Nematology, 2004, Vol. 6(5), 681-693

Radopholus arabocoffeae sp. n. (Nematoda: Pratylenchidae), a nematode pathogenic to *Coffea arabica* in Vietnam, and additional data on *R. duriophilus*

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Received: 23 February 2004; revised: 20 July 2004
Accepted for publication: 21 July 2004

Summary – *Radopholus arabocoffeae* sp. n., a new nematode pathogenic on *Coffea arabica* cv. Catimor, is described from Vietnam. Females of *R. arabocoffeae* sp. n. are characterised by the broad amphidial apertures with prominent margins. Males are characterised by the bursa extending to one third, rarely middle, of the tail. The new species belongs to the group of species with a long tail in the female. *Radopholus arabocoffeae* sp. n. is easily distinguished from *R. similis* by the bursa reaching to only one third of the tail *vs* extending to the tail terminus. *Radopholus arabocoffeae* sp. n. is differentiated from *R. bridgei* by the lateral field having three bands of equal width (*vs* middle one narrower than others), lateral field completely areolated over whole body *vs* not areolated except irregularly in neck and tail, hemizonid distinct *vs* indistinct, four lateral field incisures terminating far behind phasmid *vs* three incisures terminating at or just behind phasmid, lateral lines fusing at two thirds of tail *vs* fusing at one third of tail, longer spicule length (18-21 *vs* 15.5-18.0 μm), and male bursa usually extending to only one third of tail *vs* mid tail. *Radopholus arabocoffeae* sp. n. differs from *R. colbrani* by the rod-like *vs* round sperm, spicule length (18-21 *vs* 13-16 μm), tail length to stylet ratio (4.1-4.9 *vs* more than 5.1) and presence *vs* absence of a bursa. *Radopholus arabocoffeae* sp. n. differs from *R. duriophilus* by the rod-like *vs* kidney-shaped sperm. Males further differ from *R. duriophilus* by shorter stylet length (8.2-11.6 *vs* 11.5-15 μm), smaller distance between dorsal pharyngeal gland orifice and stylet base (1.7-3.4 *vs* 4-9.5 μm), shorter hyaline tail (2.6-3.4 *vs* 4-9.5 μm), and bursa extending to one third of tail *vs* mid-tail. Female *R. arabocoffeae* sp. n. differ from *R. duriophilus* by the broad amphidial aperture with prominent margin present *vs* absent. Males of *R. arabocoffeae* sp. n. differ from *R. musicola* by the rudimentary and amalgamated stylet base (*vs* with knobs), and inner lateral lines fusing at two thirds of the tail *vs* just posterior to the phasmid. The high level of ITS-rDNA sequence divergence of *R. arabocoffeae* sp. n. from other *Radopholus* spp. and the presence of nucleotide autapomorphies support a separate specific status of this new species. On carrot disks, the two species reproduced from 15-30°C; optimum reproduction occurring at 28°C. The reproductive capacity of *R. duriophilus* was higher than that of *R. arabocoffeae* sp. n. *Radopholus duriophilus* reproduced from single juveniles; *R. arabocoffeae* sp. n. did not. The correlation between initial densities of *Pratylenchus coffeae*, *R. duriophilus* and *R. arabocoffeae* sp. n. and the weight of *C. arabica* cv. Catimor fitted the Seinhorst model $Y = y_m$ for $Pi \leqslant T$, and $Y = y_m \cdot m + y_m(1-m)z^{(Pi-T)}$. *Coffea arabica* cv. Catimor was very susceptible for to all three nematode species tested, but especially so to *R. arabocoffeae* sp. n. The reproductive capacity of *R. arabocoffeae* sp. n. on *C. arabica* cv. Catimor was higher than *P. coffeae* or *R. duriophilus.*

Keywords – bionomics, coffee, ITS, molecular, phylogeny, rDNA, SEM, taxonomy.

Many genera and species of nematodes have been associated with coffee in several countries around the world. They include very damaging nematodes causing great losses to coffee farmers and to the local economy of developing countries (Campos *et al.*, 1990). After root-knot nematodes, the root-lesion nematodes (mainly *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 and *P. coffeae* (Zimmerman, 1898)

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Filipjev & Schuurmans Stekhoven, 1941), are recognised as being responsible for serious crop losses on both *Coffea arabica* L. and *C. canephora* Pierre *ex* Froehner. Whitehead (1968) commented on the great importance of *Radopholus similis* (Cobb, 1893) Thorne, 1949 to coffee in Java as reported by Zimmerman (1898). This nematode was considered to be the most harmful nematode to that country and second only in importance to *P. coffeae*. Holdeman (1986) cited coffee as one of the 365 host plants for *R. similis*.

Siddiqi (2000) recognised 20 valid species of *Radopholus* Thorne, 1949. Since then, *R. musicola* Stanton, Mundo-Ocampo, Baldwin & Kaplan, 2001, has been described from *Musa acuminata* in Australia (Stanton *et al.*, 2001) and Nguyen *et al.* (2003) described *R. duriophilus* Nguyen, Subbotin, Madani, Trinh & Moens, 2003 from durian (*Durio zibetinus* M.) in Vietnam. Species of the genus *Radopholus* are indigenous to Australasia where they live mostly as parasites of wild plants and forest trees. Sher (1968) reported that of the 22 species then regarded as valid, only one, *viz. R. nigeriensis* Sher, 1968, was indigenous outside of this region. Siddiqi (1991) subsequently transferred this species to the new genus, *Zygradus* Siddiqi, 1991. Since the genomic variation within a species or genus is likely to be greatest at its centre of origin, and because at that time two more new species (*R. citri* Machon & Bridge, 1996 and *R. bridgei* Siddiqi & Hahn, 1995) had been described from Indonesia, Siddiqi and Hahn (1995) stated that Australasia was probably the centre of origin of the genus *Radopholus* and therefore probably the origin of its type species, *R. similis*.

During a survey of coffee plantations in the Dak Lak province of Vietnam, two populations were collected that met the description of the genus *Radopholus* according to Siddiqi (2000). The population from *C. canephora* was attributed to *R. duriophilus*, but the morphology, morphometrics and ITS-DNA sequences of the population from *C. arabica* cv. Catimor revealed that it belonged to a new species. This species is herein described as *Radopholus arabocoffeae* sp. n. We also report on the multiplication of this new species in carrot disks and on its mode of multiplication. Finally, we demonstrate its damage potential on *C. arabica* cv. Catimor seedlings and compare it with that of *R. duriophilus* and *P. coffeae*.

