene sequences of the *A. besseyi* complex prompts an analysis of the phylogeographical pattern for the species. The results obtained by Xu *et al*. (2020) showed that *COI* haplotypes in Spain, Italy and Turkey were all found in China, suggesting the European *A. oryzae* populations may have been brought from Asia together with their rice host. These authors noted that most of the rice in Latin America has a European origin; however, there are at least two native rice species in South America, used by the indigenous inhabitants of the region to create a domesticated rice. Xu *et al*. (2020) revealed one unique *COI* haplotype (H12) from Costa Rica (Sánchez-Monge *et al*., 2017), which might support the hypothesis that native *A. oryzae* populations exist in Latin America. It is remarkable that the *COI* sequence obtained from the Louisiana sample of *A. oryzae* is identical to this Costa Rica sequence. Evidently, more *A. oryzae* samples from different countries should be analysed to understand the phylogeography of this species.

Acknowledgement

The authors thank Scott Burton and Brandon Hope, Florida Department of Agriculture and Consumer Services, USA, for their technical assistance.

References

Allen, M.W. (1952). Taxonomic status of the bud and leaf nematodes related to *Aphelenchoides fragariae* (Ritzema Bos, 1891). *Proceedings of the Helminthological Society of Washington.* 19, 108-120.

Anon. (2017). PM 7/39 (2) *Aphelenchoides besseyi*. *Bulletin OEPP/EPPO Bulletin* 47, 384-400. DOI: 10.1111/epp.12432

Bai, Z., Qin, M., Zhao, L., Han, Y., Wang, D., Xu, C. & Xie, H. (2017). [Loop-mediated isothermal amplification assay for rapid diagnosis of *Aphelenchoides besseyi*.] *Chinese Journal of Rice Science* 31, 432-440.

Bandelt, H., Forster, P. & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16, 37-48. DOI: 10.1093/oxfordjournals.molbev.a026036

Bird, A.F., Bird, B., Fortuner, R. & Moen, R. (1989). Observations on *Aphelenchoides hylurgi* Massey, 1974 feeding on fungal pathogens of wheat in Australia. *Revue de Nématologie* 12, 27-34.

Buonicontro, D.S., Roberts, D.M., Oliveira, C.M.G., Blok, V.C., Neilson, R. & Oliveira, R.D. de L. (2018). A rapid diagnostic for detection of *Aphelenchoides besseyi* and *A. fujianensis* based on real-time PCR. *Plant Disease* 102, 519-526. DOI: 10.1094/PDIS-08-17-1160-RE

Çelik, E.S. & Devran, Z. (2019). Identification and quantification of *Aphelenchoides besseyi* from rice using qPCR. *European Journal of Plant Pathology* 154, 691-703. DOI: 10.1007/s10658-019-01692-4

Çelik, E.S., Tülek, A. & Devran, Z. (2020). Development of a novel scale based on qPCR for rapid and accurate prediction of the number of *Aphelenchoides besseyi* in paddy rice. *Crop Protection* 127, 104975. DOI: 10.1016/j.cropro.2019.104975

Christie, J.R. (1942). A description of *Aphelenchoides besseyi* n. sp., the summer dwarf nematode of strawberries, with comments on the identity of *Aphelenchoides subtenuis* (Cobb, 1929) and *Aphelenchoides hodsoni* Goodey, 1935. *Proceeding of the Helminthological Society of Washington* 9, 82-84.

Christie, J.R. (1959). *Plant nematodes, their bionomics and control*. Gainesville, Florida, USA, Agricultural Experiment Stations, University of Florida.

Cuc, N.T.T. & Pilon, M. (2007). An *Aphelenchoides* sp. nematode parasitic of *Polianthes tuberosa* in the Mekong Delta. *Journal of Nematology* 39, 248-257.

Cui, R.Q., Ge, J.J., Hu, X.N., Zhao, L.R., Zhong, G.Q. & Feng, L.X. (2010). [A rapid method to detect *Aphelenchoides besseyi* by PCR.] *Plant Quarantine* 24, 10-12.

De Grisse, A.T. (1969). Redescription ou modification de quelques techniques utilisées l'étude des nematodes phytoparasitaires. *Mededelingen van de Rijksfaculteit der Landbouwwetenschappen Gent* 34, 351-369.

