Journal of Helminthology

cambridge.org/jhl

Research Paper

Cite this article: Trinh QP, Le TML, Nguyen TD, Nguyen HT, Liebanas G, Nguyen TAD (2019). *Meloidogyne daklakensis* n. sp. (Nematoda: Meloidogynidae), a new root-knot nematode associated with Robusta coffee (*Coffea canephora* Pierre ex A. Froehner) in the Western Highlands, Vietnam. *Journal of Helminthology* **93**, 242–254. https://doi.org/10.1017/S0022149X18000202

Received: 16 August 2017
Accepted: 6 February 2018
First published online: 5 April 2018

Author for correspondence:
Q.P. Trinh, E-mail: tqphap@gmail.com

CAMBRIDGE UNIVERSITY PRESS

Meloidogyne daklakensis n. sp. (Nematoda: Meloidogynidae), a new root-knot nematode associated with Robusta coffee (*Coffea canephora* Pierre ex A. Froehner) in the Western Highlands, Vietnam

Q.P. Trinh[1,2], T.M.L. Le[1,2], T.D. Nguyen[1,2], H.T. Nguyen[2,4], G. Liebanas[3] and T.A.D. Nguyen[1,3]

[1]Graduate University of Science and Technology, Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; [2]Institute of Ecology and Biological Resources, Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; [3]Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus 'Las Lagunillas' s/n, Edificio B3, 23071–Jaén, Spain and [4]Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

Abstract

The root-knot nematode species *Meloidogyne daklakensis* n. sp. was discovered on the roots of Robusta coffee (*Coffea canephora* Pierre ex A. Froehner) in Dak Lak Province, Vietnam. This species is characterized by the females having rounded or oval perineal patterns, smooth, regular, continuous striae, and reduced lateral lines. The dorsal arch is low, rounded and encloses a quite distinct vulva and tail tip. The stylet is normally straight with well-developed and posteriorly sloped knobs. The males have a rounded cap that extends posteriorly into the lip region. The procorpus is outlined distinctly, and is three times longer than the metacorpus. The metacorpus is ovoid, with a strong valve apparatus. The species closely resembles *M. marylandi*, *M. naasi, M. ovalis, M. panyuensis, M. lopezi, M. mali* and *M. baetica* in the perineal pattern of the females, and the morphology of the males and the second-stage juveniles. Nonetheless, it can be differentiated from other species by a combination of morphometric, morphological and molecular characteristics. Phylogenetic analysis was conducted based on the internal transcribed spacer (ITS) and 28S rDNA as well as the region between the cytochrome *c* oxidase I (COI) and cytochrome *c* oxidase II (COII) mitochondrial genes. Herein, this nematode is described, illustrated, and designated as a new species, *Meloidogyne daklakensis* sp. n., based on morphometric, morphological and molecular analyses.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are one of the most important pests, given their widespread distribution, host range, and potential damage to many crops (Hussey & Janssen, 2002). Wesemael *et al.* (2011) reported that more than 90 species of root-knot nematodes had been described. They are significantly affecting agricultural products in the tropical, subtropical and temperate regions (Trudgill & Blok, 2001; Hunt & Handoo, 2009). The most common known species are *M. incognita* (Kofoid & White, 1919) Chitwood, 1949; *M. javanica* (Treub, 1885) Chitwood, 1949; *M. arenaria* (Neal, 1889) Chitwood, 1949; *M. hapla* Chitwood, 1949 and *M. exigua* (Göldi, 1887).

Twenty-one species of the *Meloidogyne* genus have been recorded on coffee (Souza, 2008; Humphreys-Pereira *et al.*, 2014). In Vietnam, only *M. incognita, M. exigua* and *M. coffeicola* have been recorded on coffee (Trinh *et al.*, 2013), and *M. incognita* causes serious damage at almost all the coffee areas (Nguyen & Nguyen, 2000, 2001; Trinh *et al.*, 2009).

Morphological analysis of perineal patterns in adult females of *Meloidogyne* species is often used as a rapid diagnostic tool. The basic morphology of the perineal pattern is shown as a collage of the alternative features throughout the genus. Jepson (1987) proposed that the best way to examine the whole genus was to group species with similar morphology, and recognized six broad groups based on perineal pattern features.

However, this approach has some major limitations, including overlapping morphological characters, which can lead to misidentification (Souza, 2008). Some coffee-parasitizing nematodes, such as *M. izalcoensis*, *M. paranaensis* and *M. incognita*, have overlapping distributions of characters that are related to the perineal patterns of females (Brito *et al.*, 2004; Carneiro *et al.*, 2005).

Ribosomal coding genes, such as the small subunit 18S internal transcribed spacer (ITS) and the large subunit 28S rRNA genes, evolve relatively slowly (Blaxter, 2001; De Ley *et al.*, 2002). These genes are useful for phylogenetic studies among groups of plant-parasitic, animal-parasitic, and free-living nematodes, or between the orders within the phylum Nematoda (Blaxter, 2001). Conversely, mitochondrial genes evolve more rapidly than ribosomal genes, which makes them useful for intra-specific and population genetic studies or intra-genus and intra-family analyses (Powers & Harris, 1993; Blaxter, 2001; Plantard *et al.*, 2008).

During our surveys in 2015 in the Western Highlands of Vietnam, we found root samples of coffee with untypical symptoms, including a high number of egg masses protruding outside the roots, and numerous small, white galls gathered around adventitious roots. These symptoms differ from those caused by other root-knot nematodes present in the region. Therefore, the objective of this study was to describe the new root-knot nematode using an integrated approach, including the diagnostics of morphometric, morphological and molecular characters.

Materials and methods

Nematode population

Nematodes were extracted from galled root and soil samples of Robusta coffee that were collected in Cu M'gar District, Dak Lak Province, Western Highlands, Vietnam.

Morphological characterization

Second-stage juveniles and males were extracted from soil samples using the modified Baermann tray method (Whitehead & Hemming, 1965). Measurements of heat-killed nematodes were made on permanent slides following transarticular screw fixation (TAF) and ethanol–glycerin dehydration, according to the method described by Seinhorst (1962) and modified by De Grisse (1969).

Females were extracted from the root tissues under a stereomicroscope, using a scalpel. Perineal patterns of mature females were prepared following Hartman & Sasser (1985) and mounted in lactophenol.

Light microscopy (LM)

All slides were examined under an Axio Lab.A1 light microscope (Carl Zeiss AG, Oberkochen, Germany). Measurements, line drawings and photographs were produced using ZEN lite software and a ZEISS Axiocam ERc 5s digital camera (Carl Zeiss AG, Oberkochen, Germany). Raw photographs were edited using Adobe Illustrator CS3 (Adobe Systems, San Jose, California, USA). Comparisons of morphological and morphometric characters were based on the diagnostic keys of Hewlett & Tarjan (1983), Eisenback (1985), Jepson (1987), Karssen (2002) and Kazachenko & Mukhina (2013).

Scanning electron microscopy (SEM)

Following examination and identification, some specimens in good condition were selected for observation under a scanning electron microscope, following Abolafia (2015). The nematode was hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and examined under a Merlin scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany).

Molecular characterization

DNA was extracted from individual second-stage juveniles using the Lysis buffer protocol as described by Holterman *et al.* (2009). The extracted crude DNA was kept at −70°C until it was used. The crude DNA of each specimen was amplified using the following sets of primers: for ITS rRNA genes using the reverse primer MelR5′–TCGTAACAAGGTAGCTGTAG–3′ and forward primer MelF5′–TGCTCTCGACTGAGTTCAG–3′, modified from Vrain *et al.* (1992); for the mtDNA cytochrome *c* oxidase I (COI) genes using the Cox1 F/Cox1R primers (Cox1–5′–TGGTCATCCTGAAGTTTATG–3′/Cox1R–5′–CTACA ACATAATAAGTATCATG–3′), modified from Kiewnick *et al.* (2014); for the mtDNA cytochrome *c* oxidase II (COII) genes using the primers Cox2–5′GTCAATGTTCAGAAATTTGTG–3′/ 16R–5′–TACCTTTGACCAATCACGCT–3′ (Powers & Harris, 1993); and for the D2–D3 fragment of the 28S rRNA gene using the forward primer D2–5′–ACAAGTACCGTGAGGGA AAGTTG–3′/ and reverse primer D3–5′–TCCTCGGAAGGAA CCAGCTACTA–3′ (De Ley *et al.*, 1999). All of the polymerase chain reactions (PCR) were performed in 25 µl Hot Start PCR Master Mix (Promega, Madison, Wisconsin, USA), 1 µM of each forward and reverse primer, 5 µl crude DNA and Milli-Q water (Merck Millipore, Burlington, Massachusetts, USA). After electrophoresis in 1% TAE-buffered agarose gel (1 h, 100 V) the PCR products were visualized under UV light, excised from the gel and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, Wisconsin, USA). For direct sequencing of both DNA strands, 20 mg was used. The sequences were aligned using ClustalW in BioEdit (Hall, 1999). The phylogenetic trees were generated based on Maximum Likelihood (ML) analysis in MEGA 6 (Tamura *et al.*, 2013). The phylograms were bootstrapped 1000 times to assess the degree of support for the phylogenetic branching indicated by the optimal tree for each method.