Materials and methods

Nematodes

Female, male and juvenile specimens of the *R. arabocoffeae* sp. n. population were obtained from roots and soil of coffee, *C. arabica* cv. Catimor, sampled in the Krongnang District, Dak Lak province, Western Highland, Vietnam. This population was compared with the population of *R. duriophilus* collected from the roots and rhizosphere of *C. canephora* in Krong Ana District in the Highlands, and with a population of *Pratylenchus coffeae* collected from *C. arabica* cv. Catimor grown in Dak Lak, Vietnam.

The nematodes were extracted from soil by decantation followed by centrifugal flotation and from roots by maceration and centrifugation (Coolen & D'Herde, 1972). All populations were maintained on carrot disks (O'Bannon & Taylor, 1968) and used for further observations.

Morphology and morphometrics

Individuals from both *Radopholus* populations were heat killed, fixed in TAF (Seinhorst, 1959), and processed to and mounted in anhydrous glycerine (Hooper & Evans, 1993). From each population, morphometrics of 20 females and 20 males were taken using a drawing tube attached to an Olympus CH40 light microscope.

For scanning electron microscopy (SEM), specimens preserved in anhydrous glycerine were transferred to a drop of 4% formalin. A subsequent ultrasonic treatment (10 min) removed particles adhering to the body surface of the specimen. The nematodes were dehydrated by passing them through an ethanol gradient of 25 (overnight), 50, 75, 95 (3 h each) and 100% (overnight) at 25°C. They were critical point dried with liquid CO_2, mounted on stubs, and coated with gold-palladium (25 nm) before observation with a Jeol LSM-840 at 15 kV.

Molecular studies

DNA was extracted and amplified from both *Radopholus* populations as described by Waeyenberge *et al.* (2000). Forward primer 5′-CGTAACAAGGTAGCTGTAG-3′ and reverse primer 5′-TCCTCCGCTAAATGATATG-3′ (Ferris *et al.*, 1993) were used for amplification of the ITS regions, including the 5.8S gene plus flanking areas of the 18S and 28S genes of rDNA. Amplified products were excised from 1% TAE-buffered agarose gels using the Qiaquick Gel Extraction Kit (Qiagen-Westburg, Leusden, The Netherlands), cloned into the pGEM-T vector and transformed into JM 109 High Efficiency Competent Cells (Promega, Leiden, The Netherlands). Five colonies of each population were isolated using blue/white selection and submitted to PCR with vector primers. Amplified product was purified using a Qiagen Gel Purification Kit (Qiagen). DNA fragments

were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit according to manufacturer's instructions (PE Applied Biosystems, Foster City, CA, USA). The resulting products were purified using a Centriflex Gel Filtration Cartridge (Edge Biosystems, Gaithersbugs, MD, USA) before being analysed using an ABI Prism 310 Genetic analyser. The ITS sequence of *R. arabocoffeae* sp. n. is deposited at GenBank under accession number AY547297. The DNA sequences of both *Radopholus* populations from coffee were aligned using ClustalX 1.64 (default options) together with six sequences of *R. similis* from GenBank (Elbadri *et al.*, 2002) and two sequences of *R. duriophilus* (Nguyen *et al.*, 2003). The alignment is available upon request to the corresponding author. Equally weighted maximum parsimony (MP) analysis was performed using PAUP* 4.0 beta version (Swofford, 1998). Gaps were treated as missing data. Bootstrap analysis was calculated with 1000 replicates for MP tree. Pairwise divergences between taxa were computed by PAUP* as the absolute distance values and the percent mean distance values adjusted for missing data.

Multiplication on carrot disks

The *in vitro* multiplication of *R. arabocoffeae* sp. n. was compared with that of *R. duriophilus* in two experiments. In the first, 25 females were randomly picked with a needle and transferred into a drop of sterile water on the cortex of a peeled and ethanol-sterilised carrot disk placed in a 3.5 cm diam. Petri dish. The Petri dishes were then sealed with Parafilm and incubated in the dark at 15, 20, 25, 28, or 30°C. There were eight to 12 replicates for each species and temperature combination. The second experiment was similar to the first, but differed in the number of nematodes used and their developmental stage. One hundred mature females or juveniles of each of both nematode species were individually inoculated on carrot disks as described above and incubated in the dark at 28°C. In both experiments the nematodes were extracted from carrot disks 50 days after inoculation. The disks were macerated in 600 ml water in a Waring blender (Global Researches Inc., Winsted, CT, USA) for 30 s and the obtained suspension was passed through a 250 μm pore sieve. The nematodes were separated from the remaining carrot tissue in an automated nematode extraction apparatus (Hendrickx, 1995). Eggs, juveniles, males and females were counted separately. As the nematode numbers were not normally distributed, they were $log_{10}(x + 1)$ transformed before ANOVA. Population increase was expressed as reproduction factor (= final nematode number/number of nematodes inoculated). Means were statistically analysed using the Duncan Multiple Range Test ($P \leqslant 0.05$).

Damage potential on coffee

Coffee (*C. arabica* cv. Catimor) seedlings were grown from seeds obtained from the Coffee Research Station in Dak Lak, Vietnam. Seeds were surface sterilised (30 min in 5.25% NaOCl) and germinated in sterile vermiculite (2 months). Just before two cotyledons had fully developed, seedlings were transferred individually to a 250 cm^3 pot filled with autoclaved soil (loam and sand 70:30). The plants were kept in the glasshouse for 30 days before inoculation with mobile stages of *R. arabocoffeae* sp. n., *R. duriophilus*, or *P. coffeae* at densities of 0, 1, 2, 4, 8, 16 or 32 nematodes/cm^3 soil. Each combination of nematode species-density was replicated five times. After nematode inoculation, the nematode-treated and control plants were grown for an additional 3 months in the glasshouse with an illumination regime of 12 h light and 12 h dark at 25-30°C (day) and 22-25°C (night), and 50-80% relative humidity. The plants were watered when necessary. Each month, each plant was watered with 10 ml of a liquid fertiliser (NPK 7-4-6/1000 ml). Ninety days after inoculation, the fresh root and shoot weight were determined and the final nematode population density in both soil and roots was determined after extracting the nematodes with an automatic apparatus (Hendrickx, 1995). The influence of initial inoculum density on the plant development was examined using Seinfit, a computer program (Viaene *et al.*, 1997) estimating the equation developed by Seinhorst (1965).

Radopholus arabocoffeae* **sp. n.**

(Figs 1-3)

Measurements

See Table 1.

Description

Female

Body almost straight or slightly curved ventrally after killing by gentle heat. Labial region continuous with body; dome shaped and bearing four or five annules.

* Named after arabica coffee, the host on which the species was found.