De Jesus, D.S., Oliveira, C.M.G., Roberts, D., Blok, V., Prior, T., Balbino, H.M., MacKenzie, K.M. & Oliveira, R.D. (2016). Morphological and molecular characterisation of *Aphelenchoides besseyi* and *A. fujianensis* (Nematoda: Aphelenchoididae) from rice and forage grass seeds in Brazil. *Nematology* 18, 337-356. DOI: 10.1163/15685411-00002962

Desaeger, J. & Noling, J. (2017). *Foliar and bud nematodes in Florida strawberries*. (ENY-068). Gainesville, FA, USA, University of Florida Institute of Food and Agricultural Sciences. Retrieved from https://edis.ifas.ufl.edu/pdffiles/IN/IN118400.pdf

Devran, Z., Tülek, A., Mıstanoğlu, İ., Çiftçiğil, T.H. & Özalp, T. (2017). A rapid molecular detection method for *Aphelenchoides besseyi* from rice tissues. *Australasian Plant Pathology* 46, 43-48. DOI: 10.1007/s13313-016-0452-1

De Waele, D. (2002). Foliar nematodes: *Aphelenchoides* species. In: Starr, J.L., Cook, R. & Bridge, J. (Eds). *Plant resistance to parasitic nematodes*. Wallingford, UK, CAB International, pp. 141-151.

Duncan, L.W. & Moens, M. (2013). Migratory endoparasitic nematodes. In: Perry, R.N. & Moens, M. (Eds). *Plant nematology*, 2nd edition. Wallingford, UK, CAB International, pp. 144-178.

Esser, R.P. (1986). A water agar *en face* technique. *Proceedings of the Helminthological Society of Washington* 53, 254-255.

Fang, Y., Gu, J., Wang, X. & Li, H. (2014). Description of *Aphelenchoides stellatus* n. sp. (Nematoda: Aphelenchoididae) found in packaging wood from Japan. *Nematology* 16, 621-630. DOI: 10.1163/15685411-00002792

Favoreto, L., Faleiro, V.O., Freitas, M.A., Brauwers, L.R., Galbieri, R., Homiak, J.A., Lopes-Caitar, V.S., Marcelino-Guimarães, F.C. & Meyer, M.C. (2018). First report of *Aphelenchoides besseyi* infecting the aerial part of cotton plants in Brazil. *Plant Disease* 102, 2662. DOI: 10.1094/PDIS-02-18-0334-PDN

Fortuner, R. (1970). On the morphology of *Aphelenchoides besseyi* Christie, 1942 and *A. siddiqii* n. sp. (Nematoda, Aphelenchoidea). *Journal of Helminthology* 44, 141-152.

Franklin, M.T. & Siddiqi, M.R. (1972). *Aphelenchoides besseyi. CIH descriptions of plant-parasitic nematodes, Set 1, No. 4*. St. Albans, UK, Commonwealth Institute of Helminthology.

Godoy, F.M.C., Overstreet, C., McGawley, E.C., Hollier, C.A., Kularathna, M.T., Khanal, C. & McInnes, B. (2019). Incidence of *Aphelenchoides besseyi* in rice in Louisiana and host status of the most widely planted cultivars. *Nematropica* 49, 107-123.

Golhasan, B., Fang, Y., Li, H., Tanha Maafi, Z. & Heydari, R. (2019). Description of *Aphelenchoides tabarestanensis* n. sp. (Nematoda: Aphelenchoididae) isolated from *Pinus brutia* in northern Iran. *Nematology* 21, 159-169. DOI: 10.1163/15685411-00003204

Hockland, S. (2001). *A pragmatic approach to indentifying* Aphelenchoides *species for plant quarantine and pest management programmes*. Ph.D. Thesis, University of Reading, Reading, UK.

Hsieh, S.-H, Lin, C.-J. & Chen, P. (2012). Sexual compatibility among different host-originated isolates of *Aphelenchoides besseyi* and the inheritance of the parasitism. *PLoS ONE* 7(7), e40886. DOI: 10.1371/journal.pone.0040886

Huang, C.T. & Huang, C.S. (1974). Embryogenesis and morphometry of rice white tip nematode, *Aphelenchoides besseyi*. *Plant Protection Bulletin* 16, 56-68.

Hunt, D.J. (1993). *Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics*. Wallingford, UK, CABI Publishing.

Kanzaki, N. & Futai, K. (2002). A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within the *xylophilus* group. *Nematology* 4, 35-41. DOI: 10.1163/156854102760082186

Khan, M.R., Handoo, Z.A., Rao, U., Rao, S.B. & Prasad, J.S. (2012). Observations on the foliar nematode, *Aphelenchoides besseyi*, infecting tuberose and rice in India. *Journal of Nematology* 44, 391-398.