Results

Systematics

Phylum: Nematoda Potts, 1932
Class: Chromadorea Inglis, 1983
Order: Rhabditida Chitwood, 1933
Suborder: Tylenchina Thorne, 1949
Infraorder: Tylenchomorpha De Ley & Blaxter, 2002
Superfamily: Tylenchoidea Örley, 1880
Family: Meloidogynidae Skarbilovich, 1959
Genus: *Meloidogyne* Göldi, 1887

Meloidogyne daklakensis *n. sp.*

Description

Females. Body pearly white, pear-shaped, and anterior body portion commonly off-centre from a median plane (fig. 2c). Anterior end pointed; posterior end varying from rounded to slightly flattened. Neck usually short, not curved (bent). Stoma slit-like; lip region sclerotized and slightly offset from rest of

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

body, bearing one annulus; labial disc not fused with medial lips, elevated; lateral sectors enlarged, extension prominent; amphidial openings elongated (fig. 3a–e). Stylet short, cone base triangular and wider than shaft. Stylet tip normally straight (sometimes curved dorsally), shaft cylindrical, same diameter throughout its length (figs 1c and 2a, b). Three stylet knobs, oval and sloping posteriorly (figs 1c and 2a, b). Distance from base of stylet to dorsal pharyngeal gland orifice (DGO) *c.* 5 ± 0.2 µm. Pharyngeal lumen of procorpus wide, often showing rounded protuberances. Excretory pore situated far behind stylet knobs, within range of level of procorpus to metacorpus (figs 1c and 3b). Metacorpus large and rounded, valve apparatus oval-shaped and sclerotized. Perineal patterns of females rounded to oval; striae smooth, regular and continuous; lateral lines reduced, resulting in appearance of thick prominent lateral part in SEM; dorsal arch low, rounded and encloses distinct vulva, anus and tail tip. Phasmids indistinct. Vulva slit centrally located in the unstriated area, and longer than the distance between vulva and anus (figs 1f, 2d and 3f).

Males. Body vermiform, anterior end tapering and posterior region bluntly rounded. Lip region slightly offset from body, with a high lip region cap consisting of a large labial annulus. Medial lips and labial disc are bow-tie shaped. Lateral lips large and triangular, slightly lower than labial disc and medial lips. Amphidial openings appear as long, wide slits located between labial disc and lateral lips. Post-labial annulus divided into four lobes by longitudinal slits (figs 1a, 2i, 2k and 3g–k). Stylet robust

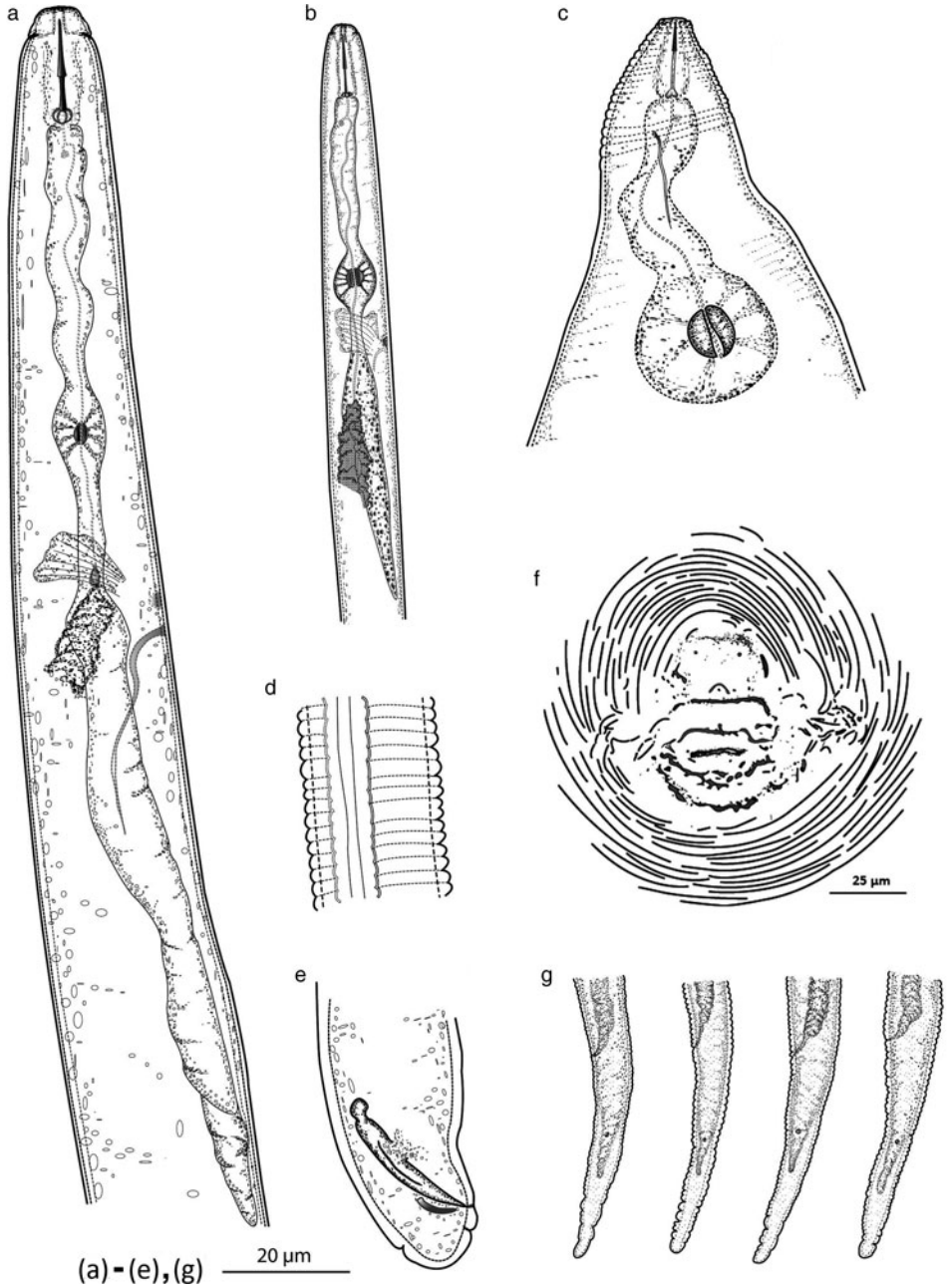

Fig. 1. *Meloidogyne daklakensis* n. sp. (a) Anterior of the male, showing pharynx and excretory canal; (b) anterior of the second-state juvenile, showing pharynx and excretory canal; (c) female anterior end; (d) lateral field of male; (e) tail region; (f) perineal pattern; (g) tail variation of juvenile.