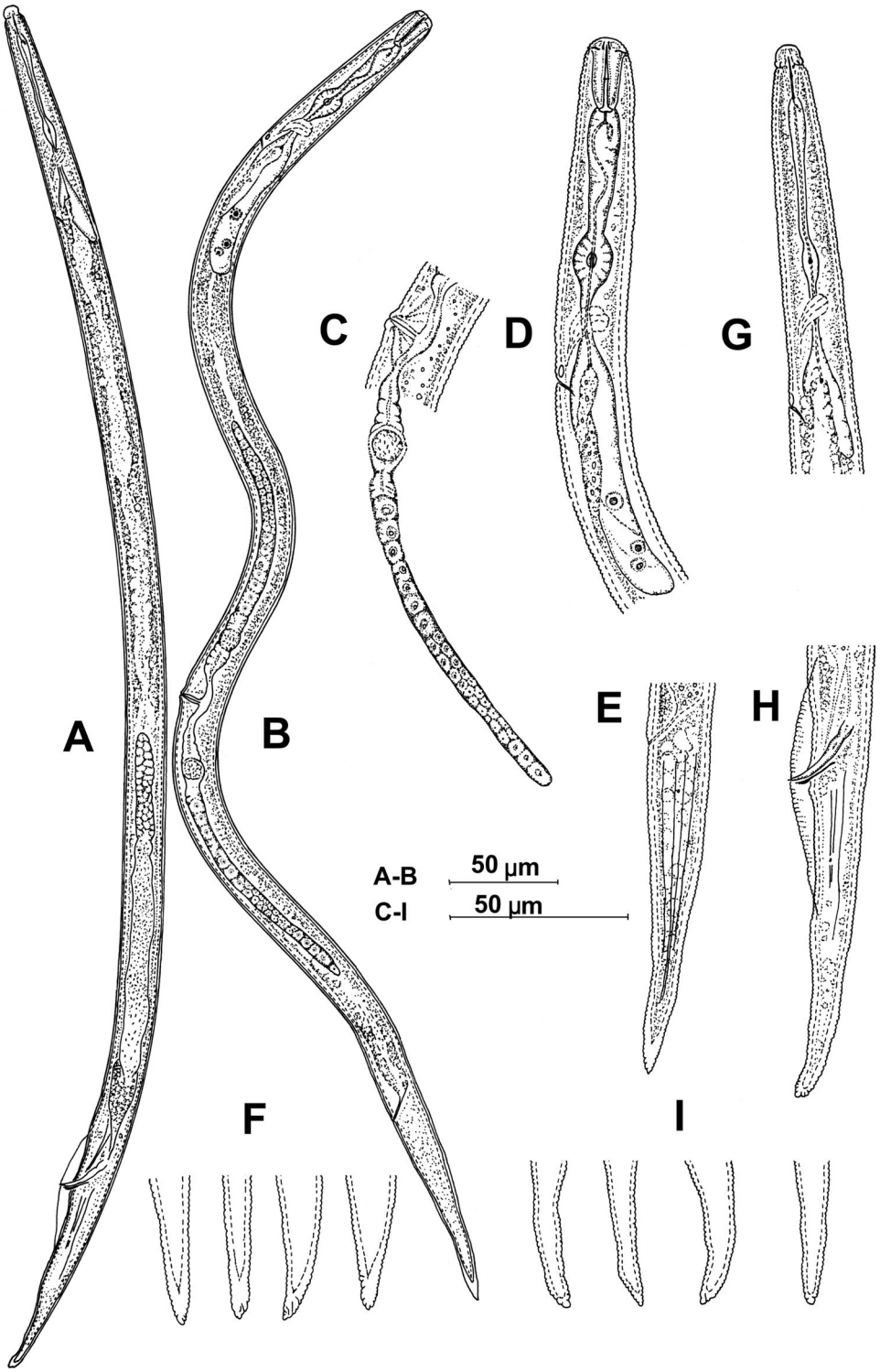

Fig. 1. Radopholus arabocoffeae *sp. n. Female, B-F. B: Entire body; C: Posterior branch of reproductive tract; D: Anterior region including pharynx; E: Tail; F: Variation in tail tip morphology – Male, A. G-I. A: Entire body; G: Anterior region including pharynx; H: Tail; I: Variation in tail tip morphology.*

