

***Cryphodera gayae* n. sp. (Nematoda: Heteroderidae), from root of *Lagerstroemia indica* in Korea**

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Abstract

A new non-cyst forming nematode, *Cryphodera gayae* n. sp., was found from the root of crapemyrtle (*Lagerstroemia indica*) in Korea. Female *C. gayae* n. sp. appears pearly white when young, has protruding vulval lips, flat to concave anus-vulva profile and has three lateral lines in J2s. Morphologically, *Cryphodera gayae* n. sp. is most similar to *C. brinkmani* but differ by the tail shape of J2 (sharply pointing with constriction at the middle of hyaline without mucro tip vs. sharply pointing with mucro-like tip), shorter stylet length of J2 (26.8–31.3 µm vs 31.6–35.4 µm), stylet knob shape of J2 (flat posteriorly vs concave), number of eggs in female (64–69 vs 15–45). *C. gayae* n. sp. differs from *C. kalesari* by the longer vulva-anus distance (51.9–54.1 µm vs 35 µm), the bigger body size of female (363.1–544.6 µm vs 272–353 µm in length), in J2, the longer tail length (59.6–76.7 µm vs 27–54 µm), longer hyaline length (31.7–47.8 µm vs 18–26 µm) and the shape of tail (sharply pointing with constriction at the middle of hyaline vs narrow rounded without constriction). From *C. sinensis*, the new species differs by the longer J2 tail length (59.6–76.7 µm vs 52.0–65.0 µm) and longer hyaline portion (31.7–47.8 µm vs 24.5–35.0 µm) of J2. *C. gayae* n. sp. and can be distinguished from the remaining four species, *C. eucalypti*, *C. podocarpi*, *C. nothophagi* and *C. coxi*, by the shape of the J2 tail terminus (sharply pointing tip with constriction at the middle of hyaline vs narrow rounded) and the shape of the vulval lips (pronounced protruding vs protruding slightly out of body contour). Molecular analysis shows that *C. gayae* n. sp. is the most similar to *C. sinensis* with the identity of sequence 95% on the rRNA LSU D2–D3 segments and the highest match with *C. brinkmani* with 88% identity on the ITS region.

Keywords

Crapemyrtle, *Cryphodera gayae* n. sp., *Lagerstroemia indica*, Morphology, Systematics, Taxonomy.

Cryphodera (Colbran, 1966) is a thick-cuticled non-cyst forming nematodes in the group of Heteroderinae. There are seven species of *Cryphodera*, all parasitic to various species of trees; they are *C. brinkmani* (Karssen and Van Aelst, 1999), *C. coxi*, *C. nothophagi*, *C. podocarpi* (Wouts, 1973), *C. eucalypti* (Colbran, 1966), *C. kalesari* (Bajaj et al., 1989) and *C. sinensis* (Zhuo et al., 2014). During

a survey of plant-parasitic nematodes in Korea, a new species of the non-cyst forming nematode was found from crapemyrtle (*Lagerstroemia indica*) root. Comparative morphological, morphometric and molecular studies of the nematode revealed that it differed from other *Cryphodera* species. The new species is described and illustrated herein as *Cryphodera gayae* n. sp.

Material and methods

Nematode isolation

Cryphodera gayae n. sp. was collected from crapemyrtle roots at Gaya (N35°42'45.2", E128°17'30.9), Gyeongsangbuk-do, Korea. Females were hand-picked from roots using forceps. Juveniles (J2) were separated from soil by the modified Baermann funnel method or released from crushed females (Southey, 1986).

Morphological study

Females and juveniles were killed and fixed by addition of 80°C FG 4:1 fixative (Southey, 1986). Nematodes were fixed for at least 24 hr and, then, processed according to the Seinhorst method (Cid Del Prado Vera and Subbotin, 2012). For light microscopic observations, specimens were mounted on Cobb slides and sealed with a paraffin ring and glycerin (Cobb, 1917). Nematodes were observed, measured and photographed with the aid of a compound microscope (BX53, Olympus) equipped with microscope digital camera (DP73, Olympus). For SEM study, nematodes were dehydrated through an ethanol series ranging from 20% through 100%, with specimens left at each step for about 1 day (Cid Del Prado Vera et al., 2012). Subsequently, the specimens were critical point-dried and mounted on studs in suitable position arrangements. Nematodes were coated with gold palladium (SC7620; Polaron) and observed under a scanning electron microscope (Jeol JSM-6390) at 10 kV.

Molecular study

For molecular analysis, DNA was extracted with DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). Two rRNA fragments, i.e. the LSU D2–D3 and ITS regions, were amplified. Primers for D2–D3 segments amplification were D2A (5'-ACAAGTACCGTGAGGG AAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCT ACTA-3'). Primers for ITS amplification were TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-AT ATGCTTAAGTTCAGCGGGT-3'). Detailed protocols of polymerase chain reaction (PCR) are as follows: after an initial 5-min denaturation step at 94°C, a 40-cycle amplification (94°C for 1 min, 56°C (D2–D3 segments) to 58°C (ITS region) for 1 min, and 72°C for 2 min) was conducted. The final extension step continued for 10 min at 72°C. In order to confirm the successful amplification of DNA by PCR, electrophoresis was performed using 0.5 × TAE buffer on 1% agarose gel. The PCR product was subsequently purified with a

PCR Purification Kit (Qiagen Inc.). The amplicons were cloned in pGEM-T Easy Vector System (Promega), and the resultant plasmid DNA was isolated with a Plasmid Midiprep System (Promega). Both strands of the PCR amplicons were cycle-sequenced with an ABI PRISM BigDye Terminator version 1.1 Cycle Sequencing Kit and electrophoresed in each direction on an ABI Prism ABI 377 Genetic Analyzer (PE Applied Biosystems). The newly obtained sequences were submitted to the GenBank database under accession numbers MN244153 and MN244164.

