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Radopholus duriophilus sp. n. (Nematoda: Pratylenchidae) from Western Highland of Vietnam

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Summary – A new species of the genus *Radopholus* associated with durian (*Durio zibetinus* M.) in the Western Highland of Vietnam is described as *Radopholus duriophilus* sp. n. The new species is close to *R. similis*, but is distinguished from *R. similis* by the position of the excretory pore located posterior to pharyngo-intestine junction (*vs* at level of pharyngo-intestine junction), oval shape sperm (*vs* rod-like), four incisures terminating far behind position of phasmid (*vs* three incisures terminating at or just behind phasmid), and bursa in male never reaching tail terminus (*vs* bursa reaching tail terminus). Females of *R. duriophilus* sp. n. differ from *R. nativus* females by stylet length (16.5-19 *vs* 19-23 μm), oval or kidney-shaped sperm (*vs* rod-like), four incisures at level of phasmid (*vs* three) and their areolated lateral field (*vs* not areolated). The position of excretory pore of both female and male is located posterior to pharyngo-intestine junction (*vs* at level or anterior to pharyngo-intestine junction). Females of *R. duriophilus* sp. n. differ from *R. clarus* females by stylet length (16.5-19 *vs* 19-21 μm) and areolated lateral field (*vs* no areolation). Females of *R. duriophilus* sp. n. differ from *R. musicola* females by their lateral field with equidistant incisures at mid-body (*vs* two deep outer folds and two faint shallow inner incisures), oval or kidney-shaped sperm (*vs* rod-like), and rounded terminus tail (*vs* sharply pointed). The species also sp. n. differ in male stylet length (11.5-15 *vs* 8.8-12 μm). Females of *R. duriophilus* sp. n. differ from *R. bridgei* females by stylet length (16.5-19 *vs* 15-17.5 μm), median bulb length (11-16.5 *vs* 11-13 μm), length of hyaline tail (3-11 *vs* not more than 4 μm), and lateral field areolated for entire body (*vs* not areolated except irregularly on neck and tail). The male differs by stylet length (11.5-15 *vs* 10-12 μm) and length of the hyaline portion (4-9 *vs* 1-4 μm). In addition, the relatively high level of ITS sequence divergence of the new species from *R. similis* populations and the presence of nucleotide autapomorphies support a separate specific status for these durian populations. Results of surveys revealed that *R. duriophilus* sp. n. is rather widely distributed in durian orchards and associated with decline and death of trees in many durian nursery gardens. Densities of nematode population reached thousands of individuals per g of root samples.

Keywords – durian, ITS, molecular characterisation, phylogeny, rDNA, RFLP, taxonomy, Vietnam.

Ryss and Wouts (1997) classified 24 species within the genus *Radopholus* Thorne, 1949. However, Siddiqi (2000) accepted only 20 species, placing several others in other genera. Since then only *R. musicola* Stanton, Mundo-Ocampo, Baldwin & Kaplan, 2001 has been described in the genus. *Radopholus similis* (Cobb, 1893) Thorne, 1949 is considered to be the most economically important species world-wide. Recently, *R. nativus* Sher, 1968, *R. bridgei* Siddiqi & Hahn, 1995 and *R. musicola* Stanton, Mundo-Ocampo, Baldwin & Kaplan, 2001, were reported as potential pests of wheat in West Australia (Riley & Kelly, 2001), turmeric roots in Indonesia (Siddiqi & Hahn, 1995) and banana in North Australia (Stanton *et al.*, 2001), respectively. Although *R. similis* is prevalent in many tropical and subtropical regions throughout the world, it has never been recorded in Vietnam. Earlier attempts to recover *R. similis* from banana growing areas of Vietnam were unsuccessful (Eroshenko *et al.*, 1985; Nguyen *et al.*, 1997; Van den Bergh *et al.*, 2000). However, in some neighbouring countries (Thailand, Indone-

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sia, Malaysia and the Philippines), the species is common and causes damage to banana and black pepper (Holdeman, 1986; Razak, 1994).

In 1998-1999, many durian (*Durio zibetinus* M.) growers in Dak Lak province, Western Highland, Vietnam were confronted with tree decline and death of young trees. Frequently the problem was associated with imported planting material. Tree decline also appeared in some orchards replanted with the same breeding source. In 1999 the Plant Quarantine Office of the Ministry of Agriculture and Rural Development (MARD) detected high population densities of a nematode in the rhizosphere and roots of diseased durian, but none or very few in unaffected plants. These nematodes met the description of the genus *Radopholus* (Siddiqi, 2000). During 2000 and 2001, surveys were carried out in durian at numerous localities in Dak Lak province. The morphology, morphometrics and molecular data of the Vietnamese *Radopholus* populations revealed that these populations belong to a new species, which in this paper is described as *Radopholus duriophilus* sp. n.