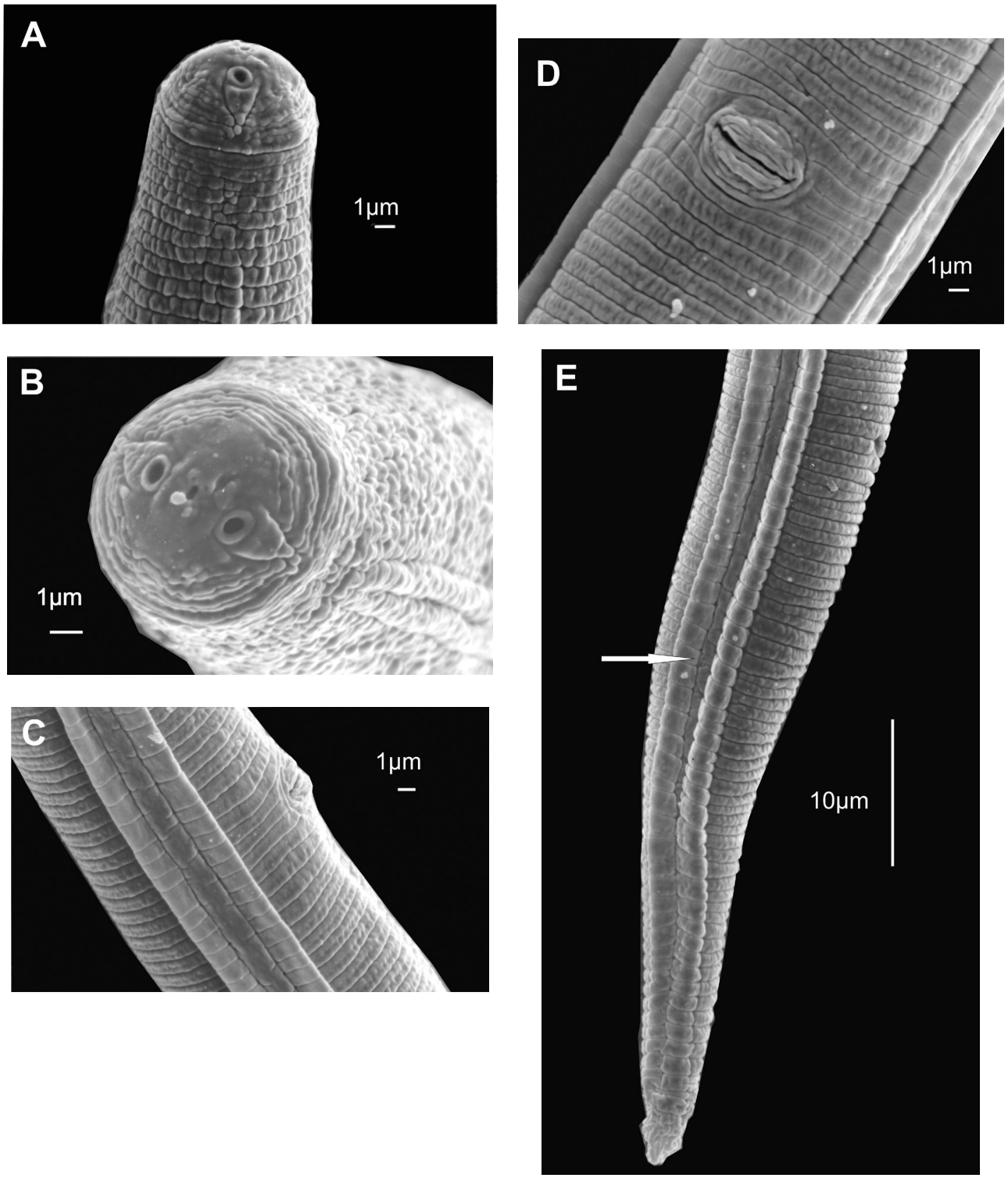

Fig. 2. *SEM photographs of* Radopholus arabocoffeae *sp. n. Female. A: Head lateral view; B: Head* en face *view; C: Vulva and lateral field; D: Vulva ventral view; E: Lateral view of tail (arrow indicates position of phasmid).*

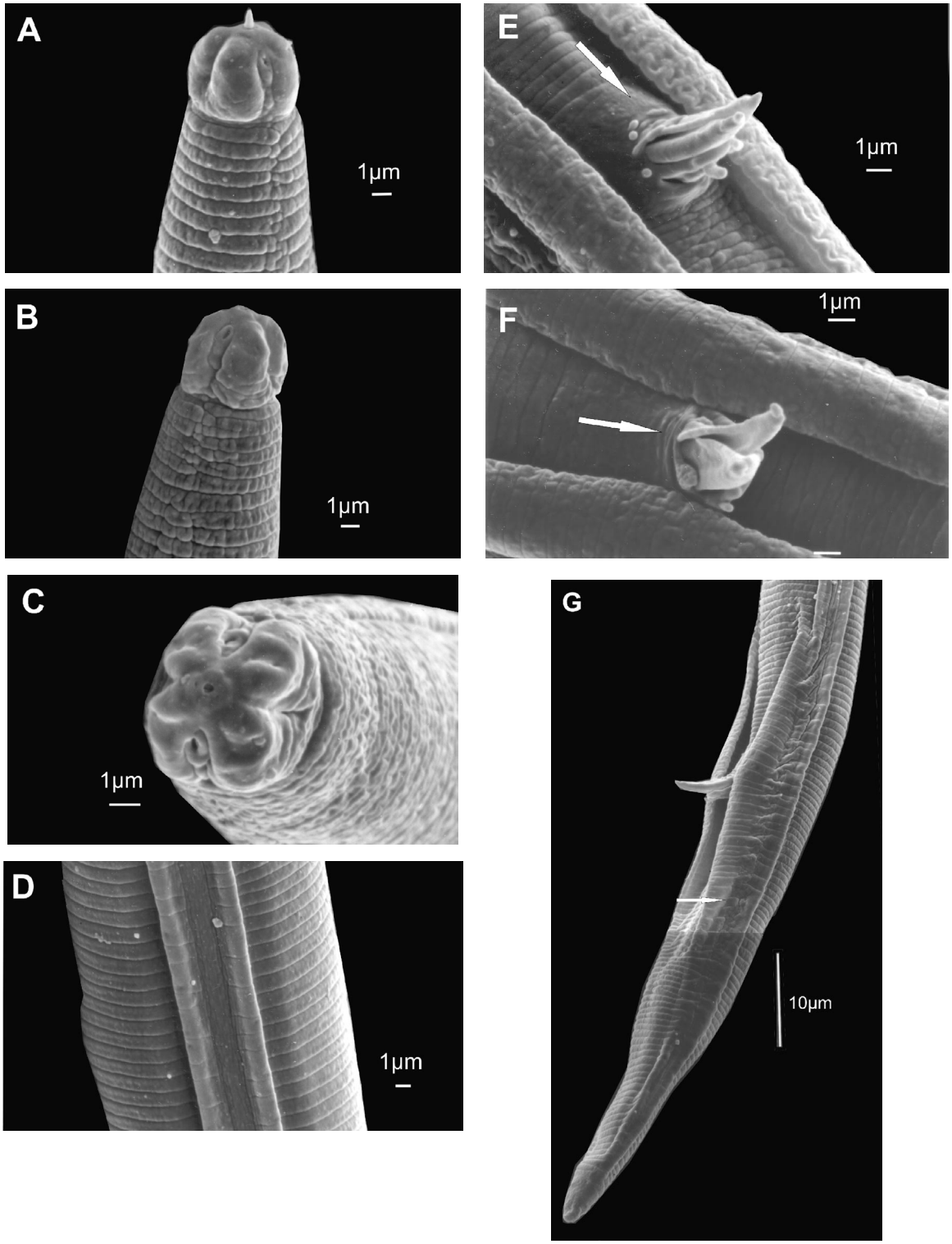

Fig. 3. *SEM photographs of* Radopholus arabocoffeae *sp. n. Male. A-B: Head lateral view; C: Head* en face *view; D: Lateral field; E-F: Ventral view of spicules (arrows indicate hypoptygma); G: Lateral view of tail (arrow indicates position of phasmid).*

Table 1. *Morphometric characters of* Radopholus arabocoffeae *sp. n. Measurements in μm and in form: mean ± standard deviation (range).*