Lin, M.S., Ding, Z.M., Wang, Z.M., Zhou, F.M. & Lin, N. (2004). Description of *Aphelenchoides besseyi* from abnormal rice with 'small grains and erect panicles' symptom in China. *Rice Science* 12, 289-294.

Lisetskaya, L.F. (1971). [*Aphelenchoides menthae* n. sp. (Nematoda: Aphelenchoididae).] *Parazity Zhivotnykh i Rastenii* 6, 123-126.

Liu, W., Wu, X., Duan, Y. & Liu, Y. (1999). A new species of genus *Aphelenchoides*, leaf nematode of American ginseng, *Aphelenchoides panaxofolia* n. sp. (Nematoda: Aphelenchoididae). *Acta Phytopathologica Sinica* 29, 360-363.

Marlatt, R.B. (1966). *Ficus elastica* a host of *Aphelenchoides besseyi in* a subtropical climate. *Plant Disease Reporter* 50, 689-691.

Marlatt, R.B. (1970). Transmission of *Aphelenchoides besseyi* to *Ficus elastica* leaves via *Sporobolus poiretii* inflorescences. *Phytopathology* 60, 543-544. DOI: 10.1094/Phyto-60-543

Massey, C.L. (1974). *Biology and taxonomy of nematode parasites and associates of bark beetles in the United States*. Agriculture Handbook No. 446. Washington, USA, USDA Forest Service.

Meyer, M.C., Favoreto, L., Klepker, D. & Marcelino-Guimarães, F.C. (2017). Soybean green stem and foliar retention syndrome caused by *Aphelenchoides besseyi*. *Tropical Plant Pathology* 42, 403-409. DOI: 10.1007/s40858-017-0167-z

Miraeiz, E., Heydari, R. & Bert, W. (2017). *Aphelenchoides gorganensis* n. sp. (Nematoda: Aphelenchoididae), a new species from Iran. *European Journal of Plant Pathology* 149, 157-169. DOI: 10.1007/s10658-017-1175-z

Mobasseri, M., Pourjam, E. & Pedram, M. (2018). Morphological and molecular characterisation of *Aphelenchoides primadentus* n. sp. (Nematoda: Aphelenchoididae) from northern Iran. *Nematology* 20, 97-109. DOI: 10.1163/15685411-00003127

Oliveira, C.J., Subbotin, S.A., Álvarez-Ortega, S., Desaeger, J., Brito, J.A., Xavier, K.V., Freitas, L.G., Vau, S. & Inserra, R.N. (2019). Morphological and molecular identification of two Florida populations of foliar nematodes (*Aphelenchoides* spp.) isolated from strawberry with the description of *Aphelenchoides pseudogoodeyi* sp. n. (Nematoda: Aphelenchoididae) and notes on their bionomics. *Plant Disease* 103, 2825-2842. DOI: 10.1094/PDIS-04-19-0752-RE

Riggs, R.D. (1991). Resistance-breaking races of plant parasitic nematodes. In: Nickle, W.R. (Ed.). *Manual of agricultural nematology*. New York, NY, USA, Marcel Dekker, pp. 827-854.

Rybarczyk-Mydłowska, K., Mooyman, P., van Megen, H., van den Elsen, S., Vervoort, M., Veenhuizen, P., van Doorn, J., Dees, R., Karssen, G., Bakker, J. *et al.* (2012). Small subunit ribosomal DNA-based phylogenetic analysis of foliar nematodes (*Aphelenchoides* spp.) and their quantitative detection in complex DNA backgrounds. *Phytopathology* 102, 1153-1160. DOI: 10.1094/PHYTO-05-12-0114-R

Sánchez-Monge, G.A., Jansen, T., Fang, Y., Couvreur, M., Karssen, G. & Bert, W. (2017). mtCOI successfully diagnoses the four main plant-parasitic *Aphelenchoides* species (Nematoda-Aphelenchoididae) and supports multiple origin of plant-parasitism in this polyphyletic genus. *European*

Journal of Plant Pathology 148, 853-866. DOI: 10.1007/s10658-016-1141-1

Schwartz, M. (1911). Die Aphelenchen der Veilchengallen und Blattflecken an Farnen und Chrysanthemum. *Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft* 8, 303-334.

Seinhorst, J.W. (1966). Killing nematodes for taxonomic study with hot f.a. 4:1. *Nematologica* 12, 178. DOI: 10.1163/187529266X00239

Shahina, F. (1996). A diagnostic compendium of the genus *Aphelenchoides* Fischer, 1894 (Nematoda: Aphelenchida) with some new records of the group from Pakistan. *Pakistan Journal of Nematology* 14, 1-32.