Materials and methods

Nematode populations

Sixteen farms were surveyed. A total of 96 samples comprising soil and roots were collected. Nematodes were extracted from soil by decantation followed by centrifugal flotation and from roots by maceration and centrifugation (Coolen & D'Herde, 1972). Three populations of *Radopholus* (Buon Ma Thuot, Curgma and Krong Ana) were successfully maintained on carrot discs (O'Bannon & Taylor, 1968) and used for further observations. From two populations (Buon Ma Thuot and Krong Ana) about 15 to 20 nematodes (juveniles and adults) were transferred to 1 M NaCl for molecular observations. The remaining nematodes were heat killed and fixed in TAF (Seinhorst, 1959).

Morphological study

Fixed nematodes were processed and mounted in anhydrous glycerine using the slow method of Hooper and Evans (1993). From each population, morphometrics of 30 females and 30 males were taken using a camera lucida 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 on the body surface of the specimen. The nematodes were dehydrated by passing them through a gradual 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 observations

DNA extraction and amplification were made as described by Subbotin *et al.* (2000). Primers rDNA1 (5′-TTGATTACGTCCCTGCCCTTT-3′) and rDNA2 (5′-TTTCACTCGCCGTTACTAAGG-3′) (Vrain *et al.*, 1992) 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 product was purified using a Qiagen Gel Purification Kit (Qiagen GmbH, Hilden, Germany). PCR product was digested with one of eight restriction enzymes, *viz Alu*I, *Rsa*I, *Cfo*I, *Bsp*143I, *ScrF*I, *Bsh*1236I, *Tru*9I and *Taq*I. (Promega, Madison, WI, USA and MBI Fermentas, St Leon-Rol, Germany). DNA fragments were sequenced using primers rDNA1, rDNA2 and 5.8SM5 (5′-GGCGCAATGTGCATTCGA-3′) with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster city, CA, USA). The resulting products were purified using a Centriflex Gel Filtration Cartridge (Edge Biosystems Inc., Gaithersbugs, MD, USA). The DNA samples were sequenced by ABI Prism 377 DNA Sequencer. The DNA sequences of durian nematode populations were aligned using ClustalX 1.64 (default options) with six ITS-rDNA sequences of *R. similis* from GenBank. The original ITS sequence of *R. duriophilus* sp. n. is deposited at GenBank under accession numbers AY257199, AY257200.

Equally weighted maximum parsimony (MP) analysis was performed using PAUP* 4.0 beta version (Swofford, 1998). A heuristic search procedure was used with the following settings: ten replicates of random taxon addition, tree-bisection-reconnection branch swapping, multiple trees retained, no steepest descent, and accelerated transformation. Gaps were treated as missing data. Bootstrap analysis was calculated with 1000 replicates for MP tree. Pair wise divergences between taxa were computed by PAUP* as the absolute distance values and the percent mean distance values adjusted for missing data.