	Female		Male
	Holotype	Paratypes	Paratypes
n	–	20	20
L	598	615.4 ± 23.3 (590-682)	571 ± 27.3 (525-619)
a	31.6	29.9 ± 2.4 (23.6-33.6)	33 ± 2.6 (25.8-38.3)
b	7.7	8 ± 0.3 (7.5-8.8)	8 ± 0.4 (7.4-8.9)
b′	4.4	4.4 ± 0.2 (4.2-4.8)	6 ± 0.4 (5-6.6)
c	8.1	8.5 ± 0.3 (8-9)	7.4 ± 0.4 (6.5-8)
c′	4.3	4.5 ± 0.5 (3-5.2)	5.6 ± 0.6 (4.2-7)
V	55.9	55.3 ± 1.5 (50.9-58.3)	–
V′*	63.8	62.7 ± 1.6 (57.6-65.6)	–
G1	158	159 ± 35.3 (114-209)	–
G2	155	139 ± 29.3 (103-176)	–
Maximum body diam.	19	21 ± 1.9 (18-25)	17 ± 1.6 (15-22)
Height of lip region	3.4	3.2 ± 0.4 (2.6-3.4)	5.4 ± 0.4 (4.3-6)
Diam. of lip region	8.6	9.1 ± 0.5 (7.7-10.3)	7.1 ± 0.5 (6-7.7)
Length of stylet	15.5	15.9 ± 0.8 (14.6-17.2)	10 ± 1.1 (8.2-11.6)
Width of stylet base	4.3	4 ± 0.4 (3.4-4.9)	–
DGO	2.6	3.1 ± 0.6 (2.6-4.3)	2.8 ± 0.5 (1.7-3.4)
Ant. end to centre of med. bulb	54	53 ± 2.2 (49-58)	49 ± 2.5 (45-53)
Length of medium bulb	12.9	12.6 ± 1 (10.3-14.2)	9.4 ± 0.9 (6.5-11.2)
Diam. of medium bulb	9.0	9.4 ± 1.1 (7.7-12.9)	4.6 ± 0.6 (3.4-5.3)
Length of pharynx	77	77 ± 2.9 (72-84)	71 ± 4.1 (61-76)
Length of gland lobe	76	80 ± 5.2 (70-90)	42 ± 5.9 (36-58)
Anterior end to excret. pore	88	84 ± 4.7 (74-90)	81 ± 4.4 (72-88)
Annule width at mid-body	1.1	1.2 ± 0.1 (1-1.5)	1.1 ± 0.1 (1-1.3)
Diam. of spermatheca	8.2	7.7 ± 0.5 (6.9-8.6)	–
Tail length	74	72 ± 3 (67-79)	77 ± 4.6 (70-84)
Body diam. at anus	17	16 ± 2.1 (14-23)	14 ± 1.3 (11-17)
h	8.2	8.4 ± 1.1 (6.9-11.2)	2.8 ± 0.4 (2.6-3.4)
Length of spicules	–	–	19.4 ± 0.7 (18-21)
Length of gubernaculum	–	–	9.8 ± 1 (7.7-11.2)
Lip region diam./height	2.5	2.9 ± 0.4 (2.5-3.7)	1.3 ± 0.1 (1.1-1.5)
Medium bulb length/diam.	1.4	1.4 ± 0.1 (1.1-1.5)	2 ± 0.5 (0.2-2.6)
Tail length/stylet length	4.8	4.6 ± 0.3 (4.1-4.9)	7.8 ± 1 (6.5-9.5)
Diam. hyaline part/length	0.5	0.7 ± 0.1 (0.4-0.9)	1 ± 0.3 (0.1-1.7)
Tail annules	56	50 ± 5.4 (40-58)	50 ± 6.4 (42-61)
Testis length	–	–	164 ± 26.4 (128-218)

* After Elbadri *et al.* (1999a, b).

Labial disc not distinct in LM, round in SEM. Amphidial apertures broad and with prominent margin. Lateral lips terminating on fourth to fifth head annule. Stylet moderately, strong knobs directed posteriorly, slightly anchor shaped. Length of stylet cone equal to length of shaft plus knobs or slightly longer. Dorsal gland opening near stylet base. Rounded or oval median bulb well developed. Pharyngeal glands in tandem, forming a long, dorsally overlapping lobe; nucleus near posterior end. Excretory pore located at level of pharyngo-intestinal junction or 0.5 body diam. posteriorly. Hemizonid one to two body annules long and zero to two annules anterior to excretory pore. Lateral field with four equidistant longitudinal incisures, completely areolated over whole body. Four incisures present at phasmid level. Three body annules terminating at vulva. Vulval lips always protuberant. Ante-

rior genital branch slightly longer than posterior branch. Spermatheca round, filled with thick, rod-shaped, sperm. Oocytes in single row anteriorly or two rows near middle of ovary; genital branch sometimes reaching pharynx. Postrectal intestine sac absent. Tail long, conoid, with 40-58 annules, terminus annulated, rarely smooth, narrow, conically rounded. Phasmids distinct, located in anterior third of tail. Lateral lines fusing at two thirds of tail length.

Male

Slender, slightly ventrally curved after killing by gentle heat. Cephalic region set off, knob-like, with zero to three annules. Labial disc not distinct. Lateral lips terminating at junction of head and body. Stylet rudimentary with amalgamated base. Median pharyngeal bulb oval and gland lobe poorly developed. Excretory pore located at pharyngo-intestinal junction. Lateral field with four equidistant incisures at mid-body; central band of lateral field sometimes narrower than outer bands. Lateral field on tail indistinct. Phasmids located at *ca* one third of tail. Thick, rod-shaped, sperm present in posterior genital tract. Postrectal intestine sac absent. Bursa usually extending to one third of tail, rarely to mid-tail. Spicule tylenchoid, with asymmetrical, oval shaped, manubrium. Gubernaculum with head more or less protruding and pronounced pair of titillae. Anterior cloacal lip with zero to three hypotygma. Tail conical, with 49 (42-61) annules, terminus rounded and annulated, rarely smooth. Hyaline part of tail narrow and short.

Type host and locality

Coffee (*Coffea arabica* cv. Catimor) roots and soil, Krongnang District, Dak Lak province, Western Highland, Vietnam.

Type material

One female holotype, nine female paratypes and six male paratypes deposited at the nematode collection of the Institute of Zoology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium. Six female and two male paratypes and fixed material from cultures deposited at the nematode collection of the Nematology Department, Institute of Ecology and Biological Resources, 18 Hoang Quoc Viet, Hanoi, Vietnam.

Diagnosis and relationships

The female of *R. arabocoffeae* sp. n. is characterised by a body length of 590-682 μm, the broad amphidial apertures with prominent margins, stylet length of 14.6-17.2 μm, excretory pore located at 74-90 μm from anterior end, distinct hemizonid present, lateral field completely areolated over whole body with four equidistant longitudinal incisures, four incisures at phasmid level, but lateral lines fusing at two thirds of the tail, c value of 8-9 and postrectal intestinal sac absent. The male is characterised by the stylet length of 8.2-11.6 μm, dorsal pharyngeal gland orifice located 1.7-3.4 μm posterior to stylet base, rudimentary stylet with amalgamated base, excretory pore located at 72-88 μm from anterior end, spicule 18-21 μm long, gubernaculum 7.7-11.2 μm long, hyaline tail 2.6-3.4 μm long and 2.6-4.3 μm wide, and bursa never extending to the tail terminus, but usually reaching to one third of the tail length, rarely mid-tail.

In having a long female tail, *R. arabocoffeae* sp. n. resembles *R. similis*, *R. bridgei* Siddiqi & Hahn, 1995, *R. colbrani* Kumar, 1980, *R. kahikateae* Ryss & Wouts, 1997, *R. musicola* Stanton, Mundo, Baldwin & Kaplan, 2001, and *R. duriophilus* Nguyen, Subbotin, Madani, Trinh & Moens, 2003.