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

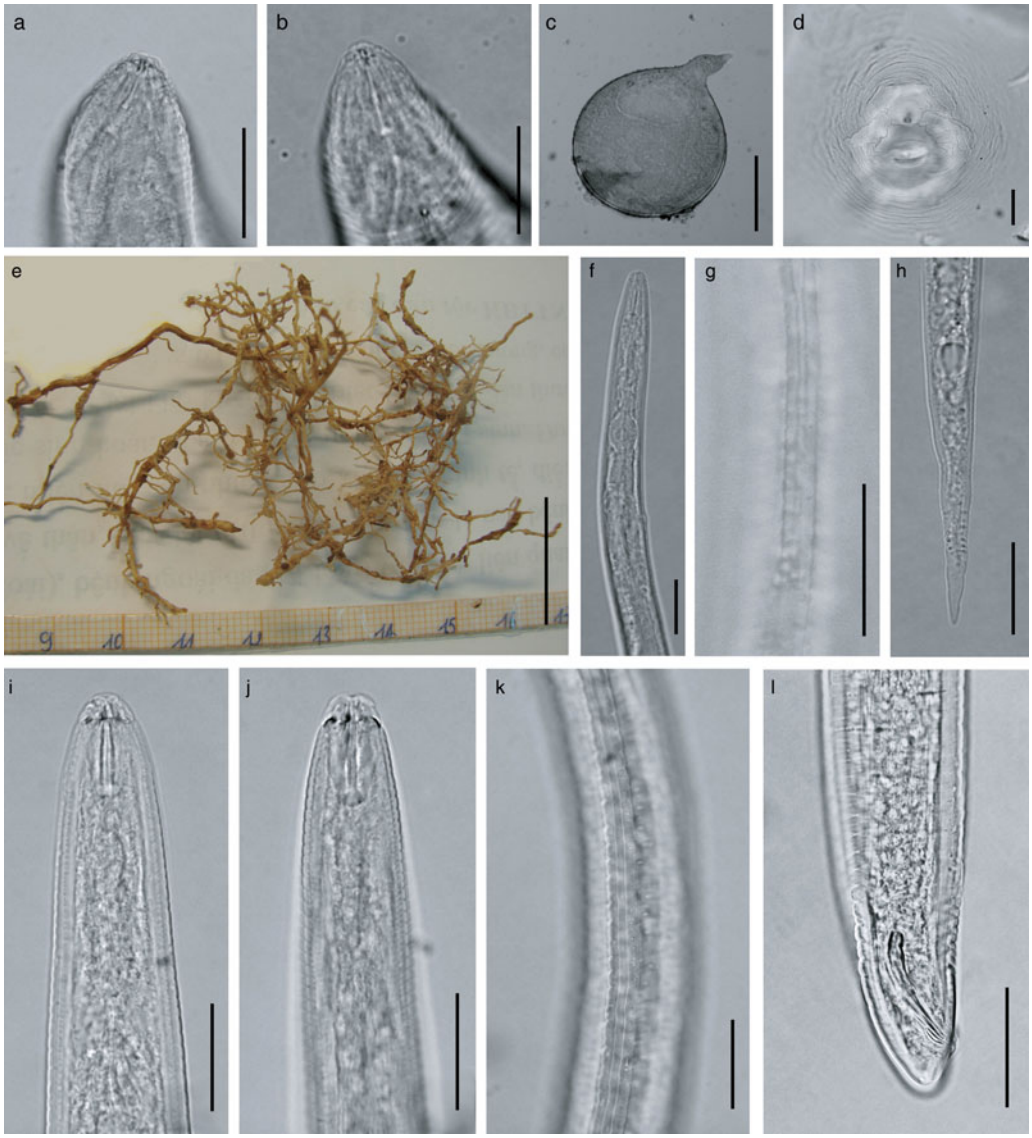

Fig. 2. Light micrographs of *M. daklakensis* n. sp. Female: (a, b) anterior end; (c) body; (d) perineal pattern; (e) coffee root. Second-stage juvenile: (f) anterior end; (g) lateral fields; (h) tail region. Male: (i, j, k) anterior end; (l) lateral fields; (m) tail region. (Scale bar: (a, b, d, f, g, h, i, k, l, m): 25 µm; (c): 200 µm; (e): 2000 µm).

and straight, one lateral knob projected, two others sloping posteriorly (figs 1a and 2i, l). Distance from base of stylet to DGO = 5 ± 1 µm. Procorpus distinctly outlined, three times longer than metacorpus. Metacorpus ovoid, with a strong valve apparatus. Excretory duct curved. Lateral field with four incisures forming three bands, areolation of whole body (figs 2k and 3k). One testis, occupying 58% of the body cavity. Spicules slightly curved ventrally, with bluntly rounded terminus. Gubernaculum short and crescent-shaped (figs 1e and 2l).

Second-stage juveniles (J2). Body slender, tapering to an elongated tail. Lip region narrower than body, weak and slightly offset from body; labial disc offset; stoma slit-like; medial lips and labial disc bow-tie shaped; labial disc rounded; amphidial openings enlarged, covered lateral lips (figs 1b and 3m–q). Stylet slender; cone weakly expanded at junction with shaft; knobs rounded (figs 1b and 2f). Distance from stylet knobs to dorsal gland orifice *c.* 4 ± 0.8 µm. Metacorpus broadly oval, valve large and heavily sclerotized. Pharyngeal–intestinal junction at level of nerve ring. Excretory pore slightly behind nerve ring, opening just behind hemizonid. Lateral field with four incisures, not areolated or prominent (figs 2g and 3o). Phasmids small, distinct. Tail elongate–conoid (figs 1g, 2h and 3q).

Taxonomic summary

Type host. Robusta coffee *(Coffea canephora* Pierre ex A. Froehner).

Type locality. Western Highlands, Dak Lak Province (12°47′12′′N, 108°05′43′′E).

Etymology. The species epithet refers to the Dak Lak Province, Western Highlands, Vietnam, where this new species was found.

Type material. Holotype (females) and paratypes that were isolated from the roots of the type host are deposited in the nematode collection of the Nematology Department at the Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology (VAST). Five female paratypes are deposited in the nematode collection of the Zoology Museum,

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

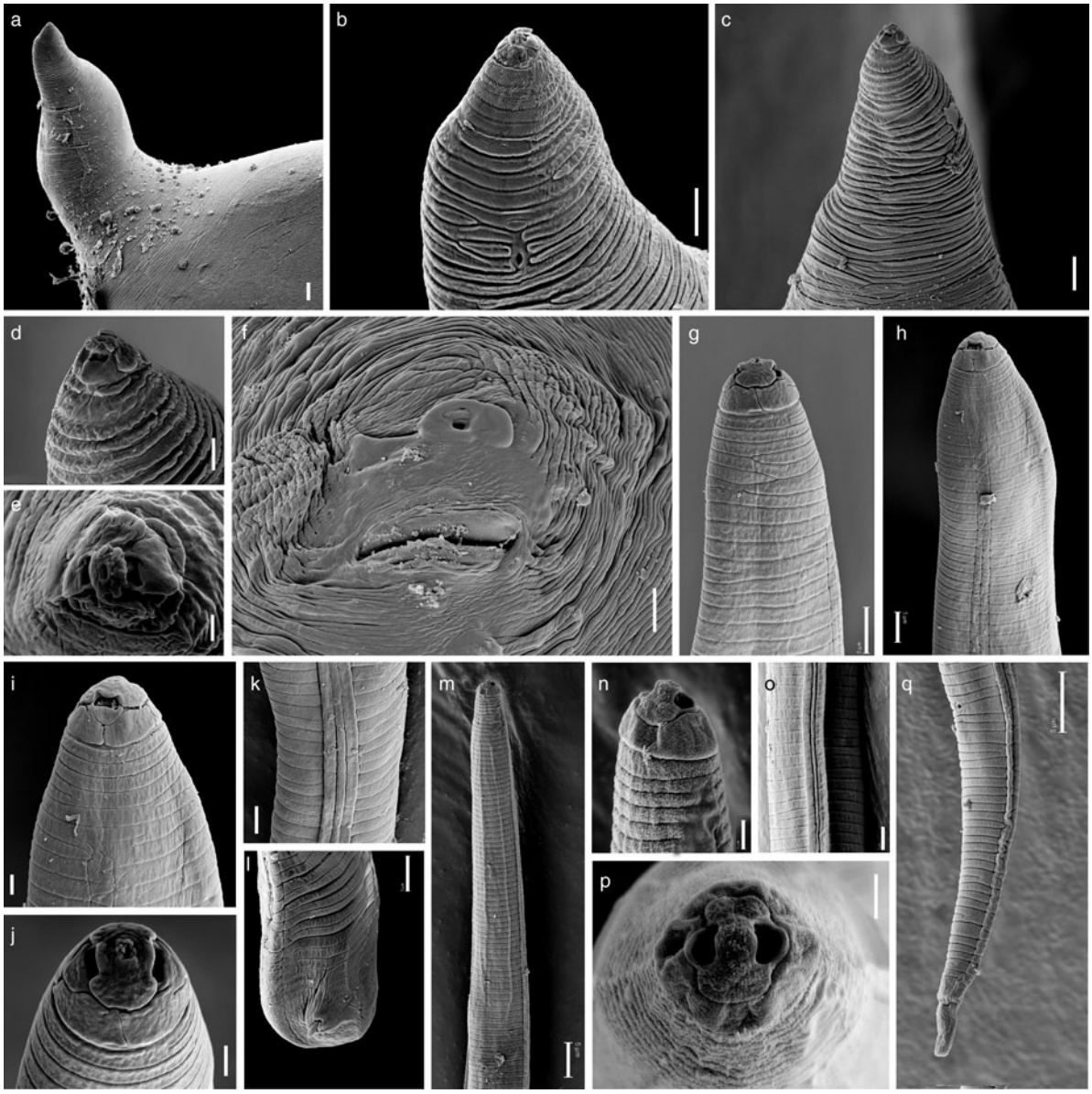

Fig. 3. Scanning electron micrographs of *M. daklakensis* n. sp. Female: (a) neck region; (b) anterior end, lateral view; (c) anterior end, ventral view; (d) en face view; (e) lip region, lateral view; (f) perineal pattern. Male: (g) anterior end, ventral view; (h) anterior end, lateral view; (i) en face view; (j) lip region, lateral view; (k) lateral fields; (l) tail region. Second-stage juvenile: (m) anterior end, ventral view; (n) anterior end, subdorsolateral view; (o) lateral field; (p) en face view; (q) posterior end. (Scale bar: (a–f): 10 µm; (g–l, q, m): 5 µm; (l, p): 1 µm, (o): 2 µm).