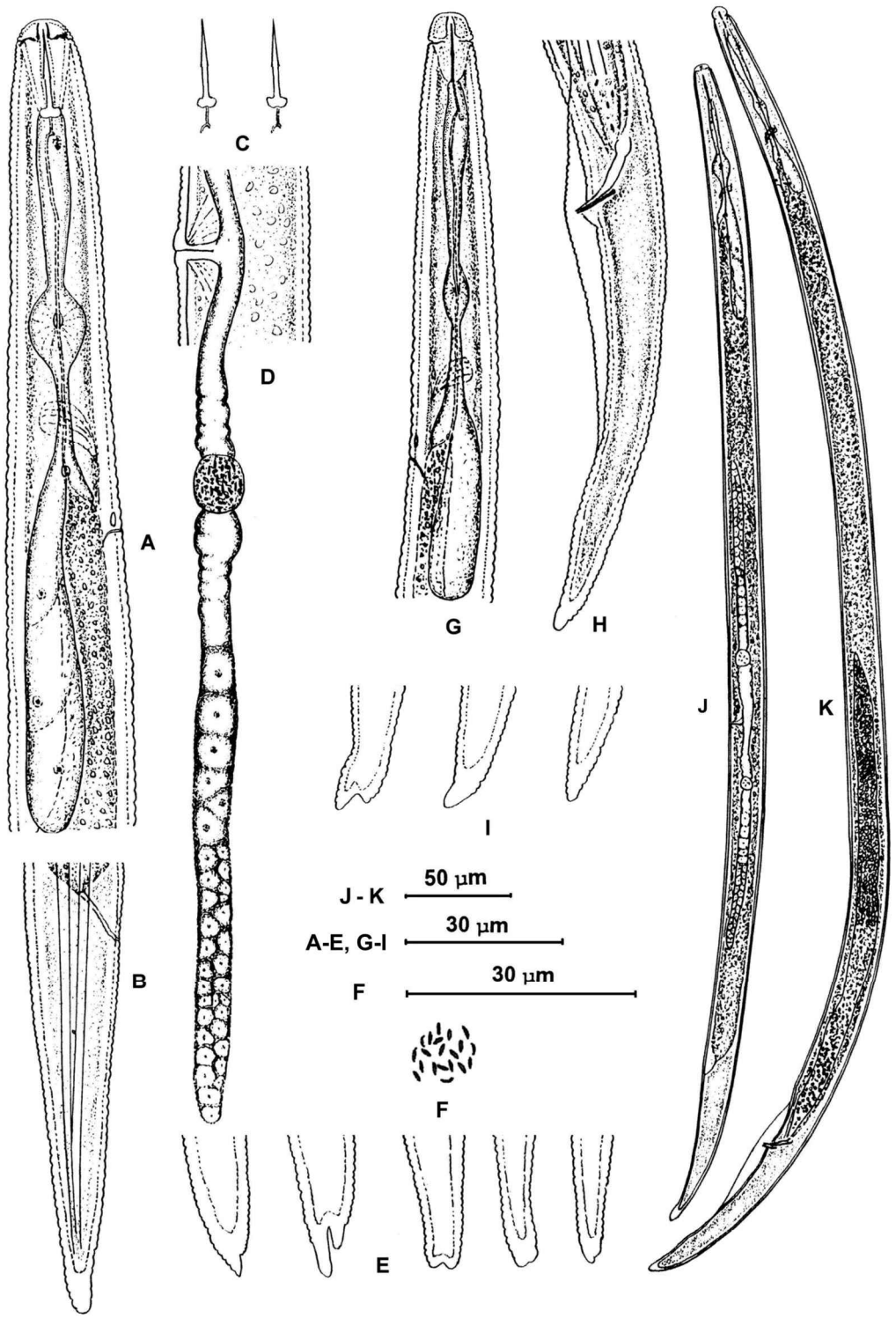

Fig. 1. Radopholus duriophilus *sp. n. Female, A-F. J: A: Anterior end including pharynx; B: Tail; C: Stylet; D: Vulva region and posterior branch of reproductive tract; E: Variation in tail tip morphology; J: Entire female body. Male, G-I. K: G: Anterior end including pharynx; H: Tail; I: Variation in tail tip morphology; K: Entire male body. F: Sperm.*