The new species differs from *R. similis* by the four incisures of the lateral field terminating far behind the position of the phasmid (*vs* three incisures terminating at or just behind phasmid), female stylet length (14.6-17.2 *vs* 16-22 μm), and absence *vs* presence of a postrectal intestinal sac (Ryss & Wouts, 1997). It is further separated by the shorter male stylet (8.2-11.6 *vs* 13.5-16.5 μm), the shorter distance between the orifice of dorsal pharyngeal gland and the stylet base (1.7-3.4 *vs* 4.5-5.5 μm), bursa reaching one third of tail, rarely half *vs* bursa reaching tail terminus. Elbadri *et al*. (1999a, b) described variation within *R. similis*. Some of the populations examined by these authors had large, rounded amphidial apertures, but no population had amphids with broad aperture and prominent margins as in the new species.

Radopholus arabocoffeae sp. n. is differentiated from *R. bridgei* by the lateral field having three bands equal in width *vs* middle one narrower than others, lateral field completely areolated over whole body *vs* not areolated except irregularly on neck and tail, hemizonid distinct *vs* indistinct, four lateral field incisures terminating far behind phasmid *vs* three incisures terminating at or just behind phasmid, lateral lines fusing at two thirds of the tail *vs* fusing at one third of the tail, longer spicule (18-21 *vs* 15.5-18.0 μm), and bursa of male extending for one third of tail, rarely half *vs* reaching mid tail.

Radopholus arabocoffeae sp. n. shares with *R. colbrani* the same host but differs by the rod-like *vs* round sperm,

longer spicules (18-21 *vs* 13-16 μm), tail length to stylet ratio of 4.1-4.9 *vs* more than 5.1, and presence *vs* absence of a bursa (see Kumar, 1980).

Females of *R. arabocoffeae* sp. n. differ from *R. kahikatea* by having four incisures at phasmid level, lateral lines fusing at two thirds of the tail *vs* two inner incisures joining anterior to or at phasmid, lower lip region (2.6-3.4 *vs* 3.5-4.5 μm), shorter stylet (14.6-17.2 *vs* 18-23 μm), shorter distance from anterior end to centre of median bulb (49-58.1 *vs* 66-82 μm), shorter distance of excretory pore to anterior end (74-90 *vs* 98-114 μm), c value of 8-9 *vs* 9.9-19.0, tail length to stylet length ratio of 4.1-4.9 *vs* 1.86-3.75. *Radopholus arabocoffeae* sp. n. males have a shorter stylet (8.2-11.6 *vs* 18-20 μm) which is rudimentary with amalgamated base *vs* stylet with distinct knobs, bursa extending to one third of tail *vs* bursa reaching to tail terminus, and excretory pore located closer to the anterior end (72-88 *vs* 91-109 μm).

Radopholus arabocoffeae sp. n. is similar morphometrically to *R. musicola*, but males differ by the rudimentary stylet and amalgamated base *vs* with knobs developed, and inner lateral lines fusing at two thirds of the tail *vs* just posterior to phasmid. In SEM photographs, the new species resembles *R. musicola* in the large, rounded, amphidial apertures, but differs by the presence of prominent margins *vs* absence.

Radopholus arabocoffeae sp. n. is close to *R. duriophilus*, but differs from that species by the rod-like *vs* kidney shaped sperm. Males of *R. arabocoffeae* sp. n. further differ from *R. duriophilus* by the shorter stylet (8.2-11.6 *vs* 11.5-15 μm), shorter distance between the dorsal pharyngeal gland orifice and the stylet base (1.7-3.4 *vs* 4-9.5μm), and shorter hyaline tail part of tail (2.6-3.4 *vs* 4-9.5 μm). The bursa of *R. arabocoffeae* sp. n. is usually shorter, reaching to one third, rarely half, of the tail *vs* mid-tail. Females of *R. arabocoffeae* sp. n. differ from those of *R. duriophilus* by the broad amphidial apertures with prominent margins *vs* margins not prominent.

Radopholus arabocoffeae sp. n. resembles *R. inanis* Colbran, 1970, *R. vangundyi* Sher, 1968, *R. neosimilis* Sauer, 1958, *R. intermedius* Colbran, 1971, *R. clarus* Colbran, 1971, *R. nelsonensis* Ryss & Wouts, 1997 by having four incisures in the lateral field, all four incisures remaining visible between the phasmid and female tail terminus. The new species, however, differs from *R. inanis* by the rod-like sperm *vs* rounded, greater b value (7.5-8.8 *vs* 5.5-6.1), smaller c value (8-9 *vs* 12-14), longer tail (67-79 *vs* 35-40 μm), longer spicule length (18-21 *vs* 14-17 μm), and the leptoderan type bursa never reaching the tail terminus, but reaching to only one third of the tail length (*vs* caudal alae subterminal).

Radopholus arabocoffeae sp. n. differs from *R. vangundyi* by the b′ value (4.2-4.8 *vs* 3.3-4.0 in females and 5-6.6 *vs* 3.6-4.6 in males), smaller c value (8-9 *vs* 12-16), shorter stylet length (14.6-17.2 *vs* 17-19 μm) in females and (8.2-11.6 *vs* 12-15 μm) in males, and longer spicule length (18-21 *vs* 14-18 μm).

Females of *R. arabocoffeae* sp. n. differ from *R. neosimilis* by the domed lip region *vs* anteriorly flattened, and the c value (8-9 *vs* 12-15). Males differ by their leptoderan bursa never reaching the tail terminus, but extending to one third of the tail length *vs* caudal alae enclosing tail, c value (6.5-8 *vs* 12-15), c′ value (4.2-7 *vs* 2.9-3.7), and stylet length (8.2-11.6 *vs* 12-16 μm).

Radopholus arabocoffeae sp. n. differs from *R. intermedius* by the body length of the female (590-682 *vs* 371-498 μm), b value (7.5-8.8 *vs* 5.1-6.6), c value (8-9 *vs* 11-14), tail length (67-79 *vs* 32-39 μm), stylet length (14.6-17.2 *vs* 11-13 μm), and the rod-like sperm *vs* rounded to oval.

Female *R. arabocoffeae* sp. n. differ from *R. clarus* by the b value (7.5-8.8 *vs* 5.9-7.3), lower c value (8-9 *vs* 12-16), shorter stylet length (14.6-17.2 *vs* 19-21 μm), longer distance of excretory pore from anterior end (74-90 *vs* 65-71 μm), and longer tail (67-79 *vs* 36-54 μm). Males differ by the b value (7.4-8.9 *vs* 6.4), b′ value (5-6.6 *vs* 4.2-4.4), smaller c value (6.5-8 *vs* 15-16), shorter stylet length (8.2-11.6 *vs* 14 μm), longer spicules (18-21 *vs* 16-17 μm), and leptoderan bursa type never reaching the tail terminus, but extending to one third of the tail length *vs* caudal alae enveloping.