Phylogenetic study

For phylogenetic analyses, the sequences of *C. gayae* n. sp. were compared with GenBank nematode sequences using the BLASTn homology search program. The closest sequences were selected for phylogenetic analyses. Outgroup taxa for each data set were chosen according to previous molecular phylogenetic analyses of cyst and non-cyst forming nematodes (Zhuo et al., 2014; Van den Berg et al., 2016; Subbotin et al., 2017, 2018). The newly obtained and published sequences for each gene were aligned using ClustalW (Thompson et al., 1994) with default parameters. Sequence alignments were manually edited using BioEdit (Hall, 1999). The alignment quality was examined visually and optimized manually by adjusting the ambiguous nucleotide positions. Models of base substitution were evaluated using MODELTEST 3.7 (Huelsenbeck and Ronquist, 2001) combined with PAUP 4.0 (Swofford, 2003). The Akaike-supported model, the base frequency, the proportion of invariable sites and the gamma distribution shape parameters and substitution rates in the AIC were then used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.2.7 (Ronquist et al., 2012) running the chain for 2.0×10^6 generations and setting the “burn-in” at 2,500. The Markov Chain Monte Carlo method was used within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) and generated a 50% majority-rule consensus tree. Posterior probabilities are given on appropriate clades. Trees were visualized using TreeView (Page, 1996).

Results and discussion

Systematics

Cryphodera gayae n. sp.
(Figs. 1–5; Table 1).
Measurements Table 1.

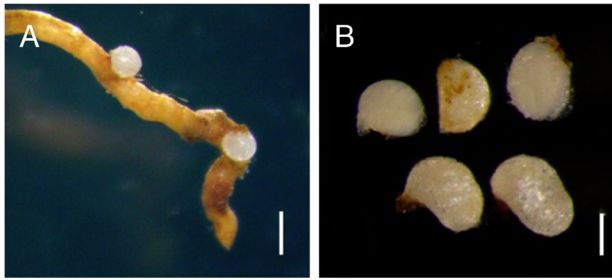


Figure 1: Stereo microscopic photos of *Cryphodera gayae* n. sp. A: females attached on crapemyrtle (*Lagerstroemia indica*) root; B: females (scale bars: A=500µm, B=200µm).

Description

Female

The body is globose, varies in size (513.4–778.3µm). Cuticle is annulated on entire body (Fig. 3A). Female is pearly white when young and becoming yellowish. Cuticle does not transform to a cyst and is partially covered by some tissue materials (Fig. 1B). Head region is not set off, labial disk is distinct and neck is distinct, usually bent laterally. Stylet is 30.1µm long. Median bulb is nearly rounded to oval, with distinct valve plates situated centrally (Fig. 3D). Except for the stylet and median bulb, most internal structures are difficult

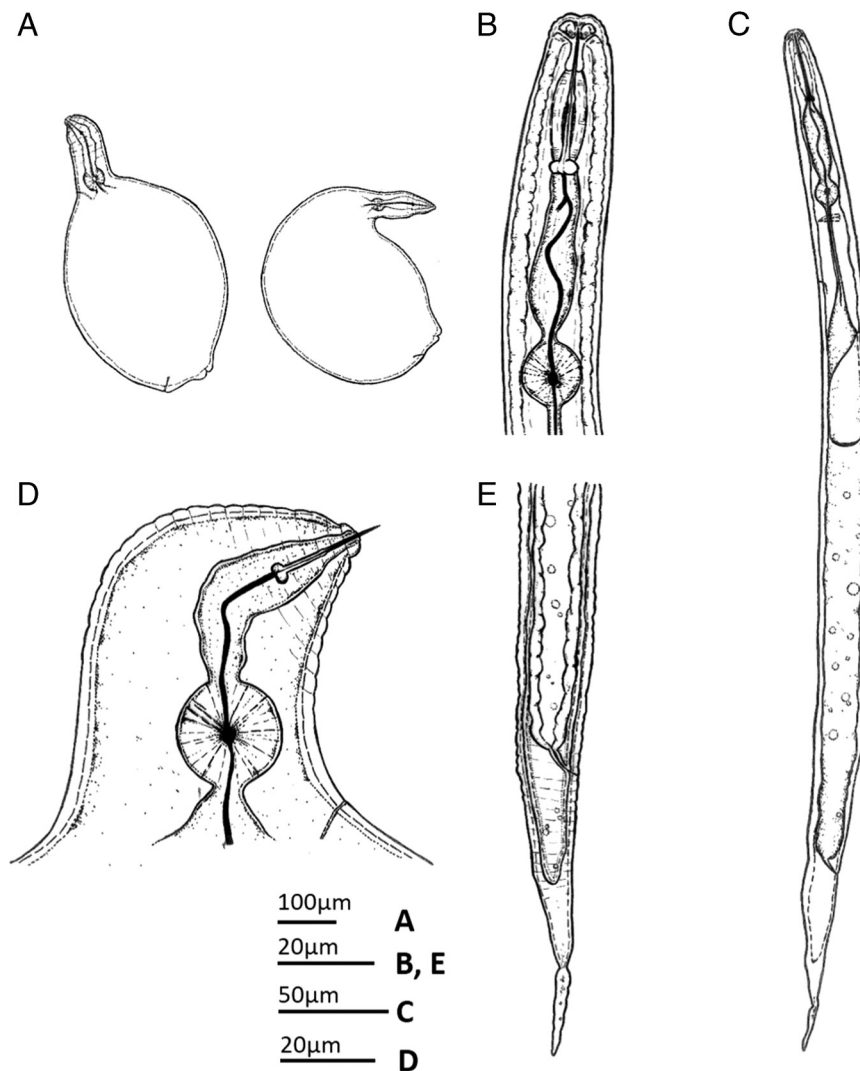


Figure 2: Drawings of *Cryphodera gayae* n. sp. A: female; B: anterior region of J2; C: entire of J2; D: anterior region of female; E: tail region of J2.