Siddiqi, M.R. & Hawksworth, D.L. (1982). Nematodes associated with galls on *Cladonia glauca*, including two new species. *The Lichenologist* 14, 175-184. DOI: 10.1017/S0024282982000310

Siddiqi, M.R., Husain, S.I. & Khan, A.M. (1967). *Seinura propora* n. sp. and *Aphelenchoides aligarhiensis* n. sp. (Nematoda: Aphelenchoididae) from north India. *Nematologica* 13, 287-290. DOI: 10.1163/187529267X00166

Singh, S.D. (1967). On two new species of the genus *Aphelenchoides* Fischer, 1894 (Nematoda: Aphelenchoididae) from north India. *Journal of Helminthology* 41, 63-70.

Singh, S.P. (1977). *Aphelenchoides wallacei* sp. n. and *Aphelenchoides jonesi* sp. n. (Nematoda: Aphelenchoididae) from inside the roots of papaya and eggplant. *Indian Journal of Nematology* 5, 207-213.

Sites Jr, J.W. & Marshall, J.C. (2004). Operational criteria for delimiting species. *Annual Review of Ecology Evolution and Systematics* 35, 199-227. DOI: 10.1146/annurev.ecolsys.35.112202.130128

Steiner, G. & Buhrer, E.M. (1932). Miscellaneous notes on nemic diseases. *Plant Disease Reporter* 16, 137.

Subbotin, S.A. & Chitambar, J.J. (2018). *Plant parasitic nematodes in sustainable agriculture of North America: Vol. 2 – Northeastern, midwestern and southern USA*. Cham, Switzerland, Springer Nature, 6330. DOI: 10.1007/978-3-319-99588-5

Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. & Baldwin, J.G. (2006). Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8, 455-474. DOI: 10.1163/156854106778493420

Tanha Maafi, Z., Subbotin, S.A. & Moens, M. (2003). Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. *Nematology* 5, 99-111. DOI: 10.1163/156854102765216731

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The CLUSTAL_X Windows Interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876-4882. DOI: 10.1093/nar/25.24.4876

Tzeng, C.Y. & Lin, Y.Y. (2005). The intraspecific variation of *Aphelenchoides besseyi* populations in Taiwan. *Plant Pathology Bulletin* 14, 67-75.

Wang, Z., Bert, W., Gu, J., Couvreur, M. & Li, H. (2019). *Aphelenchoides medicagus* n. sp. (Tylenchina: Aphelenchoididae) found in *Medicago sativa* imported into China from the USA. *Nematology* 21, 709-723. DOI: 10.1163/15685411-00003247

Wu, G.-L., Kuo, T.-H., Tsay, T.-T., Tsai, I.J. & Chen, P.J. (2016). Glycoside hydrolase (GH) 45 and 5 candidate cellulases in *Aphelenchoides besseyi* isolated from bird's-nest fern. *PLoS ONE* 11(7), e0158663. DOI: 10.1371/journal.pone.0158663

Xu, X., Qing, X., Xie, J.L., Yang, F., Peng, Y.L. & Ji, H.L. (2020). Population structure and species delimitation of rice white tip nematode, *Aphelenchoides besseyi* (Nematoda: Aphelenchoididae), in China. *Plant Pathology* 69, 159-167. DOI: 10.1111/ppa.13113

Yang, J.I. & Yu, G.Y. (2019). A loop-mediated isothermal amplification assay for the plant-parasitic nematode *Aphelenchoides besseyi* in rice seedlings. *Journal of Nematology* 51, e2019-80. DOI: 10.21307/jofnem-2019-080

Ye, W., Giblin-Davis, R.M., Braasch, H., Morris, K. & Thomas, W.K. (2007). Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43, 1185-1197. DOI: 10.1016/j.ympev.2007.02.006

Yokoo, T. (1948). [*Aphelenchoides oryzae* Yokoo n. sp. a nematode parasite to rice plant.] *Annals of the Phytopathological Society of Japan* 13, 40-43.

Yu, P.C. & Tsay, T.T. (2004). Occurrence of a foliar nematode disease of fern in Taiwan. *Plant Pathology Bulletin* 13, 35-44.

Zhou, K., Cui, R., Ye, W., Luo, M., Wang, H., Hu, X. & Liao, J. (2010). Morphological and molecular characterization of *Aphelenchoides fujianensis* n. sp. (Nematoda: Aphelenchoididae) from *Pinus massoniana* in China. *Zootaxa* 2509, 39-52. DOI: 10.11646/zootaxa.2509.1.3