Radopholus arabocoffeae sp. n. differs from *R. nelsonensis* by the shorter stylet length (14.6-17.2 *vs* 25-27 μm), longer tail (67-79 *vs* 17-19 μm) and smaller c value (8-9 *vs* 19-33).

Molecular characterisation

The length of the alignment for *Radopholus* sequences was 604 bp. Sequence divergence ranged from 0.2 to 6.3%. The sequences of *R. arabocoffeae* sp. n. differed from those of *Radopholus* spp. in 21-31 substitutions (3.5-5.2%). Maximum parsimony analysis revealed 37 parsimony informative characters. The single unrooted maximum parsimonious tree is given in Figure 4. The branch with *R. arabocoffeae* sp. n. was supported by seven autapomorphies (unique substitutions).

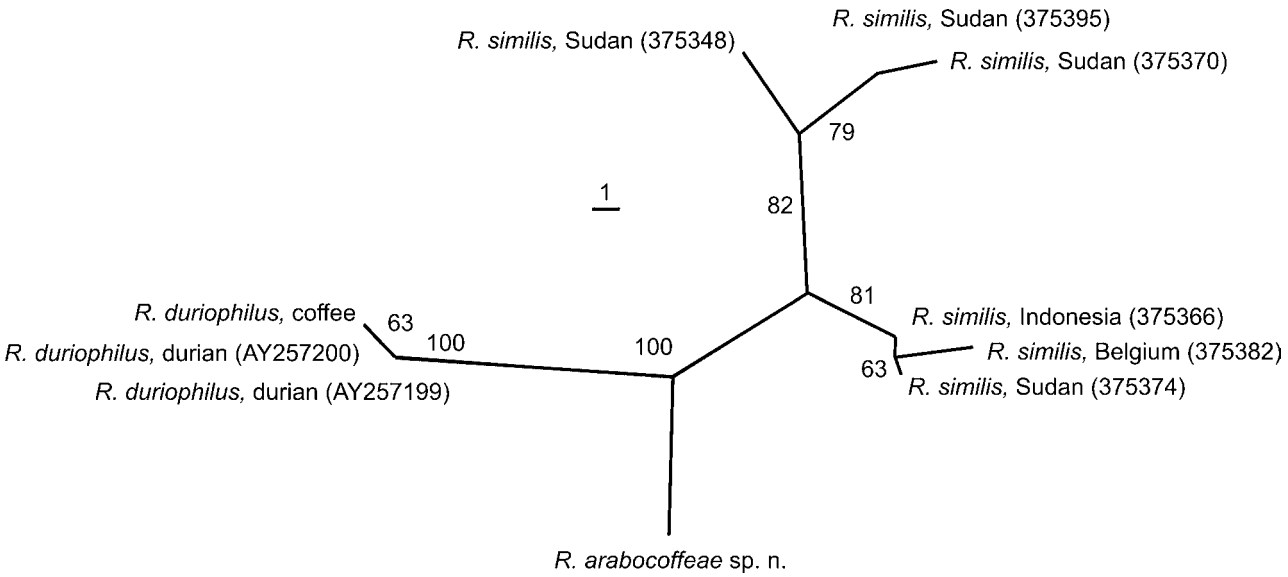

Fig. 4. *Single unrooted tree constructed on basis of nucleic acid sequences using the maximum likelihood method.*

Table 2. *Reproduction of* Radopholus duriophilus *and* R. arabocoffeae *sp. n. on carrot disks 50 days after inoculation with 25 females and incubation at different temperatures.*

Species	Temp.	Final population					Rf
	(°C)	Females	Juveniles	Males	Eggs	Total	
R. duriophilus	15	153 A*	33 A	29 A	19 A	234 A*	9.344 A*
	20	964 B*	468 B*	128 B	1784 B	2748 B	109.92 B
	25	10 212 D*	8984 D*	1524 C*	13 400 D*	34 120 D*	1364.8 D*
	28	13 196 E*	18 196 E*	2936 C*	17 336 D*	51 664 E*	2066.56 E*
	30	7008 C	5300 C	2348 C	7376 C*	22 032 C*	881.28 C*
R. arabocoffeae sp. n.	15	127 a*	21 a	22 a	16 a	186 a*	7.44 a*
	20	580 b*	776 b*	84 b	1908 b	2488 b	99.52 b
	25	7408 d*	5596 d*	2828 c*	7376 d*	23 208 d*	928.32 d*
	28	10 064 e*	12 064 e*	2816 c*	10 236 d*	35 180 e*	1407.2 e*
	30	6308 c	4688 c	2244 c	5824 c*	19 064 c*	762.56 c*

Data are averages of five replicates. Data were transformed to $log_{10}(x+1)$ before analysis of variance; untransformed data are presented. Per species, means in the column followed by the same letter do not differ significantly according to Duncan's Multiple Range Test ($P \leqslant 0.05$). Two populations were compared by T-Test: *indicates significant difference between *R. duriophilus* and *R. arabocoffeae* sp. n. per combination of stage and temperature ($P \leqslant 0.05$).

Identity of the Krong Ana *Radopholus* population

In both morphology and morphometrics, the *Radopholus* population isolated from *C. canephora* in Krong Ana, Vietnam, almost completely matched the description of *R. duriophilus* (Nguyen *et al.*, 2003). The only difference in morphology noted was in the stylet knob shape, which in the *Radopholus* population from *C. canephora* is directed anteriorly whereas in the type population of *R. duriophilus* the knobs are rounded, although sometimes with a dorsal projection. This difference is herein interpreted as intraspecific variation.

Multiplication on carrot disks

Both *R. duriophilus* and *R. arabocoffeae* sp. n. showed the lowest reproduction at 15°C; however, the high reproduction factor obtained for each of the species prove that 15°C is not the lower limit for their reproduction (Table 2).

Table 3. *Reproduction of single females or single juveniles of* Radopholus duriophilus *and* R. arabocoffeae *sp. n. on carrot disks at 28°C.*

Population character	Single female		Single juvenile	
	R. duriophilus	*R. arabocoffeae* sp. n.	*R. duriophilus*	*R. arabocoffeae* sp. n.
Number of juveniles/disk	4264 a	2832 b	139	0
Number of females/disk	4664 a	2172 b	166	0
Number of males/disk	1944 a	196 b	38	0
Females as % of total	29.2 a	23.2 b	30.9	0
Males as % of total	12.2 a	2.1 b	25.9	0
Juveniles as % of total	26.7 a	30.2 a	7.1	0
Eggs as % of total	31.9 a	44.5 b	36.1	0

Data are averages from five replicates. Data were transformed to $log_{10}(x + 1)$ before T-Test. Comparisons between single female inoculations of *R. duriophilus* and *R. arabocoffeae* sp. n. followed by the same letter are not significantly different ($P \leqslant 0.05$).