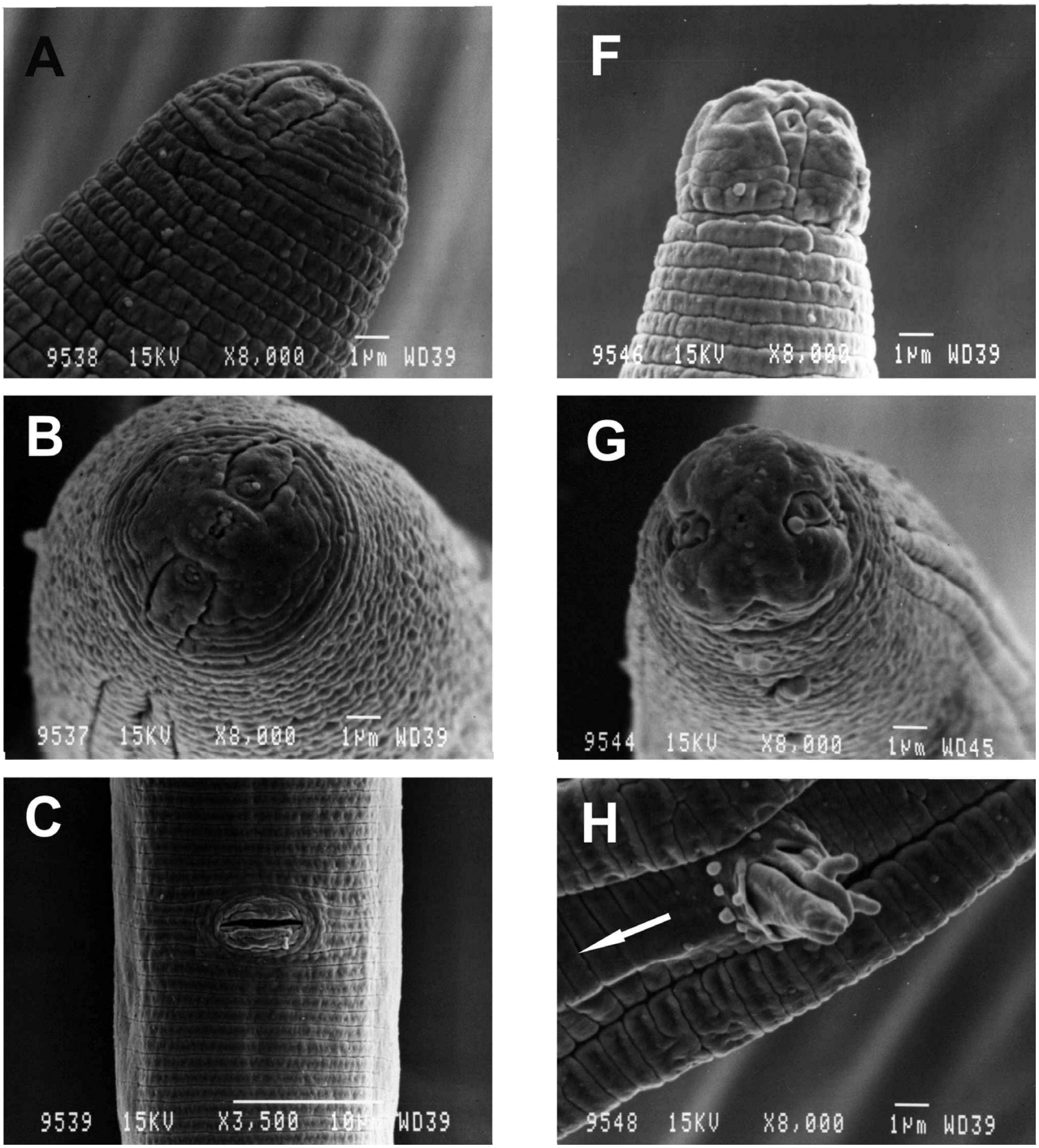

Fig. 2. *SEM photographs of* Radopholus duriophilus *sp. n. Female, A-E. A: Head lateral view; B: Head* en face *view; C: Vulva ventral view; D: Vulva lateral view and lateral field; E: Tail, phasmid and lateral incisures (arrow). Male, F-J. F: Head lateral view; G: Head* en face *view; H-I: ventral view of spicules (arrow indicates direction of head); J: Lateral view of tail (arrow indicates position of phasmid).*