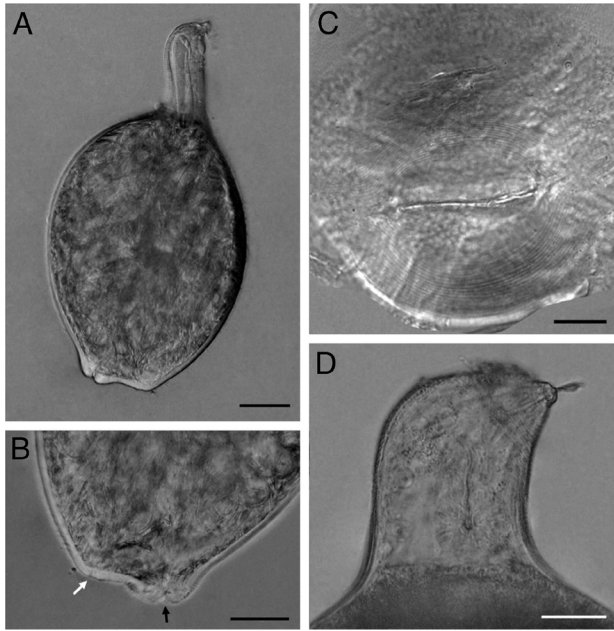


Figure 3: Light microscope photos of female of *Cryphodera gayae* n. sp. A: entire of body; B: vulva-anus region (white arrow = anus; black arrow = vulva); C: perineal pattern; E: anterior region (scale bars: A=50 μ m, B–D=20 μ m).

to observe in adult females. Excretory pore is located posterior to metacarpus. Vulva is located sub-terminal to terminal, near the anus. Vulval lips are pronounced with protrusion. Vulva-anus region is slightly concave to flat (Fig. 3B). Mature females contain less than 100 eggs in various stages of development. Perineal patterns are difficult to prepare flat due to a prominent protuberance. Perineal pattern is ovoid with fine, smooth and continuous striae. Vulva is surrounded by circles of striae (Fig. 3C).

Male

Not found.

Second-stage juvenile (J2)

The body is cylindrical, tapering posteriorly, straight or slightly ventrally curved after fixation (Fig. 4A). The lip region is offset, a labial disc with four annuli (Fig. 5A). Stylet is long, cone is straight, and shaft is cylindrical. Stylet knobs are well developed with

distinct flat or droop to posterior. Body annuli is 1.5–1.6 μ m in width at mid-body and three incisures are visible in lateral position (Fig. 4E). Tail is long conoid, gradually tapering to a sharply pointing terminus. Hyaline terminal section is 38.4 (31.7–47.8) μ m long, occupying 49.4 to 62.4% of tail length. Particularly, nearly 65% of J2 specimens of the present population exhibited a distinct constriction around middle of the hyaline region (Fig. 4C).

Diagnosis and relationships

Cryphodera gayae n. sp. has the general characteristics of the genus *Cryphodera*. Diagnostic characters of *C. gayae* n. sp. with other species are compiled (Tables 2 and 3). The new species having morphological characters of protruding vulval lips, flat to concave anus-vulva profile, three lateral lines in J2s, appears most similar to *C. brinkmani* but differs by the shorter stylet length of J2 (26.8–31.3 μ m vs 31.6–35.4 μ m), stylet knob shape of J2 (flat posteriorly vs concave), egg numbers in mature female (64–69 vs 15–45). Also, J2 of *C. brinkmani* exhibited a small mucro at the tip of the tail (Vovlas et al., 2013) but *C. gayae* n. sp. has sharply pointing tip with constriction at the middle of hyaline without mucro-like tip. *C. gayae* n. sp. differs from *C. kalesari* by the longer vulva-anus distance (51.9–54.1 μ m vs 35 μ m), the bigger body size of female (363.1–544.6 μ m vs 272–353 μ m in length), in J2, the longer tail length (59.6–76.7 μ m vs 27–54 μ m), longer hyaline length (31.7–47.8 μ m vs 18–26 μ m) and the shape of tail (sharply pointing tip with constriction at the middle of hyaline vs narrow rounded without constriction). From *C. sinensis*, the new species differs by the longer J2 tail length (59.6–76.7 μ m vs 52.0–65.0 μ m) and longer hyaline portion (31.7–47.8 μ m vs 24.5–35.0 μ m) of J2. *C. gayae* n. sp. can be distinguished from the remaining four species of *Cryphodera*, namely, *C. eucalypti* (Colbran, 1966), *C. podocarpi* (Wouts, 1973; Luc et al., 1978), *C. nothophagi* (Wouts, 1973; Luc et al., 1978) and *C. coxi* (Wouts, 1973; Luc et al., 1978) by the shape of the J2 tail terminus (sharply pointing tip with constriction at the middle of hyaline vs narrow rounded) and the shape of the vulval lips (pronounced protruding vs protruding slightly out of body contour).

Molecular profiles and phylogenetic status

The sequenced LSU D2–D3 segments and ITS region are 792 and 1,028 bp, respectively. A BLASTn