Both species reproduced in highest numbers at 28°C. At that temperature, the reproduction of *R. duriophilus* was higher than that of *R. arabocoffeae* sp. n. The optimum temperature of *R. arabocoffeae* sp. n. is similar to the optimum observed for *R. similis* (Holdeman, 1986; Fallas & Marban-Mendoza, 1994; Fallas & Sarah, 1995; Elbadri *et al.*, 2001). Significant differences in multiplication between *R. arabocoffeae* sp. n. and *R. duriophilus* were also found at 15, 25, 28 and 30°C, although there was no significant difference at 20°C. These differences were significant for almost all developmental stages.

At 28°C both *Radopholus* species reproduced easily from single females. *Radopholus duriophilus* had a higher reproductive fitness than *R. arabocoffeae* sp. n., whereas *R. arabocoffeae* sp. n. produced many more males (Table 3). When starting from single juveniles, only *R. duriophilus* reproduced. Reproduction of *Radopholus* species has been the subject of debate. Loos (1962), and Rivas and Roman (1985) reported that they did not obtain progeny from single *R. similis* juvenile inoculations and concluded that *R. similis* reproduced solely by amphimixis. Brooks and Perry (1962), however, showed that *R. similis* was able to develop up to three generations from a single egg inoculation and proposed that *R. similis* might reproduce *via* parthenogenesis. Kaplan and Opperman (2000) came to the conclusion that the rod-shaped structures they observed in the spermatheca of *R. similis* females did not resemble sperm and did not result from mating, but were spermatids produced by the ovotestis of the hermaphrodite female. The authors concluded that hermaphroditism appears to afford the burrowing nematode with a reproductive strategy enabling individual nematodes to produce progeny without the assistance of males. Stanton *et al.* (2001) also observed rod-shaped structures in freshly moulted, apparently virgin, females of *R. musicola* and commented on the apparently hermaphroditic nature of this species.

Table 4. *Estimates of parameters of Seinhorst model of fresh weight (g)* Coffea arabica *cv. Catimor grown in 250 cm³ pots after inoculation with three species of coffee nematodes.*

Nematode species	Plant part	Parameter[1)]					R^2
		m	T	z	y_m	$y_m \cdot m$	
Pratylenchus coffeae	Total	0.50	0	0.8	4.864	2.432	0.94
	Root	0.55	0	0.65	1.978	1.088	0.76
	Shoot	0.45	0.18	0.85	2.886	1.299	0.94
Radopholus duriophilus	Total	0.25	0.8	0.75	4.864	1.216	0.94
	Root	0.20	0.5	0.70	1.978	0.396	0.93
	Shoot	0.25	0.51	0.75	2.886	0.722	0.83
R. arabocoffeae sp. n.	Total	0.10	0	0.70	4.864	0.486	0.96
	Root	0.15	0.22	0.60	1.978	0.297	0.92
	Shoot	0.10	0	0.70	2.886	0.289	0.95

[1)] The Seinhorst model is of the form: $Y = y_m$ for $Pi \leqslant T$, and $Y = y_m \cdot m + y_m \cdot z^{(Pi-T)}$ for $Pi > T$; $y_m =$ yield (g fresh weight) without nematode damage, $m =$ a constant so that $y_m \cdot m$ equals the minimum yield, $z =$ parameter determining the slope of the curve, $T =$ damage threshold density (nematodes per plant at inoculation).

Damage potential

The relationship between the initial densities of *R. duriophilus*, *R. arabocoffeae* sp. n. and *P. coffeae* and the weight of *C. arabica* cv. Catimor 90 days after inoculation fitted the Seinhorst model ($R^2 > 0.76$)

Table 5. *Reproduction of three species of coffee nematodes on* Coffea arabica *cv. Catimor seedlings in 250 cm³ pots under glasshouse conditions.*

Inoculum density (nematodes/cm³ soil)	Reproduction factor (Rf [a])		
	Pratylenchus coffeae	*Radopholus duriophilus*	*R. arabocoffeae* sp. n.
0	0 a	0 a	0 a
1	1.27 a	1.67 a	3.18 b
2	0.64 a	0.94 a	2.46 b
4	0.40 a	0.65 b	2.18 c
8	0.25 a	0.22 a	0.94 b
16	0.09 a	0.12 a	0.59 b
32	0.05 a	0.08 a	0.36 b

[a] Rf: final nematode number / number of nematodes inoculated. Data are averages of five replicates. Row means followed by the same letter are not significantly different according to Duncan Multiple Range Test ($P \leqslant 0.05$).

(Table 4). Differences in tolerance were observed between the three species and for three growth parameters. For *P. coffeae*, the tolerance limit (T) equalled zero for both the total weight and root weight; for *R. arabocoffeae* sp. n., T was equal to zero for the parameters total weight and shoot weight. The T-values for *R. duriophilus* were low for the total weight (0.8 nematodes/cm³ soil), root weight (0.5 nematodes/cm³ soil) and shoot weight (0.51 nematodes/cm³ soil). *Radopholus arabocoffeae* sp. n. caused the highest damage to coffee seedlings as demonstrated by the minimum yield for the parameters total weight (0.486), root weight (0.297) and shoot weight (0.289). The order of minimum yield for all of the parameters ranked: *P. coffeae* > *R. duriophilus* > *R. arabocoffeae* sp. n. Clearly, *C. arabica* cv. Catimor is very susceptible to all species examined, but especially to *R. arabocoffeae* sp. n.

On *C. arabica* cv. Catimor both *P. coffeae* and *R. duriophilus* increased in number only at the lowest initial density ($Pi = 1$ nematode/cm³ soil); at higher densities the reproduction factor dropped below zero and decreased further with increasing density (Table 5). *R. arabocoffeae* sp. n. increased in number up to an inoculum density of four nematodes per cm³ of soil; as for the other species increasing densities caused decreasing multiplication. This poor multiplication is caused by the detrimental effect of *Pratylenchus* and *Radopholus* species on the roots of the host plant. Species of these genera reduce the root mass, which eventually rots, rendering plants incapable of supporting large nematode populations (Loof, 1991). On coffee, *P. coffeae* causes lesions on the roots with consequent destruction of the whole root system (Monteiro & Lordello, 1974).

Acknowledgements

The senior author gratefully acknowledges the financial support by a VLIR scholarship for the Master of Science in Nematology programme at Ghent University, Belgium. This work was partly supported by the Vietnamese National Centre for Natural Sciences and Technology. The authors thank Mrs Rita Van Driessche, Department of Biology at Ghent University for her assistance in the SEM preparation and Mr Binh and Mr Hoa, from the Tay Nguyen Institute of Agriculture and Forests, for their assistance in sampling.

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