Ghent University, K.L. Ledeganckstraat 35, Ghent, Belgium. The ITS, D2D3, COI and COII sequences are deposited in GenBank under the accession numbers (KU243339, MG266058), (KU243338, MG266060), (MG431339, MG341340) and (KU243337, MG431341), respectively.

Pathology. The coffee roots infected with *M. daklakensis* sp. n. had relatively small galls (1–2 mm diameter). Each gall contained a single female, with the exposure of an egg mass outside of the gall (fig. 2e).

Differential diagnosis

Meloidogyne daklakensis n. sp. is closest to *M. marylandi* Jepson & Golden, 1987, *M. naasi* Franklin, 1965, *M. ovalis* Riffle, 1963, *M. panyuensis* Liao, Yang, Feng & Karssen, 2005, *M. lopezi* Humphreys-Pereira, Flores Chaves, Gomez, Salazar, Gomez-Alpizar & Elling, 2014, *M. mali* Itoh, Ohshima & Ichinohe, 1969 and *M. baetica* Castillo, Vovlas, Subbotin & Troccoli, 2003 (the perineal patterns are characterized by a round/oval-shaped, moderately high dorsal arch, and smooth, regular and continuous striae). However, the species can be distinguished from each other by certain features (tables 1 and 2).

Meloidogyne daklakensis n. sp. differs from *M. marylandi* Jepson & Golden, 1987 in that females have shorter body length (548–709 vs 717–956 µm) and stylet length (14–15 vs 15.4–23 µm); males have shorter body length (1085–1365 vs 1616–2340 µm) and spicule length (18–29 vs 28.7–39.3 µm); and second-stage juveniles have shorter body length (280–373 vs 478–574 µm), smaller body diameter (11–16 vs 15–22 µm) and shorter tail length (32–54 vs 53.2–62.3 µm).

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

Table 1. Morphometric indices of the females, males and second-stage juveniles (J2) of *M. daklakensis* n. sp. All measurements are in μm and in the form: mean ± SD (range).

Character	Holotype (Female)	Paratypes		
		Female	Male	Second-stage juvenile (J2)
n	1	10	10	20
L	666	645 ± 54 (548–709)	1228 ± 98 (1085–1365)	333 ± 27 (280–373)
a	1.5	1.7 ± 0.3 (1.4–2.6)	39.1 ± 2.5 (35.3–42.3)	27 ± 2 (23–32)
b	–	–	10.76 ± 0.8 (9.4–12.1)	4.5 ± 0.5 (3.4–5.4)
b′	–	–	5.9 ± 0.4 (6.1–6.3)	2.9 ± 0.3 (2.2–3.4)
c	–	–	104 ± 15.5 (84–124)	7.4 ± 1 (5.8–10.8)
c′	–	–	0.6 ± 0.1 (0.5–0.6)	4.8 ± 0.6 (3.9–6.2)
Maximum body diameter	355	378 ± 45 (322–462)	31 ± 3 (27–34)	12 ± 1 (11–15)
Neck length	162	147 ± 21 (118–172)	–	–
Ratio of body length to length of neck	2.9	4.4 ± 1.04 (2.9–6)	–	–
Stylet length	14.6	14.5 ± 0.5 (14–15)	19 ± 1 (17–20)	14 ± 1 (11–16)
Stylet knob width	–	–	4 ± 0.2 (3.9–4.1)	–
Stylet knob height	–	–	2.5 ± 0.5 (2–3)	–
DGO	4	5 ± 0.2 (4.5–5.5)	5 ± 1 (4–7)	4 ± 0.8 (2–5)
Distance from anterior end to excretory pore	18	19 ± 1 (18–21)	136 ± 17 (107–161)	66 ± 6 (55–78)
Distance from anterior end to posterior end of metacorpus	66	66 ± 2 (62–67)	75 ± 11 (54–87)	48 ± 6 (34–57)
Metacorpus width	27	28 ± 1 (26–30)	–	–
Distance from anterior end to nerve ring	–	–	102 ± 10 (86–117)	59.3 ± 6 (44–69)
Distance from anterior end to pharyngeal–intestinal valve	–	–	208 ± 20 (175–228)	116 ± 12 (97–143)
Vulva–anus distance	17	15 ± 4 (10–21)	–	–
Vulva slit length	27	21 ± 3 (16–25)	–	–
Vulva width	9	8 ± 3 (5–13)	–	–
Tail length	–	–	12 ± 2 (10–15)	50 ± 23 (32–54)
Spicule length	–	–	31 ± 2.1 (28–34)	–
Testis length	–	–	712 ± 98 (537–827)	–
Hyaline tail terminus	–	–	–	12 ± 2 (9–17)

Meloidogyne daklakensis n. sp. differs from *M. naasi* Franklin, 1965 in that females have a perineal pattern with indistinct phasmids vs large phasmids, and second-stage juveniles have shorter body length (280–373 vs 418–465 μm); b = 3.4–5.4 vs 7–8; c = 7.4 vs 6.2.

Meloidogyne daklakensis n. sp. differs from *M. ovalis* Riffle, 1963 in that females have indistinct phasmids vs large phasmids; males have shorter body length (1085–1365 vs 1300–1800 μm) and spicule length (18–29 vs 31–38 μm); and second-stage juveniles have shorter body length (280–373 vs 350–430 μm); a = 27 vs 22; c = 7.4 vs 8.

Meloidogyne daklakensis n. sp. differs from *M. panyuensis* Liao *et al.*, 2005 in that in females the distance from anterior end to excretory pore is shorter (18–21 vs 32.5–37.5 μm) and DGO = 4.5–5.5 vs 8.8–12.5 μm; males have shorter body length (1085–1365 vs 1710–2050 μm), DGO = 4–7 vs 3–4.5 μm, and shorter spicule length (18–29 vs 25–35 μm); and second-stage juveniles have shorter body length (280–373 vs 353–455 μm), c =5.8–10.8 vs 21–29, shorter distance from anus to posterior end (32–54 vs 48–63 μm), and longer hyaline part (9–17 vs 7.5–10 μm).

Meloidogyne daklakensis n. sp. differs from *M. lopezi* Humphreys-Pereira *et al.*, 2014 in that females have shorter body length (548–709 vs 717–956 μm), stylet length (14–15 vs 15.4–23 μm) and distance from anterior end to excretory pore (18–21 vs 22–59 μm), and the perineal pattern has moderately smooth, regular and continuous dorsal striae and reduced lateral lines vs coarse, fine or wavy striae and weak lateral lines; males have shorter body length (1085–1365 vs 1616–2340 μm), smaller body diameter (27–34 vs 37–47 μm), shorter spicule length (18–29 vs 28.7–38.3 μm), DGO = 4–7 vs 1.9–3.4 μm), lip region offset from body, with a high lip region cap consisting of a large labial annulus and a prominent post-labial annulus vs lacking annulation; and second-stage juveniles have shorter body length (280–373 vs 478–574 μm), smaller body diameter (11–15 vs

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

Table 2. Morphological differences between *M. daklakensis* n. sp. and some closely related *Meloidogyne* species. All measurements are in μm and in the form: mean ± SD (range).