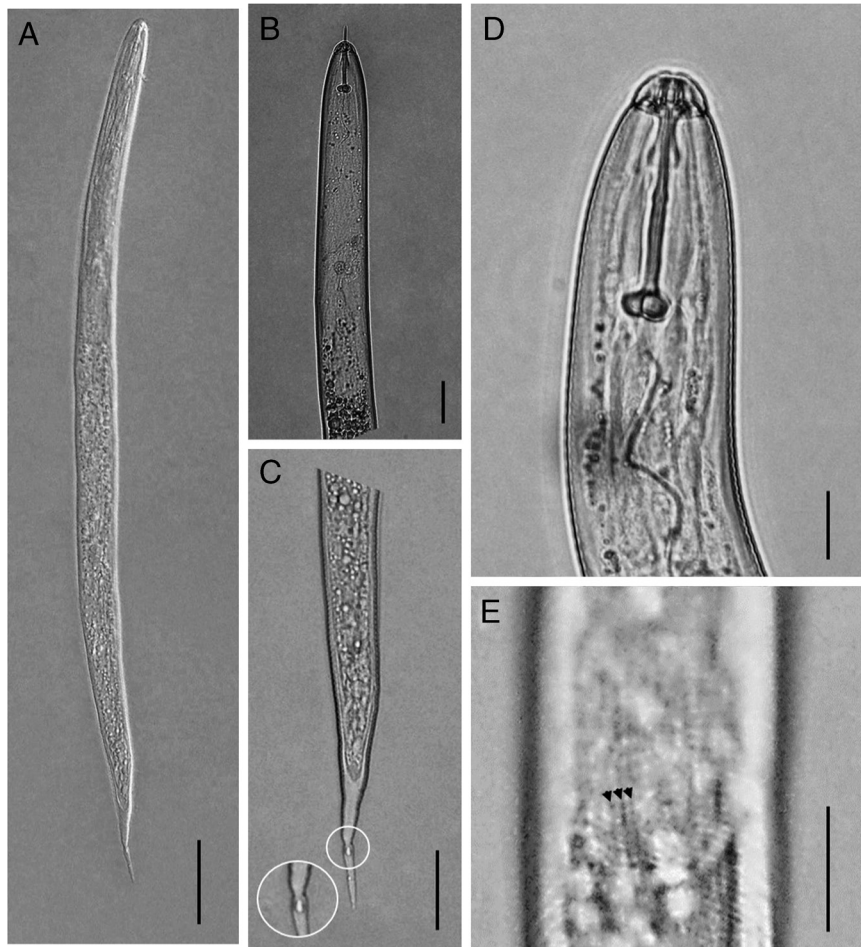


Figure 4: Light microscope photos of second-stage juvenile (J2) of *Cryphodera gayae* n. sp. A: entire body of second-stage juvenile (J2); B: anterior region of J2; C: tail region of J2. Note (*inset*) the middle of hyaline in tail with a constriction; D: head of J2; E: Lateral lines (arrowed) (scale bars: A=50 μ m, B=20 μ m, C=20 μ m, D=10 μ m, E=10 μ m).

search of *C. gayae* n. sp. on the LSU D2–D3 segments revealed high-scoring matches with some *Cryphodera* species, the most similar to *C. sinensis* (GenBank accession number JX566455), from

ramie in Hunan Province in China. The identity of sequence was 95% (750/789), with four insertions/deletions (0.1%). A BLASTn search of *C. gayae* n. sp. on the ITS region also revealed similarities with

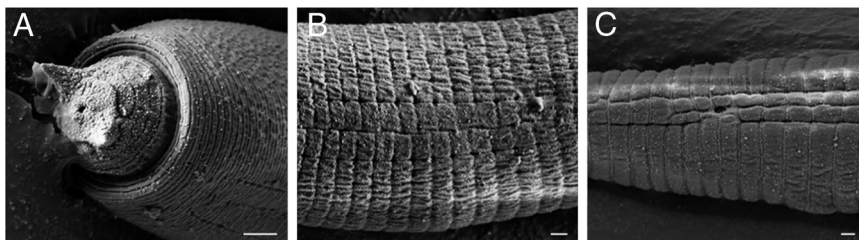


Figure 5: SEM photos of second-stage juvenile (J2) of *Cryphodera gayae* n. sp. A: cephalic region; B: lateral field; C: phasmid (scale bars: A=2 μ m, B, C=1 μ m).

Table 1. Morphometric measurements of *Cryphodera gayae* n. sp.

Stage	Character	Paratype		
		Holotype	Range (min.–max.)	Mean ± SD
Female				
	<i>n</i>		10	
	L (including neck)	462.7	363.1–544.6	471.5±53.8
	L (excluding neck)	380.3	430.6–255.5	367.8±51.4
	W (width)	286.5	166.6–329.1	259.3±46.2
	Neck	82.5	77.2–164.8	103.6±26.9
	L (inc. neck)/W ratio	1.6	1.6–2.2	1.8±0.2
	Stylet length	30.7	28.2–31.9	30.1±1.0
	Anterior end to median bulb	65.0	53.9–93.7	70.3±14.7
	Length of medium bulb	28.3	22.6–31.9	25.9±2.9
	Width of medium bulb	25.2	20.6–29.9	24.3±3.2
	Vulva-nus distance	46.9	35.8–51.6	44.7±4.3
	Vulval slit length	–	51.9–54.1	52.6±1.3
	Excretory pore from anterior end	80.1	72.6–124.5	92.4±17.5
Second stage (J2)				
	<i>n</i>		20	
	L (body length)	469.1	424.2–525.9	482.8±28.5
	<i>a</i> (L/body diam.)	22.7	18.5–22.7	20.1±1.4
	<i>b</i> (L/esophago-intestinal valve)	3.9	3.4–4.4	3.9±0.3
	<i>b</i> ′ (L/end of esophageal)	2.7	2.2–3.0	2.7±0.2
	<i>c</i> (L/tail length)	7.2	6.2–7.4	7.0±0.3
	<i>c</i> ′ (tail length/tail diam.)	4.4	3.3–5.0	4.2±0.4
	Stylet length	29.8	26.8–31.3	29.3±1.2
	Tail length	64.8	59.6–76.7	69.4±4.4
	Max. body diam.	20.6	20.3–28.3	24.1±2.5
	Hyaline length	39.0	31.7–47.8	38.4±3.9
	Excretory pore from anterior end	111.93	101.43–132.66	118.6±8.9
Egg				
	<i>n</i>		10	
	L		92.8–144.2	114.1±15.1
	W		31.4–39.7	36.1±2.8
	L/W ratio		2.9–3.9	3.2±0.3

All measurements are in μ m.

some *Cryphodera* species, but the identities were about 85%. The highest match was *C. brinkmaini* (JQ965678), with only 88% identity (618/703) and 23

insertions/deletions (3%). Compared with *C. sinensis* (JX566457), *C. gayae* n. sp. has 83% identity (892/1,075) and 85 insertions/deletions (7%).