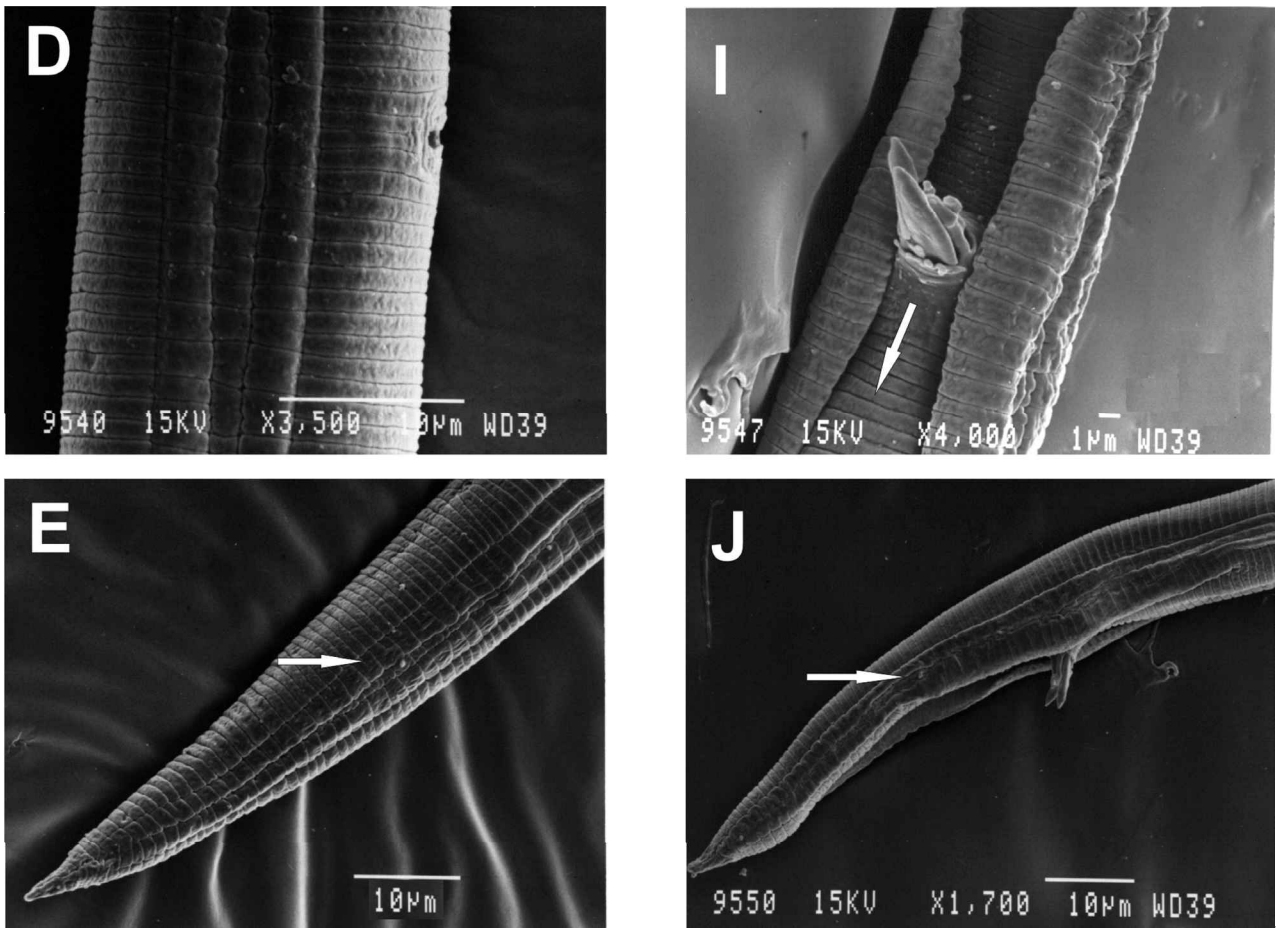

Fig. 2. *(Continued).*

Radopholus duriophilus **sp. n.**[*]
(Figs 1, 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body almost straight or slightly curved ventrally after killing by heat. Cephalic region slightly set off and more or less hemispherical with four or five annules. Labial disc not distinct in LM, hexagonal in SEM. Lateral lips terminating within second and third head annule. Stylet moderately strong with rounded knobs; dorsal knob sometimes projected. Cone slightly longer than shaft plus knobs. Rounded or oval median bulb well developed. Pharyngeal glands in tandem and forming a long, dorsally overlapping lobe. Excretory pore located posteriorly to level of pharyngo-intestinal junction at a distance of half to full body diameter and zero to two annules posterior to hemizonid, hemizonid one to two body annules wide. Lateral field completely areolated for entire body, with four equidistant incisures. Four incisures at level of phasmid. Three annules terminating at vulva. Two genital branches equally developed; spermathecae round to oval and of equal size, filled with small oval or kidney-shaped sperm. Oocytes located in one or two rows; genital branches sometimes reaching pharynx, one or both branches may be reflexed. Postrectal intestine sac absent. Tail conical, tapering and with shallow to deeply forked tip; terminus annulated, rarely smooth, narrow, conoid

[*] Specific name meaning 'who likes durian'.

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

	Buon Ma Thuot			Curgma		Krong Ana	
	Holotype	Paratypes					
	Female	Female	Male	Female	Male	Female	Male
n		30	30	30	30	30	30
L	622	603 ± 67 (500-720)	614 ± 34 (550-667)	588 ± 50 (490-765)	652 ± 54 (555-750)	669 ± 68 (552-840)	647 ± 30 (585-690)
Maximum body diam.	28	23 ± 2 (19-26)	18 ± 2 (16-22)	19 ± 2 (16-23)	18 ± 2 (14-22)	23 ± 4 (16-31)	18 ± 2 (14-22)
Height of lip region	4.5	4 ± 0.2 (3.5-4.2)	6 ± 0.5 (5-7)	4 ± 0.4 (3-5)	6 ± 0.5 (5-7)	4 ± 0.7 (3-6)	6 ± 0.6 (5-7)
Diam. of lip region	10	10 ± 0.6 (9-11)	8.5 ± 0.7 (7-10)	9.4 ± 0.6 (8-11)	8 ± 0.6 (7-9)	10.3 ± 0.8 (8-11.5)	8.2 ± 0.7 (6.5-10)
Length of stylet	18	17.7 ± 0.8 (16.5-19)	13.5 ± 0.9 (12-15)	17.4 ± 0.8 (16.5-19)	13 ± 0.7 (12-14)	18 ± 0.7 (17-19)	13.6 ± 1.1 (11.5-15)
Width of stylet base	3.5	3.7 ± 0.3 (3.5-4)		3.7 ± 0.3 (3-4)		4.2 ± 0.4 (3.5-5)	
DGO	4	3-5 (4.4 ± 0.8)	5-9.5 (7 ± 1.4)	3.5-5.5 (4.5 ± 0.6)	4-9 (6.7 ± 1.1)	3-5.5 (4.6 ± 0.4)	5-9 (6.9 ± 1)
Ant. end to centre of med. bulb	54	45-60 (52.2 ± 4.8)	41-57 (51.8 ± 3.7)	45-62 (53.2 ± 3.8)	46-62 (56 ± 2.9)	48-70 (58 ± 4.7)	42-62.5 (55.5 ± 4.8)
Length of median bulb	16	14 ± 1 (12-15)	11.7 ± 0.9 (9.5-13)	13 ± 1 (11-15)	11.5 ± 0.8 (10-13)	14 ± 1.2 (12-16.5)	11.5 ± 1.1 (10-14)
Diam. of median bulb	11	9.5 ± 1.1 (8-12)	5.5 ± 0.5 (5-6.5)	8.7 ± 0.9 (7-10.5)	5.6 ± 0.6 (4-7)	10 ± 1 (8-12)	5.6 ± 0.5 (5-6)
Length of pharynx	81	77 ± 7.8 (64-87)	76.5 ± 6.1 (64-85)	75.7 ± 4.5 (65-86)	80.5 ± 4.5 (72-89)	82 ± 6 (72-92)	79 ± 6.1 (65-91)
Length of gland lobe	85	78.5 ± 7.5 (67-89)	64.6 ± 6.4 (52-78)	82.7 ± 7.1 (67-95)	60 ± 6 (50-74)	81.4 ± 8.6 (57-98)	62.5 ± 6.1 (55-78)
Anterior end to excret. pore	86	87 ± 8 (73-102)	92 ± 6 (79-100)	85.6 ± 5.5 (74-97)	96.5 ± 5.3 (86-110)	91.5 ± 8.3 (79-110)	94.6 ± 6.6 (79-104)
Annule width at mid-body	1.2	1.4 ± 0.2 (1.1-1.8)	1.3 ± 0.1 (1-1.5)	1.3 ± 0.1 (1.1-1.7)	1.4 ± 0.1 (1.1-1.7)	1.5 ± 0.2 (1.2-2)	1.3 ± 0.1 (1.2-1.5)
Diam. of spermatheca	10	9.7 ± 1.7 (8-13)		8.3 ± 0.8 (7-10)		10 ± 1.3 (8-12.5)	
Tail length	73.5	73 ± 6.7 (60-85)	76.5 ± 5.2 (65-86)	69.5 ± 8 (48-94.5)	80.5 ± 7 (69-93)	77 ± 7.8 (64-92)	81.5 ± 5.3 (73-99)
Body diam. at anus	18	16.5 ± 1.9 (12.5-19)	14.2 ± 0.9 (12-16)	14.9 ± 1.7 (12-19)	14.5 ± 1.3 (12.5-19)	17.3 ± 3 (12.5-24)	14.5 ± 0.9 (12.5-16)
h	9.5	8.8 ± 0.9 (8-10.5)	6.1 ± 1.4 (4-8)	7.8 ± 1.6 (3-11)	5.9 ± 1.6 (4-9.5)	7.6 ± 1.2 (4.5-9)	6.5 ±1.2 (5-9)
Length of spicules			17.9 ± 0.8 (16.5-19)		17.5 ± 0.5 (17-18.5)		18.4 ± 0.6 (17-19)
Length of gubernaculum			9.3 ± 0.5 (8.5-10.5)		9.2 ± 0.6 (8-10)		9.7 ± 0.5 (8.5-11)
a	22.2	26.3 ± 3.4 (22.5-32.7)	34.3 ± 3.5 (26.5-40)	30.5 ± 2.9 (26-38)	37 ± 4 (29.5-44.5)	29.7 ± 3.8 (24-42)	36.7 ± 4.4 (27.5-45)
b	7.7	7.8 ± 0.5 (6.8-8.8)	5.2 ± 0.6 (4.5-7.8)	7.8 ± 0.5 (6.5-9)	6.1 ± 1.9 (4.8-11.5)	8 ± 0.5 (7-9)	6 ± 2 (5-12)
b′	4.2	4.3 ± 0.3 (4-5)	8 ± 0.8 (5-9)	4.1 ± 0.2 (3.7-4.8)	8 ± 0.7 (5-9)	4.5 ± 0.3 (4-5.5)	8.1 ± 0.8 (5-10)