Character	*M. daklakensis* n. sp.	*M. mali*	*M. lopezi*	*M. baetica*	*M. marylandi*	*M. panyuensis*
Females						
Body length	645 ± 54 (548–709)	848 ± 93.4 (684–1044)	833 ± 80 (717–956)	911 ± 163 (775–1263)	717–956	–
Neck length	147 ± 21 (118–172)	166 ± 43.7 (90–252)	212 ± 20.3 (171–253)	–	–	–
Ratio of body length to length of neck	4.4	5.1	–	–	–	–
Maximum body diameter	401 ± 41 (354–461)	660 ± 95.8 (540–864)	521 ± 68.2 (368–598)	523 ± 88 (469–559)	–	–
Stylet length	14.5 ± 0.5 (14–15)	15 (13–17)	18 ± 1.9 (15.4–23)	17.5 ± 0.8 (17–19)	15.4–23	13
DGO	5 ± 0.2 (4.5–5.5)	5.5 (4–7)	4.5 ± 0.9 (2.9–6.2)	–	2.9–6.2	10 (8.8–12.5)
Vulva–anus distance	15 ± 4 (10–21)	17 ± 1.8 (14–22)	20.1 ± 2.0 (16.6–24.9)	21 ± 1.6 (19–25)	16.6–24.9	–
Vulva slit length	21 ± 3 (16–25)	22 ± 3.5 (17–29)	26 ± 2.5 (21.4–30.8)	20 ± 2.7 (17–24)	21.4–30.8	–
Perineal pattern	High rounded or oval dorsal. Striae smooth, regular and continuous. No lateral lines. Low, rounded dorsal arch, enclosing quite distinct vulva and tail tip, but phasmids are indistinct	Low, flat, oval dorsal arch, with smooth, finely spaced striae. Large phasmids, lateral field clearly marked with single or double incisures	Moderately high dorsal arch, mostly rounded and sometimes squarish. Dorsal striae coarse and smooth, fine or wavy. Weak lateral lines on both sides of the depressions. Punctations and striae absent in perineum	Typically formed of striae and ridges of the cuticle, the latter being more pronounced nearer the vulva and anus. The dorsal arch encloses the usually quite distinct phasmids, and the anus, situated at the centre	High dorsal arch, mostly rounded and sometimes squarish. Dorsal striae variable, mainly coarse and smooth, but can be fine or wavy. Weak lateral lines	Perineal pattern ovoid to oval shaped, smooth to moderately coarse striae, dorsal arch relatively low, lateral lines indistinct, tail terminus area with irregular striae
Juveniles						
Body length	333 ± 27 (280–373)	418 (390–450)	532 ± 23.5 (478–574)	403 ± 136 (394–422)	478–574	353–455
Body width	12 ± 1 (11–15)	14.5 (14–16)	18 ± 2.0 (15–22)	13.5 ± 0.5 (13–14)	15–22	–
Stylet length	14 ± 1 (11–15)	14 (12–15)	11.3 ± 0.9 (9.1–12.9)	13.5 ± 0.5 (13–14)	9.1–12.9	–
DGO	4 ± 0.8 (2–5)	4.7 (4–6)	–	4.0 ± 0.7 (3–5)	–	–
a	26.9 ± 2.5 (23.3–31.5)	28.5 27–31	30.2 ± 3.7 (24.4–6.7)	29 ± 1.2 (28–32)	–	–
b	4.5 ± 0.5 (3.4–5.4)	7.2 (6–8)	–	–	–	–
b’	2.9 ± 0.3 (2.2–3.4)	–	–	–	–	–
c	7.4 ± 1 (5.8–10.7)	13.3 (12–15)	9.1 ± 0.6 (8.4–10.5)	7.9 ± 0.4 (7.3–8.4)	13–16	21–29
c’	4.8 ± 0.6 (3.8–6.2)	3.7 (3–5)	–	–	–	–
Tail length	50 ± 23 (32–54)	31 (30–34)	58.5 (53.2–62.3)	50 ± 2.3 (47–54)	53.2–62.3	55 (48–63)

Males							
Body length	1228 ± 98 (1085–1365)	1447 (1270–1630)	1963 (1616–2340)	1811 ± 406 (1545–2205)	1616–2340		1710–2050
Stylet length	19 ± 1 (17–20)	20 (18–22)	23.4 ± 0.97 (21.6–24.4)	17 ± 1.6 (16–19)	21.6–24.4		17–20
DGO	5 ± 1 (4–7)	8 (6–13)	2.5 ± 0.4 (1.9–3.4)	4.0 ± 0.5 (3.5–5.5)	–		3–4.5
a	39.1 ± 2.5 (35.3–42.3)	38 (31–44)	46.2 ± 2.8 (42.2–50)	62 ± 7.0 (58–75)	–		–
b	10.76	15 (11–21)	–	12.4 ± 2.2 (9.8–15.7)	–		–
c	–	40 (32–58)	–	–	–		–
Spicule length	24 ± 3.5 (18–29)	32 (28–35)	33.9 ± 2.8 (28.7–38.3)	27 ± 4.0 (24–36)	28.7–39.3		25–35
Testis length	712 ± 98 (537–827)	788 (540–970)	903 ± 170 (517–1133)	–	517–1133		–

15–22 µm), longer stylet length (11–16 vs 9.1–12.9 µm), shorter distance from anterior end to excretory pore (55–78 vs 95–109 µm), and shorter tail length (32–54 vs 53.2–62.3 µm).

Meloidogyne daklakensis n. sp. differs from *M. mali* Itoh *et al.*, 1969 in that females have smaller maximum body diameter (548–709 vs 684–1044 µm), reduced lateral lines vs clearly visible lateral lines, and indistinct phasmids vs large phasmids; males have shorter body length (1085–1365 vs 1270–1630 µm) and spicule length (18–29 vs 28–35 µm); and second-stage juveniles have shorter body length (280–373 vs 390–450 µm), smaller maximum body diameter (11–15 vs 14–16 µm) and longer tail length (32–54 vs 30–34 µm).

Meloidogyne daklakensis n. sp. differs from *M. baetica* Castillo *et al.*, 2003 in that females have the excretory pore situated within the range from level of procorpus to level of metacorpus vs constantly anterior to the level of stylet knob, indistinct vs quite distinct phasmids, shorter body length (548–709 vs 755–1263 µm) and stylet length (14–15 vs 17–19 µm), smaller maximum body diameter (322–462 vs 469–559 µm), and smaller distance from vulva to anus (10–21 vs 19–25 µm); males have shorter body length (1085–1365 vs 1545–2205 µm); and second-stage juveniles have shorter body length (280–373 vs 394–422 µm) and shorter length from anterior end to excretory pore (55–78 vs 85–100 µm).

Molecular characterization

Amplification of the ITS rDNA region of *M. daklakensis* n. sp. yielded a single fragment approximately 600 bp in length (gene sequence was 516 bp). The ITS sequences of the new species are most similar to those of *M. panyuensis*, *M. mali* and *M. marylandi*, by 82%, 81% and 79%, respectively. Phylogenetic trees generated based on the Maximum Likelihood (ML) method show that *M. daklakensis* n. sp. is closest to *M. panyuensis* with high bootstrap (89%), and separated from other *Meloidogyne* species (fig. 4).

The D2D3 aligned sequence of *M. daklakensis* n. sp. was 727 bp. The D2D3 sequence of *M. daklakensis* n. sp. is similar to those of *M. ichinohei, M. camellia* and *M. mali* by 86%, 84% and 83%, respectively. The phylogenetic relationships between *M. daklakensis* n. sp. and other *Meloidogyne* species based on the 28S ribosomal RNA sequences indicated that *M. daklakensis* n. sp. was grouped with *M. ichinohei* and *M. camellia* with moderate bootstrap value (84%). *Meloidogyne daklakensis* n. sp. was clearly separated from other related *Meloidogyne* species.

On the other hand, the COI sequence length of *M. daklakensis* n. sp. was 364 bp. The Basic Local Alignment Search Tool (BLAST) search for the COI sequence of *M. daklakensis* n. sp. revealed the highest identical match with the sequences of *M. ichinohei* and *M. mali*, by 92% and 86%, respectively. The phylogenetic tree of COI sequences placed *M. daklakensis* n. sp. in a clade together with *M. ichinohei* (bootstrap value = 93%), and they were also a sister group of *M. mali* with 99% bootstrap (fig. 7). The COII–16S sequence length of *M. daklakensis* n. sp. was 526 bp, which was most similar to *M. mali* and *M. marylandi*, by 77% and 75%, respectively. The COII–16S phylogenetic tree showed that *M. daklakensis* n. sp. belonged to a clade together with *M. mali* and *M. marylandi* (99% support). The new species was clearly separated from other root-knot nematodes (fig. 7).

Discussion

Molecular markers are an important tool for identification of nematodes and remain essential for accurate diagnosis of root-

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

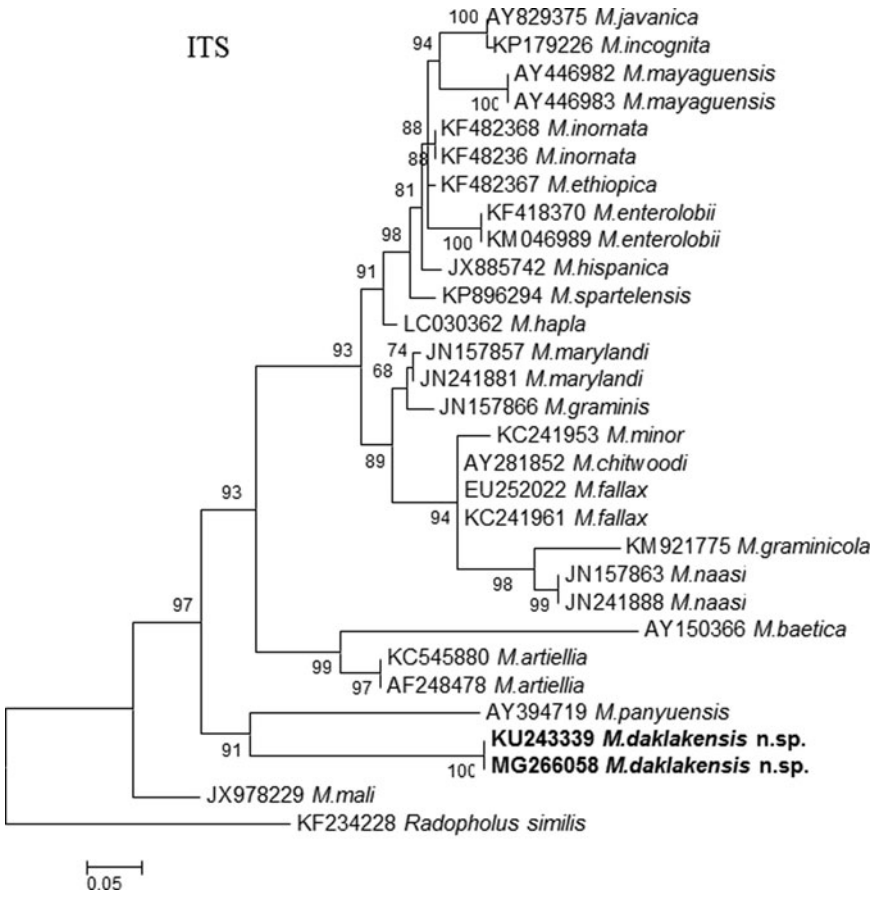

Fig. 4. Phylogenetic relationships of *M. daklakensis* n. sp. with other root-knot nematodes, from the analysis of the ITS sequences. Numbers to the left of the branches are bootstrap values for 1000 replications.