Table 2. Comparative morphometrics (in μm) of female in species of *Cryphodera* with ranges in brackets.

	<i>C. gayae</i> <i>n. sp.</i>	<i>C. sinensis</i>	<i>C. eucalypti</i>	<i>C. kalesari</i>	<i>C. brinkmani</i>	<i>C. podocarp</i> <i>i</i>	<i>C. nothophagi</i>	<i>C. coxi</i>
	Present study	Zhuo et al. (2014)	Colbran (1966)	Wouts (1973)	Bajaj et al. (1989)	Karssen and Van Aelst (1999)	Wouts (1973)	Wouts (1973)
Body length (L)	471.5 $\pm$ 53.8 (363.1–544.6)	460.9 $\pm$ 53.1 (362.9–526.8)	400–636	353 (286–428)	311 $\pm$ 21 (272–353)	450 $\pm$ 52 (384–544)	341 (261–423)	331 (218–455)
Body diameter	259.3 $\pm$ 46.2 (166.6–329.1)	240.1 $\pm$ 51.6 (172.4–314.0)	201–274	210 (147–290)	205.5 $\pm$ 51 (112–288)	238 $\pm$ 35 (160–339)	226 (148–305)	184 (116–369)
Number of lip annulus	–	–	–	1	2–3	–	2 more or less	2 more or less
Vulval slit length	52.6 $\pm$ 1.3 (51.9–54.1)	51.3 $\pm$ 6.8 (42.8–58.3)	45 (44–49)	–	35	51 $\pm$ 3.8 (44–60)	–	–
Anus-vulva profile	Flat to concave	Distinctly concave	Flat to concave	Concave	Distinctly concave	Flat to concave	Flat	Flat

Data referred to Colbran (1966), Wouts (1973), Bajaj et al. (1989), Karssen and Van Aelst (1999), Siddiqi (2000), Sturhan (2010), Zhuo et al. (2014). –, not available.

Table 3. Comparative morphometrics (in μm) of second-stage juveniles (J2) in species of *Cryphodera* with ranges in brackets.

	<i>C. gayae</i> n. sp.	<i>C. sinensis</i>	<i>C. eucalypti</i>	<i>C. kalesari</i>	<i>C. brinkmani</i>	<i>C. podocarpi</i>	<i>C. nothophagi</i>	<i>C. coxi</i>
		Zhuo et al. (2014)	Colbran (1966)	Wouts (1973)	Bajaj et al. (1989)	Karsen and Van Aelst (1999)	Wouts (1973)	Wouts (1973)
Body length (L)	482.8 $\pm$ 28.5 (424.2–525.9)	435.2 $\pm$ 25.4 (388.8–474.5)	379–461	428 $\pm$ 6	383 $\pm$ 19 (353–424)	393 $\pm$ 23 (450–541)	450 $\pm$ 4	457 $\pm$ 4
<i>a</i> = L/max. width	20.1 $\pm$ 1.4 (18.5–22.7)	22.6 $\pm$ 1.5 (19.4–25.9)	22.0–26.0	24.8 $\pm$ 0.6	22 $\pm$ 1.5 (18–26)	23.2 $\pm$ 1.3 (20.3–24.6)	24.5 $\pm$ 0.3	25.3 $\pm$ 0.4
<i>b</i> = L/esophageal length	3.9 $\pm$ 0.3 (3.4–4.4)	3.9 $\pm$ 0.2 (3.7–4.4)	3.5–4.8	3.7 $\pm$ 0.06	3.84 $\pm$ 0.38 (3.2–5.0)	–	3.7 $\pm$ 0.05	3.7 $\pm$ 0.04
<i>c</i> = L/tail length	7.0 $\pm$ 0.3 (6.2–7.4)	7.8 $\pm$ 0.5 (6.4–8.6)	7.9–9.8	8.3 $\pm$ 0.15	8.4 $\pm$ 1.2 (7–13)	7.1 $\pm$ 0.4 (6.4–7.8)	8.2 $\pm$ 0.08	8.5 $\pm$ 0.08
Number of lip annuli	4	3	3	3	3	4	4	4
Stylet length	29.3 $\pm$ 1.2 (26.8–31.3)	29.1 $\pm$ 0.8 (28.0–31.0)	– ^a	32.9 $\pm$ 0.3 (31–35)	26.5 $\pm$ 1 (24–29)	33.3 $\pm$ 1.0 (31.6–35.4)	33.9 $\pm$ 0.2	34.4 $\pm$ 0.2
Stylet knobs	Flat posteriorly	Project distinctly anteriorly	Concave	Flat or pointing slightly anteriorly	Flat to concave	Concave	Pointing distinctly anteriorly	Pointing slightly anteriorly
DGO	4.6 $\pm$ 0.9 (3.3–6.1)	5.4 $\pm$ 0.7 (4.5–6.5)	4.3–5.0	5.5 $\pm$ 0.17	3–4	5.8 $\pm$ 0.6 (4.4–7.0)	6.0 $\pm$ 0.26	6.1 $\pm$ 0.28
Number of lateral line	3	3	3	3	3	3	3 (occasionally 4) ^b	3