Table 1. *(Continued).*

	Buon Ma Thuot			Curgma		Krong Ana	
	Holotype	Paratypes					
	Female	Female	Male	Female	Male	Female	Male
c	8.5	8.3 ± 0.6 (7.5-9.5)	8 ± 0.4 (7.5-9)	8.5 ± 1.1 (6-12)	8.1 ± 0.5 (7.5-9.5)	8.7 ± 0.5 (7.5-10)	8 ± 0.4 (7-9)
c′	4.1	4.5 ± 0.5 (3.9-5.6)	5.5 ± 0.5 (4.5-6)	4.7 ± 0.7 (3.3-6.8)	5.5 ± 0.6 (3.5-6.5)	4.7 ± 0.7 (3.3-6.8)	5.6 ± 0.4 (5-6.5)
V	57	56.1 ± 1.9 (52-59)		56.4 ± 2.3 (50.8-59.5)		56.5 ± 1.9 (53.8-60)	
Lip region diam./height	2.2	2.7 ± 0.7 (2.5-5)	1.4 ± 0.2 (1.2-2)	2.5 ± 0.3 (2-3.5)	1.4 ± 0.1 (1.2-1.6)	2.5 ± 0.4 (1.7-3)	1.4 ± 0.1 (1.1-1.7)
Median bulb length/diam.	1.5	1.5 ± 0.15 (1.2-1.8)	2.1 ± 0.2 (1.7-2.5)	1.5 ± 0.2 (1.1-1.9)	2 ± 0.3 (1.6-3.1)	1.4 ± 0.15 (1.2-1.8)	2 ± 0.2 (1.8-2.4)
Tail length/stylet length	4.1	4.2 ± 0.3 (3.5-4.5)	5.7 ± 0.5 (4.8-6.5)	4 ± 0.5 (2.8-5.5)	6 ± 0.5 (5-7.5)	4.2 ± 0.4 (4-5.1)	6 ± 0.7 (5-7.3)
Width hyaline part/length	0.6	0.6 ± 0.1 (0.5-0.9)	0.8 ± 0.2 (0.5-1.6)	0.7 ± 0.3 (0.5-2)	0.7 ± 0.2 (0.4-1.2)	0.8 ± 0.1 (0.6-1)	0.7 ± 0.1 (0.6-1)
Tail annules	39	43.7 ± 5.5 (36-53)	50 ± 4 (40-58)	40.3 ± 6 (26-53)	51.5 ± 5.7 (39-61)	40 ± 3.2 (35-47)	45.6 ± 4.5 (40-61)

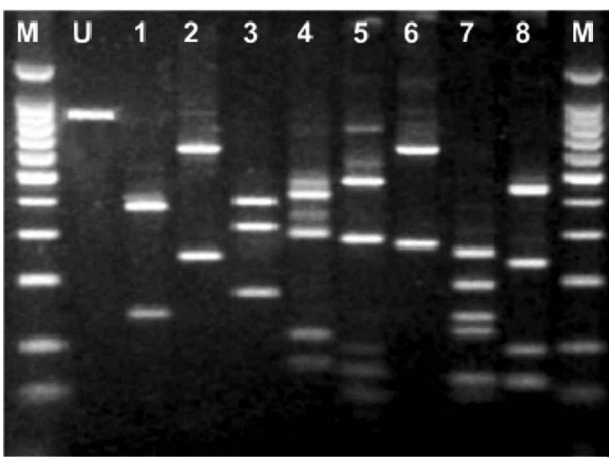

Fig. 3. *RFLP patterns of PCR products of ITS (internal transcribed spacer) of* Radopholus duriophilus *sp. n. M: 100 bp marker, U: unrestricted PCR product, 1:* Alu*I, 2:* Rsa*I, 3:* Cfo*I, 4:* Bsp143*I, 5:* ScrF*I, 6:* Bsh*1236I, 7:* Tru*9I,* Taq*I.*

rounded. Phasmids distinct, located in anterior third of tail, lateral lines fusing at two thirds of distance to tail tip.