knot nematodes (Perry & Moens, 2013). Furthermore, DNA-based methods can be applied to various stages of the nematodes to distinguish individual species from mixed populations (Onkendi & Moleleki, 2013). The 28S region is relatively conserved with high similarity among the three most common tropical root-knot nematode species, *M. incognita*, *M. arenaria* and *M. javanica*. Therefore, conserved ribosomal DNA is not useful for identifying these species (Ye *et al.*, 2015; Janssen *et al.*, 2017). However, it can be a useful tool for identification of *M. marylandi*, *M. graminis* and *M. naasi*, or *M. enterolobii*, *M.*

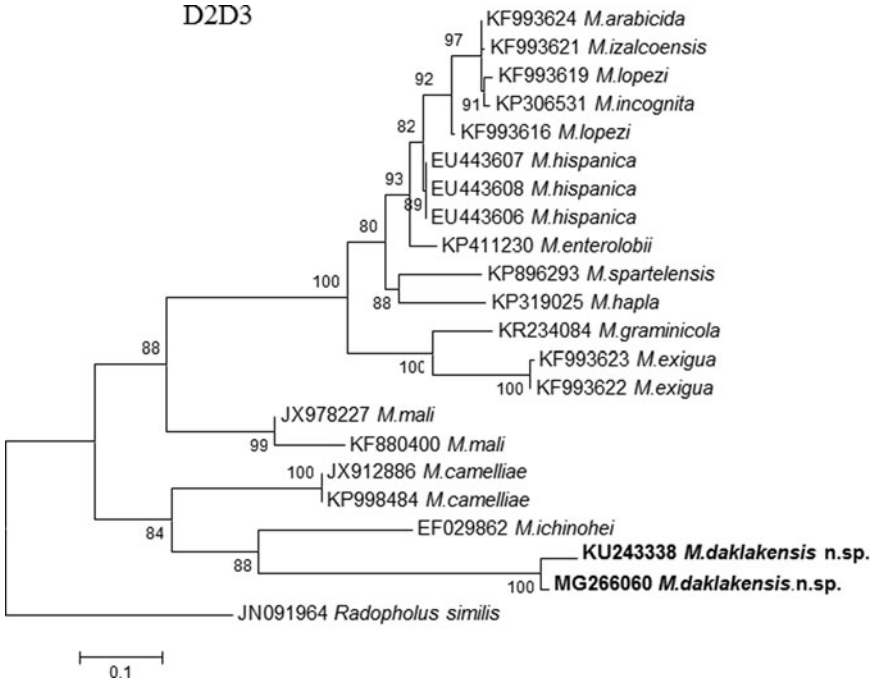

Fig. 5. Phylogenetic relationships of *M. daklakensis* n. sp. with other root-knot nematodes, from the analysis of the D2D3 (28S–rRNA) sequences. Numbers to the left of the branches are bootstrap values for 1000 replications.

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

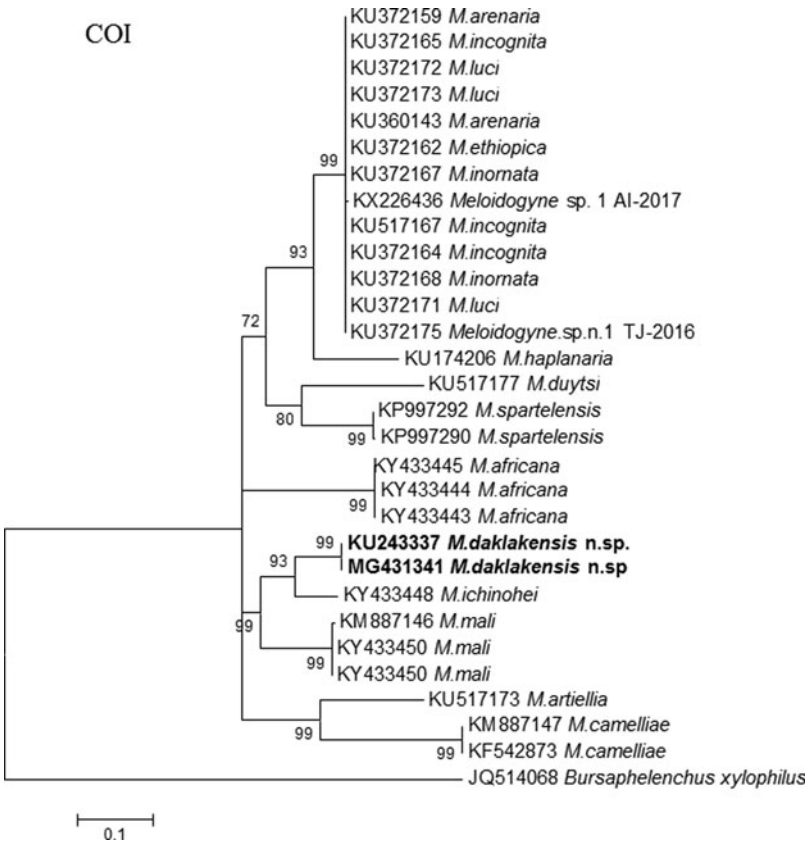

Fig. 6. Phylogenetic relationships of *M. daklakensis* n. sp. with other root-knot nematodes, from the analysis of the COI–rRNA sequences. Numbers to the left of the branches are bootstrap values for 1000 replications.

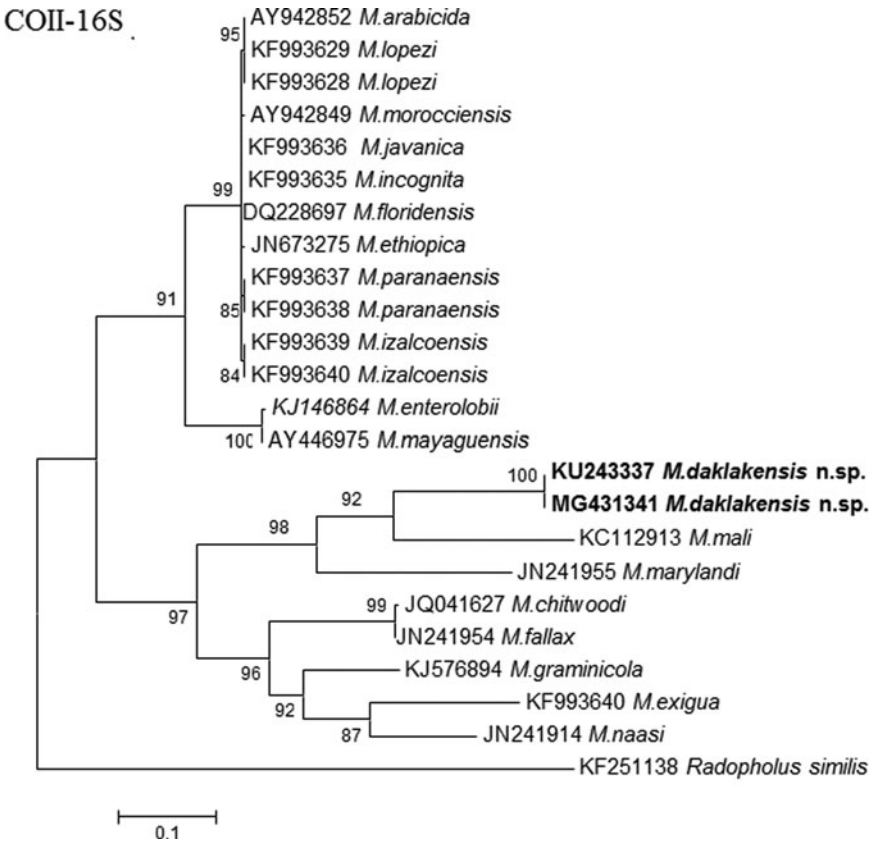

Fig. 7. Phylogenetic relationships of *M. daklakensis* n. sp. with other root-knot nematodes, from the analysis of the alignment of the COII–16S–rRNA sequences. Numbers to the left of the branches are bootstrap values for 1000 replications.