Tail length	69.4 ± 4.4 (59.6–76.7)	56.0 ± 3.2 (52.0–65.0)	–	46.5 ± 6 (27–54)	46.5 ± 6 (27–54)	69.7 ± 4.4 (61.3–76.5)	68.9 ± 0.1	55.3 ± 0.8	53.7 ± 0.8
Hyaline portion of tail	38.4 ± 3.9 (31.7–47.8)	28.3 ± 2.3 (24.5–35.0)	–	21.5 ± 2.5 (18–26)	21.5 ± 2.5 (18–26)	37.2 ± 3.6 (30.3–42.3)	36.0 ± 0.1	30.1 ± 0.7	30.6 ± 0.5
Tail terminus	Sharp point with constriction at the middle of hyaline	Point with mucro-like tip	Buntly rounded	Narrow rounded	Narrow rounded	Point with mucro-like tip	Narrow rounded	Narrow rounded	Narrow rounded

DGO, distance of dorsal esophageal gland orifice to base of stylet; Data referred to Colbran (1966), Wouts (1973), Bajaj et al. (1989), Karsen and Van Aelst (1999), Siddiqi (2000), Sturhan (2010), Zhuo et al. (2014). –, not available; ^aColbran (1966) reported stylet length for J2 of *C. eucalypti* was 26–35.9 mm. Wouts (1973) mentioned that the minimum 26 mm belonged to J2 of *Heterodera*. Bajaj et al. (1989) and Karsen and Van Aelst (1999) accepted the point of Wouts (1973). Thus, the value of *C. eucalypti* described by Colbran (1966) is omitted here; ^bWouts (1973) described four incisures in the field of *C. nothophagi* J2. Sturhan (2010) re-examined paratype J2 of *C. nothophagi*, which revealed three incisures in the field, with the inner incisure occasionally diverging into two. Thus, the point of Sturhan (2010) is accepted here.

The molecular phylogenetic relationships of the new species are shown in Figures 6 and 7. Figure 6 represents a phylogenetic tree based on LSU D2–D3 segments. The average nucleotide composition is as follows: 22.34% A, 22.47% C, 29.58% G and 25.61% T. Using *Scutellonema brachyurus* as the outgroup taxa, the molecular phylogeny strongly supports monophyly of *Cryphodera* (100% posterior probability). Figure 7 represents a phylogenetic tree based on ITS region. The average nucleotide composition is as follows: 21.11% A, 22.91% C, 27.17% G and 28.81% T. Using *Hoplolaimus columbus* as outgroup taxa, all *Cryphodera* sequences reside within a well-supported monophyletic clade with 100% support. The sequence of *C. gayae* n. sp. clade is in separate position to *C. sinensis* founded from ramie (Zhuo et al., 2014).

Type locality and habitat

Cryphodera gayae n. sp. was collected from roots of crapemyrtle (*Lagerstroemia indica* L.), Gaya district (N35°42'45.2", E128°17'30.9), Gyeongsangbuk-do, Korea in 2019. The soil type is sandy loam, and the local climate is marine west coast of Köppen climate classification.

Type specimens

Holotype (female and J2): isolated from roots from type locality and habitat. Slides T-721t and T-722t deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland. Paratype (female and J2): same data and repository as holotype. Slides T-7228p and T-7229p.

Etymology

The specific epithet refers to the Gaya dynasty before 400AD in ancient history of Korea. The species habitat is the geographical capital region of Gaya. Gaya was a confederacy of territorial polities nobility in the Nakdong river basin of southern Korea.

Key to the species of *Cryphodera*, based on second-stage juveniles:

1. — Three lip annules (lateral)..... 2
 - Four to five lip annules (lateral)..... 4
2. — Stylet ≤ 29 μm, knobs anteriorly flat
 - *C. kalesari*
 - Stylet > 29 μm..... 3
3. — Tail of J2 with narrow rounded
 - *C. eucalypti*