Male

Slender, slightly ventrally curved. Cephalic region set off, knob like with three to four annules. Labial disc not distinct. Lateral lips terminating within third head annule. Stylet thin, rudimentary with amalgamated base. Median pharyngeal bulb oval and gland lobe poorly developed. Excretory pore at base of pharynx. Lateral field with four equidistant incisures at mid-body; central band of lateral field sometimes narrower than outer bands. Four incisures at level of phasmid. Oval or kidney-shaped sperm in genital tracts. Postrectal intestine sac absent. Bursa leptoderan, never reaching tail terminus. Spicule tylenchoid with asymmetrical, oval shaped head. Gubernaculum with head more or less prominent and pronounced pair of titillae. Five to eight hypotygma on anterior cloacal aperture. Tail shape conical, sometimes forked, terminus rounded and annulated, rarely narrow and smooth.

TYPE HOST AND LOCALITY

Found in association with durian (*Durio zibetinus* M.). Buon Ma Thuot City, Dak Lak province, Western Highland, Vietnam.

TYPE MATERIAL

One holotype, nine female paratypes, and six male paratypes deposited in the nematode collection of the Institute of Zoology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium; six female and two male paratypes and fixed material from cultures deposited in the

nematode collection of the Nematology Department, Institute of Ecology and Biological Resources, 18 Hoang Quoc Viet, Hanoi, Vietnam.

Diagnosis and relationships

Radopholus duriophilus sp. n. is characterised by the female cephalic region hemispherical with four to five annules, female stylet length of 16.5-19 μm, excretory pore located posterior to pharyngo-intestine junction in both sexes, oval or kidney-shaped sperm, completely areolated lateral field with four incisures terminating far behind position of phasmid, female tail conoid and tapering, often with irregularly forked terminus, male cephalic region knob-like with three to four annules, male stylet 12-15 μm long, male tail long and conoid, hyaline portion 4-8 μm long, bursa never reaching tail terminus.

Radopholus duriophilus sp. n. is morphologically close to *R. similis* but differs from this species by the following distinctive characters: excretory pore located posterior to pharyngo-intestine junction (*vs* at level of pharyngo-intestine junction), oval-shaped sperm (*vs* rod-like), four incisures terminating far behind position of phasmid (*vs* three incisures terminating at or just behind phasmid) and bursa in male never reaching tail terminus (*vs* bursa reaching tail terminus).

Radopholus duriophilus sp. n. is differentiated from *R. nativus* by a shorter female stylet length (16.5-19 *vs* 19-23 μm), oval or kidney-shaped sperm (*vs* rod-like sperm) and four incisures at level of female phasmid *(vs* three). It is further separated from *R. nativus* by the excretory pore of both males and females being located posterior to the pharyngo-intestine junction (*vs* at level of, or anterior to, pharyngo-intestine junction) and its areolated lateral field (*vs* not areolated).

From *R. clarus* Colbran, 1971, *R. duriophilus* sp. n. is differentiated by a shorter stylet length (16.5-19 *vs* 19-21 μm) and an areolated lateral field (*vs* no areolation). From *R. musicola*, *R. duriophilus* sp. n. differs by the female lateral field having four equidistant incisures at mid-body (*vs* two deep outer folds and two faint, shallow, inner incisures), oval or kidney-shaped sperm (*vs* rod-like), rounded tail terminus (*vs* sharply pointed) and by male stylet length (12-15 *vs* 8.8-12 μm).

From *R. bridgei*, *R. duriophilus* sp. n. differs by female stylet length (16.5-19 *vs* 15-17.5 μm), length of pharyngeal median bulb (11-16.5 *vs* 11-13 μm), hyaline tail length (3-11 *vs* not more than 4 μm), lateral field areolated over entire body (*vs* not areolated except irregularly on neck and tail), male stylet length (12-15 *vs* 10-12 μm) and length of hyaline tail (4-8 *vs* 1-4 μm).