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

hapla, *M. chitwoodi* and *M. fallax* (Onkendi & Moleleki, 2013; Ye *et al.*, 2015). In order to confirm identification of *Meloidogyne* at species level, mitochondrial DNA is usually used (Tigano *et al.*, 2005; Landa *et al.*, 2008). The combined analyses of 18S and 28S nuclear rDNA sequences showed the haplotypes of *Meloidogyne* species on coffee (Herrera *et al.*, 2011). The mitochondrial DNA has a faster rate of evolution than the corresponding nuclear genes, creating sufficient nucleotide variation for species-level analyses (Hugall *et al.*, 1994; Blok & Powers, 2009). Extensive geographical sampling of the COI can be conducted to assess interspecific variability (Kiewnick *et al.*, 2014; Janssen *et al.*, 2016). The mitochondrial haplotypes are strongly linked and consistent with traditional esterase isozyme patterns, which suggests that different parthenogenetic lineages can be identified reliably using mitochondrial haplotypes (Janssen *et al.*, 2016). The COII is a highly conserved region, and therefore it was easy to distinguish different *Meloidogyne* species (Blouin, 2002; Tigano *et al.*, 2005; Onkendi & Moleleki, 2013). As an alternative to the morphological identification of *Meloidogyne* spp., esterase patterning has been used for diagnosing *Meloidogyne* spp. from a wide range of samples and has been proved to be species-specific for a number of species (Carneiro & Almeida, 2001; Carneiro & Cofcewics, 2008). Isozyme electrophoresis patterning has discriminated between all of these cryptic species, but this technique is restricted to females (Carneiro & Cofcewics, 2008). Isozyme electrophoretic profiles, often using esterase (EST) and malate dehydrogenase (MDH), have been established for a number of species and can provide a useful routine diagnostic test, particularly for morphologically variable species (Blok & Powers, 2009). Certain limitations have also been associated with biochemical analysis, such as the absence of enzymatic patterns for all the species of *Meloidogyne* and the dependence on the nematode development phase (Blok & Powers, 2009). Other limitations can include the occurrence of intraspecific variations and the difficulty in resolving the same esterase phenotype between species. In addition, weak bands on the polyacrylamide gel may require a larger number of females per well to resolve (Carneiro *et al.*, 2000). Furthermore, some *Meloidogyne* species have been misidentified based on the esterase and malate dehydrogenase isozyme phenotypes, such as *M. arenaria* and *M. morocciensis* (Carneiro & Cofcewics, 2008), and *M. ulmi* and *M. mali* (Ahmed *et al.*, 2013).

The mtDNA variation, although limited, is strongly structured, with as much divergence between two lineages of *M. arenaria* as between either of them and *M. javanica* (Pagan *et al.*, 2015). The low diversity of mtDNA suggests that these parthenogenetic lineages arose from distinct but closely related sexually mature females, a pattern observed in other parthenogenetic complexes (Hugall *et al.*, 1994). The molecular analysis based on amplification of specific genome regions stands out among the tested methods because of its speed and accuracy, particularly in the case of identifying morphologically similar nematode species or a mixture of species in the samples. The molecular method is also very useful for routine analysis in nematology laboratories, facilitating the processing of a large number of samples simultaneously (Oliveira *et al.*, 2011; Ahmed *et al.*, 2013; Baidoo *et al.*, 2016).

In this study, at the molecular level, phylogenetic trees based on the ITS, 28S rDNA genes and mtDNA (COI and COII) of *M. daklakensis* n. sp. indicated that the new species is closest to *M. marylandi*, *M. naasi*, *M. panyuensis*, *M. mali* and *M. ichinohei*, but they are clearly separated, with high genetic distance. Molecular and morphological characters strongly support the case that *M. daklakensis*. n. sp. is a new species that is clearly distinct from other *Meloidogyne* species.

Acknowledgments. We thank Prof. Dr Maurice Moens for his constructive advice. We are also grateful to the reviewers for their valuable comments.

Financial support. This research was supported by the National Foundation for Science & Technology Development (NAFOSTED) of Vietnam (Number code: 106–NN.03–2013.56) and the World Academy of Sciences (TWAS) (Code: 14–246 RGA).

Conflict of interest. None

Ethical standards. Specimens were collected under the license for the collection of biological material (4555) granted by the Department of Nematology, Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology (VAST).

References

Abolafia J (2015) A low-cost technique to manufacture a container to process meiofauna for scanning electron microscopy. *Microscopy Research and Technique* **78**, 771–776.

Ahmed M, van de Vossenberg BTLH, Cornelisse C and Karssen G (2013) On the species status of the root-knot nematode *Meloidogyne ulmi* Palmisano & Ambrogioni, 2000 (Nematoda, Meloidogynidae). *ZooKeys* **362**, 1–27.

Baidoo R, Joseph S, Mengistu TM, Brito JA, McSorley R, Stamps RH and Crow WT (2016) Mitochondrial haplotype-based identification of root-knot nematodes (*Meloidogyne* spp.) on cut foliage crops in Florida. *Journal of Nematology* **48**, 193–202.

Blaxter ML (2001) Molecular analysis of nematode evolution. pp. 1–24 *in* Kennedy MW and Harnett W (*Eds*) *Parasitic nematodes: molecular biology, biochemistry and immunology*. Wallingford, UK, CABI.

Blok VC and Powers TO (2009) Biochemical and molecular identification. pp. 98–112 *in* Perry RN, Moens M and Starr J (*Eds*) *Root knot nematodes*. 1st edn. London, CABI.

Blouin MS (2002) Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. *International Journal for Parasitology* **32**, 527–531.

Brito J, Powers TO, Mullin PG, Inserra RN and Dickson DW (2004) Morphological and molecular characterization of *Meloidogyne mayaguensis* isolates from Florida. *Journal of Nematology* **36**, 232–240.

Carneiro RMDG and Almeida MRA (2001) Técnica de eletroforese usada no estudo de enzimas dos nematoides das galhas para identificação de espécies. *Nematologia Brasileira* **25**, 35–44.

Carneiro RMDG and Cofcewics ET (2008) Taxonomy of coffee parasitic root knot nematodes, *Meloidogyne* spp. pp. 87–122 *in* Souza RM (*Ed.*) *Plant parasitic nematodes of coffee*. 1st edn. Netherlands, Springer Netherlands.

Carneiro RMDG, Almeida MRA and Quénéhervé P (2000) Enzyme phenotypes of *Meloidogyne* spp populations. *Nematology* **2**, 645–654.

Carneiro RMDG, Randig O, Almeida MRA and Goncalves W (2005) Identificacao e caracterizacao de species de *Meloidogyne* em cafeeiro nos Estados de Sao Paulo e Minas Gerais atraves dos fenotipos de esterase e SCAR–multiplex–PCR. *Nematologia Brasiliera* **29**, 233–241.

Castillo P, Vovlas N, Subbotin S and Troccoli A (2003) A new root-knot nematode, *Meloidogyne baetica* n. sp. (Nematoda: Heteroderidae), parasitizing wild olive in southern Spain. *Phytopathology* **93**, 1093–1102.

Chitwood BG (1949) Root-knot nematodes – Part I. A revision of the genus *Meloidogyne* Göldi, 1887. *Proceedings of the Helminthological Society of Washington* **16**, 90–104.

De Grisse AT (1969) Redescription ou modification de quelques techniques utilisées dans l'étude de nématodes phytoparasitaires. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent* **34**, 351–369.

De Ley P, Felix MA, Frisse LM, Nadler SA, Sternberg PW and Thomas WK (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* **1**, 591–612.

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press

De Ley IT, De Ley P, Vierstraete A, Karssen G, Moens M and Vanfleteren J. (2002) Phylogenetic analyses of *Meloidogyne* small subunit rDNA. *Journal of Nematology* **34**, 319–327.

Eisenback JD (1985) Detailed morphology and anatomy of second-stage juveniles, males, and females of the genus *Meloidogyne* (root-knot nematodes). pp. 47–78 *in* Sasser JN and Carter CC (*Eds*) *An advanced treatise on* Meloidogyne, Vol. 1. Raleigh, North Carolina State University Graphics.

Franklin MT (1965) A root-knot nematode, *Meloidogyne naasi* n. sp., on field crops in England and Wales. *Nematologica* **11**, 79–86.