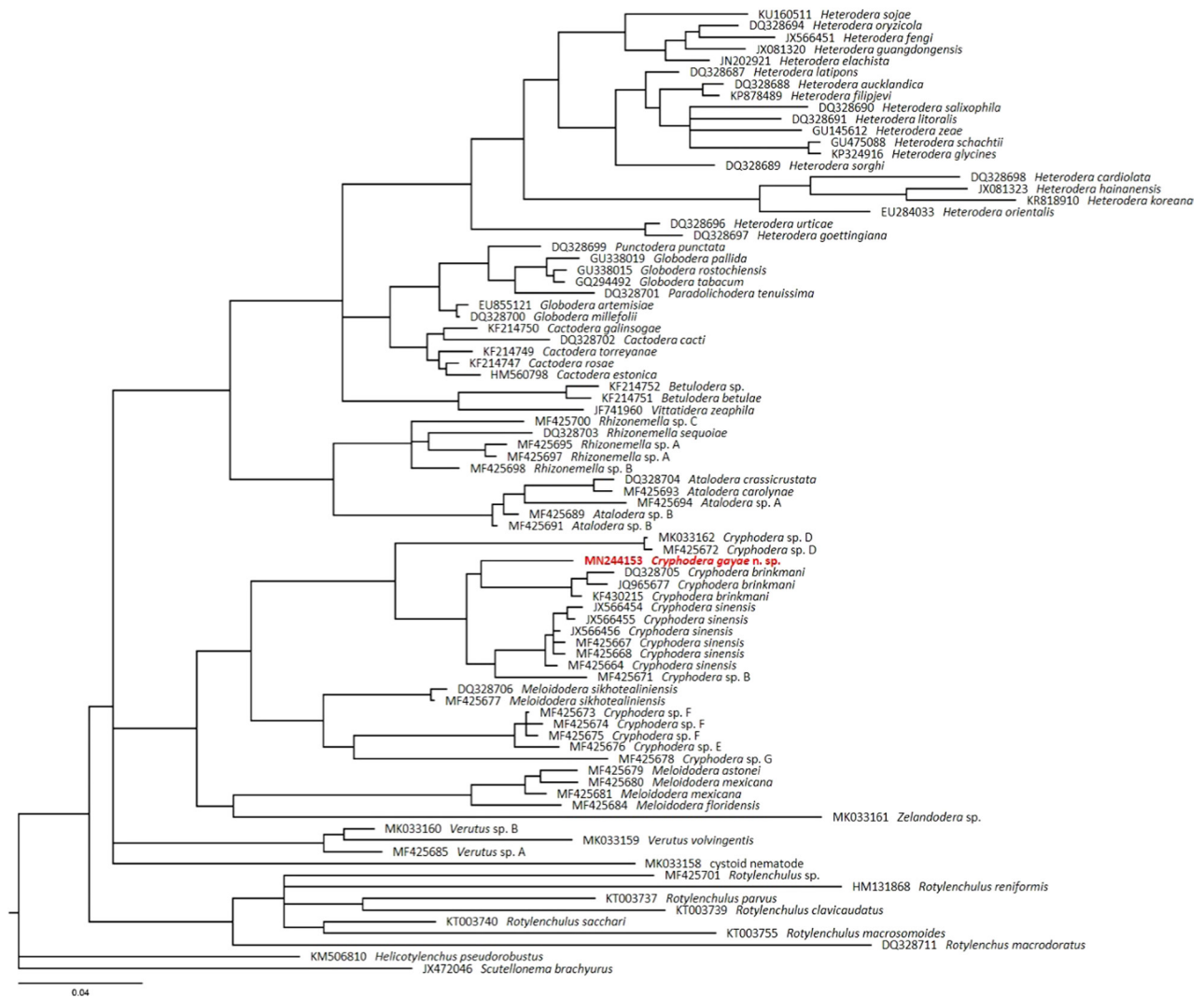


Figure 6: Phylogenetic relationships within population and species of *Heteroderidae*. Bayesian 50% majority-rule consensus tree from two runs as inferred from the analysis of the D2–D3 of 28 S rDNA gene sequences under the GTR+I+G model (1nL=15776.80; AIC=31899.59; freqA=0.2234; freqC=0.2247; freqG=0.2958; freqT=0.2561; R(a)=0.3202; R(b)=2.082; R(c)=1.008; R(d)=0.2953; R(e)=4.3966; R(f)=1; Pinva=0.3467; Shape=0.7997). Posterior probability values more than 70% are given in appropriate clades. Newly sequenced sample is indicated by red color and bold font.

- Tail of J2 with point, mucro-like tip *C. sinensis*
- 4. — Four lip annules 5
- Five lip annules *C. podocarp*
- 5. — Three lateral lines near mid-body 6
- Four lateral lines near mid-body *C. nothophagi*
- 6. — Tail length < 60 μm, tail tip narrow rounded *C. coxi*
- Tail length ≥ 60 μm, tail tip pointed 7

- 7. — Tail of J2 with mucro-like tip; stylet = 33.3 μm, DGO=5.8 *C. brinkmani*
- Tail of J2 with constriction at the middle of hyaline; style L=29.8, DGO=4.6 *C. gayae* n. sp.

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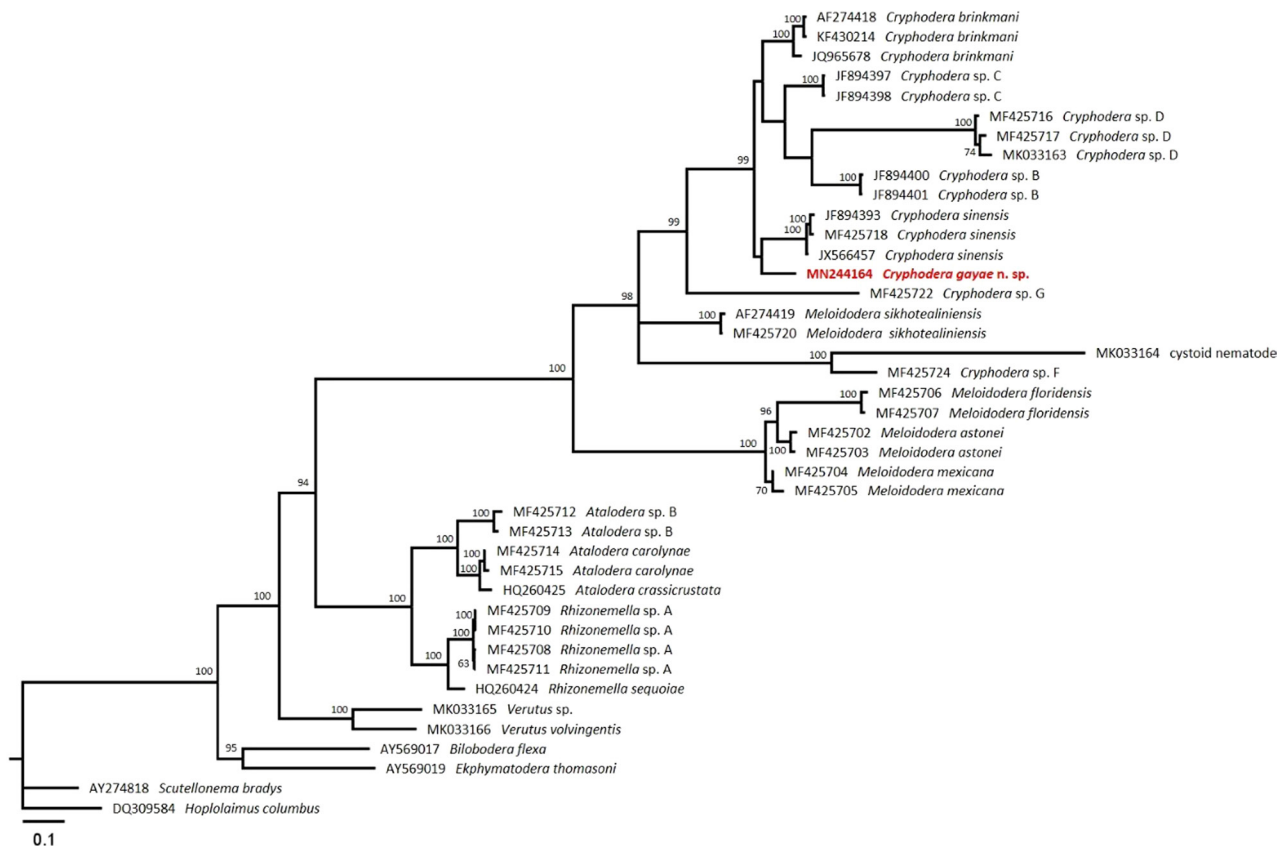