Table 2. *Length (bp) of restriction fragments of the ITS of rDNA regions for* Radopholus duriophilus *sp. n. based on RFLP and sequence data.*

Enzyme	Restriction fragments
*Alu*I	377, 375, 144
*Rsa*I	625, 248, 23
*Cfo*I	393, 316, 176, 11
*Bsp143*I	415, 298, 111, 72
*ScrF*I	469, 288, 70, 43, 26
*Bsh*1236I	621, 275
*Tru*9I	258, 194, 143, 119, 67, 64, 51
*Taq*I	441, 233, 98, 65, 59

Molecular characterisation

PCR amplification of the ITS regions of two populations of *R. duriophilus* sp. n. yielded a single product with a length of 896 bp. The ITS sequences of these two samples differed in one nucleotide only. All studied enzymes cut the PCR products. The RFLP patterns are presented in Fig. 3. Heterogeneity of the ITS regions was revealed by *Bsp*143I. The exact lengths of restriction fragments calculated using sequence information are presented in Table 2. Comparison of RFLP profiles obtained in our study with those of *R. similis* published by Fallas *et al.* (1996) and Elbadri *et al.* (2002) revealed that *R. duriophilus* sp. n. is distinguished from *R. similis* by RFLP obtained after digestion with *Tru*9I.

The length of the entire ITS region for *Radopholus* species varied from 599-601 nucleotides. The length of the alignment for *Radopholus* sequences was 602 positions. Sequence divergence ranged from 0.3 to 6.2%. The sequences of *R. duriophilus* sp. n. differed from those of *R. similis* in 28 to 37 substitutions (4.7-6.2%), whereas sequences within *R. similis* differed in two to 23 substitutions (0.3-3.8%). MP analysis revealed 35 parsimony informative characters. The single unrooted maximum parsimonious tree is given in Fig. 4. The distribution of *R. similis* populations in two main branches on the tree is congruent with the one presented by Elbadri *et al.* (2002). The branch with *R. duriophilus* sp. n. was supported by 24 autapomorphies (unique substitutions).

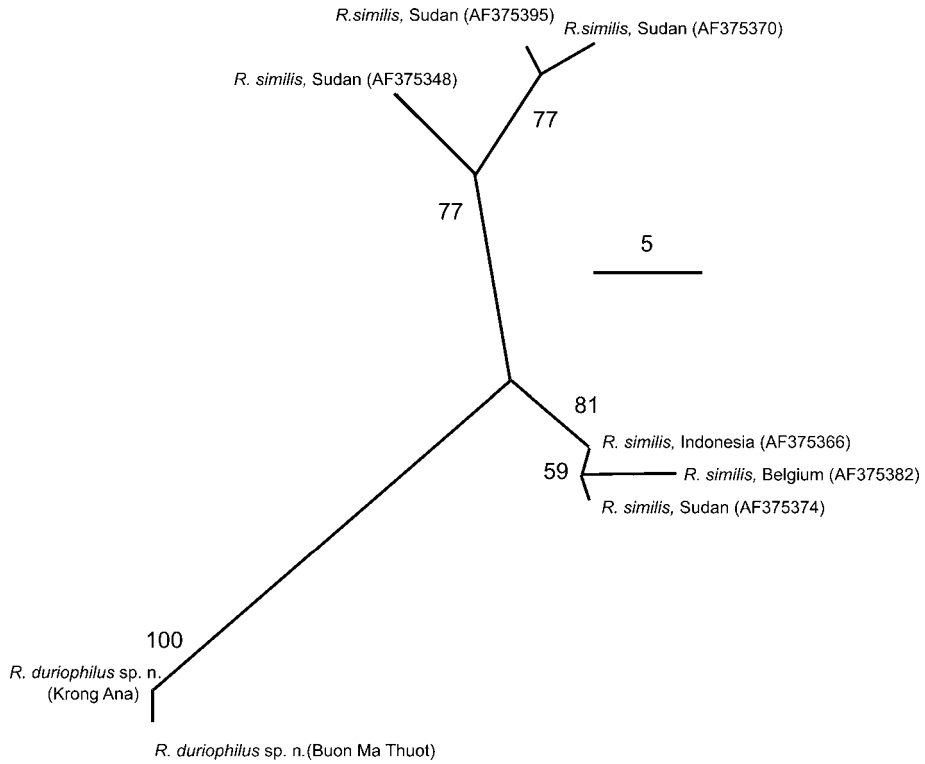

Fig. 4. *Single unrooted parsimony tree obtained from analysis of the alignment of eight* Radopholus *sequences. (Tree length = 56, CI = 0.9107, HI = 0.0893, RI = 0.9020, RC = 0.8214.) Bootstraps are given on appropriate clades.*

Distribution and economic importance

Out of the 96 durian trees sampled, 11 were found to be infested with *R. duriophilus* sp. n., population densities ranging from 35 to 215 individuals / 5 g root and 12 to 62 individuals / 250 ml soil. The orchards had been treated with carbofuran during the previous year. Before that treatment the nematode density had reached thousands of individuals per g of root, the average proportion of dead durian trees in nurseries and recently replanted fields being estimated at *ca* 20%. In some fields up to 40% of the trees had died. Infected and dead trees were not only observed in newly planted durian regions, but also in traditional durian regions. The problem, however, was less important in the latter.

Durian production is a speciality for Vietnam and some other countries in South East Asia. In Vietnam the area planted to durian is 2500-3000 ha in warmer regions with basalt soils. Durian production is mainly used for domestic consumption. Indigenous cultivars have low quality and productivity and to overcome this problem cultivars were recently imported from Thailand.

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