Göldi EA (1887) Relatorio sobre a molestia do cafeeiro na provincia do Rio de Janeiro. Extrahido do VIII Vol. dos. Archivos do Museu Nacional, Rio de Janeiro. *Imprensa Nacional*, pp. 1–121.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Hartman KM and Sasser JN (1985) Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. pp. 69–77 *in* Barker KR, Carter CC and Sasser JN (*Eds*) *An advanced treatise on* Meloidogyne. *Vol. II: Methodology*. A cooperative publication of the Department of Plant Pathology and the United States Agency for International Development. Raleigh, North Carolina State University Graphics.

Herrera I, Bryngelsson T, Monzón A and Geleta M (2011) Identification of coffee root-knot nematodes based on perineal pattern, SCAR markers and nuclear ribosomal DNA sequences. *Nematologia Mediterranea* **39**, 101–110.

Hewlett TE and Tarjan AC (1983) Monographs. Synopsis of the genus *Meloidogyne Goeldi, 1887*. *Nematropica* **13**, 79–102.

Holterman HM, Oggenfuss M, Kiewnick S and Frey JE (2009) Variation present in the COI gene of *Meloidogyne* and its potential for molecular identification. *Journal of Nematology* **41**, 340.

Hugall A, Moritz C, Stanton J and Wolstenholme DR (1994) Low, but strongly structured mitochondrial DNA diversity in root knot nematodes (*Meloidogyne*). *Genetics* **136**, 903–912.

Humphreys-Pereira DA, Flores-Chaves L, Gómez M, Salazar L, Gómez-Alpízar L and Elling AA (2014) *Meloidogyne lopezi* n. sp. (Nematoda: Meloidogynidae), a new root-knot nematode associated with coffee *(Coffea arabica* L.) in Costa Rica, its diagnosis and phylogenetic relationship with other coffee-parasitising *Meloidogyne* species. *Nematology* **16**, 643–661.

Hunt DJ and Handoo ZA (2009) Taxonomy, identification and principal species. pp. 55–97 *in* Perry RN, Moens M and Starr JL (*Eds*) *Root-knot nematodes*. Wallingford, UK, CABI.

Hussey RS and Janssen GJW (2002) Root-knot nematodes: *Meloidogyne* species. pp. 43–70 *in* Starr JL, Cook R and Bridge J (*Eds*) *Plant resistance to parasitic nematodes*. Wallingford, UK, CABI.

Itoh Y, Ohshima Y and Ichinohe M (1969) A root-knot nematode, *Meloidogyne mali* n. sp., *on apple-tree from Japan (Tylenchida: Heteroderidae). Applied Entomology and Zoology* **4**, 194–202.

Janssen T, Karssen G, Verhaeven M, Coyne D and Bert W (2016) Mitochondrial coding genome analysis of tropical root-knot nematodes (*Meloidogyne*) supports haplotype based diagnostics and reveals evidence of recent reticulate evolution. *Scientific Reports* **6**. doi: 10.1038/srep22591.

Janssen T, Karssen G, Topalović O, Coyne D and Bert W (2017) Integrative taxonomy of root-knot nematodes reveals multiple independent origins of mitotic parthenogenesis. *PLoS ONE* **12**(3), e0172190.

Jepson SB (1987) *Identification of root-knot nematodes (*Meloidogyne *species)*. Wallingford, UK, CABI, 265 pp.

Jepson SB and Golden AM (1987) *Meloidogyne marylandi* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitizing grasses. pp. 263–265 *in* Jepson SB (*Ed.*) *Identification of root-knot nematodes (*Meloidogyne *species)*. Wallingford, UK, CABI.

Karssen G (2002) *The plant-parasitic nematode genus* Meloidogyne *Göldi, 1892 (Tylenchida) in Europe*. Leiden, Netherlands, Brill, 157 pp.

Kazachenko IP and Mukhina TI (2013) *Root-knot nematodes of genus* Meloidogyne *Goeldi (Tylenchida: Meloidogynidae) of the world*. Vladivostok, Russia, Institute of Biology and Soil Sciences, 306 pp.

Kiewnick S, Holterman M, van den Elsen S, van Megen H, Frey JE and Helder J (2014) Comparison of two short DNA barcoding loci (COI and COII) and two longer ribosomal DNA genes (SSU & LSU rRNA) for specimen identification among quarantine root-knot nematodes (*Meloidogyne* spp.) and their close relatives. *European Journal of Plant Pathology* **140**, 97–110.

Kofoid CA and White WA (1919) A new nematode infection of man. *Journal of the American Medical Association* **72**, 567–569.

Landa BB, Rius JEP, Vovlas N, Carneiro RMDG, Maleita CMN, Abrantes IMO and Castillo P (2008) Molecular characterization of *Meloidogyne hispanica* (Nematoda, Meloidogynidae) by phylogenetic analysis of genes within the rDNA in *Meloidogyne* spp. *Plant Disease* **92**, 1104–1110.

Liao J, Yang W, Feng Z and Karssen G (2005) Description of *Meloidogyne panyuensis* sp. n. (Nematoda: Meloidogynidae), parasitic on peanut (*Arachis hypogaea* L.) in China. *Russian Journal of Nematology* **13**, 107–114.

Nguyen NC and Nguyen VT (2000) *Plant parasitic nematodes in Vietnam*. Hanoi, Vietnam, Science and Technology Publishing House, 403 pp. (in Vietnamese).

Nguyen NC and Nguyen VT (2001) Plant parasitic nematodes associated with coffee crop in some North and Tay Nguyen Highland provinces of Vietnam. pp. 188–195 *in Selected works on ecological and bioresource studies (1996–2000)*. Hanoi, Vietnam, Hanoi Agricultural Publishing House (in Vietnamese).

Oliveira CMG, Monteiro AR and Blok VC (2011) Morphological and molecular diagnostics for plant parasitic nematodes: working together to get the identification done. *Tropical Plant Pathology* **36**, 65–73.

Onkendi EM and Moleleki LN (2013) Distribution and genetic diversity of root-knot nematodes (*Meloidogyne* spp.) in potatoes from South Africa. *Plant Pathology* **62**, 1184–1192.

Pagan C, Coyne D, Carneiro R, Kariuki G, Luambano N, Affokpon A and Williamson VM (2015) Mitochondrial haplotype-based identification of ethanol-preserved root-knot nematodes from Africa. *Phytopathology* **105**, 350–357.

Perry R and Moens M (2013) *Plant nematology*. 2nd edn. Wallingford, UK, CABI, 568 pp.

Plantard O, Picard D, Valette S, Scurrah M, Grenier E and Mugniéry D (2008) Origin and genetic diversity of Western European populations of the potato cyst nematode (*Globodera pallida*) inferred from mitochondrial sequences and microsatellite loci. *Molecular Ecology* **17**, 2208–2218.

Powers TO and Harris TS (1993) A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology* **25**, 1–6.

Riffle JW (1963) *Meloidogyne ovalis* (Nematoda: Heteroderidae), a new species of root-knot nematode. *Proceedings of the Helminthological Society of Washington* **30**, 287–292.

Seinhorst JW (1962) On the killing, fixation and transferring to glycerin of nematodes. *Nematologica* **8**, 29–32.

Souza RM (2008) *Plant-parasitic nematodes of coffee*. Berlin, Germany, Springer International Publishing, 340 pp.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729.

Tigano MS, Carneiro RMDG, Jeyaprakash A, Dickson DW and Adams BJ (2005) Phylogeny of *Meloidogyne* spp. based on 18S rDNA and the intergenic region of mitochondrial DNA sequences. *Nematology* **7**, 851–862.

Treub M (1885) Onderzoekingen over seren–ziek suikerriet gedaan in 'slands plantentuin te Buitenzorg. *Meded. Pl. Tuin*, Batavia 2.

Trinh PQ, de la Peña E, Nguyen CN, Nguyen HX and Moens M (2009) Plant-parasitic nematodes associated with coffee in Vietnam. *Russian Journal of Nematology* **17**, 73–82.

Trinh PQ, Pham TKD and Nguyen NC (2013) Emerging *Meloidogyne* species (root-knot nematodes) threats to coffee in the Western Highlands in Vietnam. *Proceedings of the 2nd VAST–KAST workshop on biodiversity and bop–active compounds*, 313–318.

Trudgill DL and Blok VC (2001) Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual Review of Phytopathology* **39**, 53–77.

Vrain TC, Wakarchuk DA, Lévesque AC and Hamilton RI (1992) Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15**, 563–573.

Wesemael WML, Viaene N and Moens M (2011) Root-knot nematodes (*Meloidogyne* spp.) in Europe. *Nematology* **13**, 3–16.

Whitehead AG and Hemming JR (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* **55**, 25–38.

Ye W, Zeng Y and Kerns J (2015) Molecular characterisation and diagnosis of root-knot nematodes (*Meloidogyne* spp.) from turfgrasses in North Carolina, USA. *PLoS ONE* **10**(11), e0143556.

https://doi.org/10.1017/S0022149X18000202 Published online by Cambridge University Press