Figure 7: Phylogenetic relationships within population and species of *Heteroderidae*. Bayesian 50% majority-rule consensus tree from two runs as inferred from the analysis of the ITS region gene sequences under the GTR+I+G model (1nL = 18933.04; AIC = 38056.30; freqA = 0.2111; freqC = 0.2291; freqG = 0.2717; freqT = 0.2881; R(a) = 1.1195; R(b) = 3.0260; R(c) = 1.5414; R(d) = 0.6272; R(e) = 3.0260; R(f) = 1; Pinva = 0.1554; Shape = 2.1166). Posterior probability values more than 70% are given in appropriate clades. Newly sequenced sample is indicated by red color and bold font.

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References

- Bajaj, H. K., Walia, R. K., Dabur, K. R. and Bhatii, D. S. 1989. *Cryphodera kalesari*, a new heteroderid nematode species from Haryana, India. *Systematic Parasitology* 14:113–6.
- Cid Del Prado Vera, I. and Subbotin, S. A. 2012. *Belonolaimus maluceroi* sp. n. (Tylenchida: Belonolaimidae) from a tropical forest in Mexico and key to the species of *Belonolaimus*. *Nematropica* 42:201–10.
- Cid Del Prado Vera, I., Ferris, H., Nadler, S. A. and Lamothe Argumedo, R. 2012. Four new species of *Tripylina* Brzeski, 1963 (Enoplida: Tripylidae) from Mexico, with an emended diagnosis of the genus. *Journal of Nematode Morphology and Systematics* 15:71–86.
- Cobb, N. A. 1917. Notes on nemas. *Contribution to a Science Nematology* 5:117–28.
- Colbran, R. C. 1966. Studies on plant and soil nematodes. 12. The eucalypt cystoid nematode *Cryphodera eucalypti* n.g., n. sp. (Nematoda: Heteroderidae), a parasite of eucalypts in Queensland. *Queensland Journal of Agricultural and Animal Sciences* 23:41–7.
- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. *Nucleic Acids Symposium Serial* 41:95–8.
- Huelsenbeck, J. P. and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–5.

- Karssen, G. and Van Aelst, A. 1999. Description of *Cryphodera brinkmani* n. sp. (Nematoda: Heteroderidae), a parasite of *Pinus thunbergii* Parlatores from Japan, including a key to the species of the genus *Cryphodera* Colbran, 1966. *Nematology* 1:121–30.
- Larget, B. and Simon, D. L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16:750–9.
- Luc, M., Taylor, D. P. and Cadet, P. 1978. Description of a new tropical Heteroderidae, *Hylonema invorense* n. gen., n. sp., and a new outlook on the family Heteroderidae (Nematoda: Tylenchida). *Revue de Nématologie* 1:73–86.
- Page, R. D. 1996. TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Bioscience* 12:357–8.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. and Huelsenbeck, J. P. 2012. MRBAYES 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61:539–42.
- Siddiqi, M. R. 2000. Tylenchida, parasites of plants and insects, CABI, London, p. 785.
- Southey, J. F. 1986. Laboratory methods for work with plant and soil nematodes. Her Majesty's Stationery Office, London.
- Sturhan, D. 2010. Notes on morphological characteristics of 25 cyst nematodes and related Heteroderidae. *Russian Journal of Nematology* 18:1–8.
- Subbotin, S. A., Akanwari, J., Nguyen, C. N., Cid Del Prado Vera, I., Chitambar, J. J., Inserra, R. N. and Chizhov, V. N. 2017. Molecular characterisation and phylogenetic relationships of cystoid nematodes of the family Heteroderidae (Nematoda: Tylenchida). *Nematology* 19:1065–81.
- Subbotin, S. A., Vau, S. and Inserra, R. N. 2018. Molecular characterisation and phylogenetic relationships of *Verutus volvingentis* Esser, 1981 with other cystoid nematodes of the family Heteroderidae (Nematoda: Tylenchida), and some morphological details of its immature life stages. *Russian Journal of Nematology* 26:145–57.
- Swofford, D. L. 2003. PAUP: phylogenetic analysis using parsimony (and other methods). Version 4. Sunderland, MA.
- Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–80.
- Van den Berg, E., Palomares-Rius, J. E., Vovlas, N., Tiedt, L. R., Castillo, P. and Subbotin, S. A. 2016. Morphological and molecular characterisation of one new and several known species of the reniform nematode, *Rotylenchulus* Linford & Oliveira, 1940 (Hoplolaimidae: Rotylenchulinae), and a phylogeny of the genus. *Nematology* 18:67–107.
- Vovlas, N., Trisciuzzi, N., Troccoli, A., Luca, F. D., Cantalapiedra-Navarrete, C. and Castillo, P. 2013. Integrative diagnosis and parasitic habits of *Cryphodera brinkmani* a non-cyst forming heteroderid nematode intercepted on Japanese white pine bonsai trees imported into Italy. *European Journal of Plant Pathology* 135:717–26.
- Wouts, M. W. 1973. A revision of the family Heteroderidae (Nematoda: Tylenchoidea). I. The family Heteroderidae and its subfamilies. *Nematologica* 18:439–46.
- Zhuo, K., Wang, H. H. and Peng, D. L. 2014. *Cryphodera sinensis* n. sp. (Nematoda: Heteroderidae), a non-cyst-forming parasitic nematode from the root of ramie *Boehmeria nivea* in China. *Journal of Helminthology* 88:468–80.