New Cyst Nematode, Heterodera sojae n. sp. (Nematoda: Heteroderidae) from Soybean in Korea

Article *in* Journal of Nematology · December 2016

DOI: 10.21307/jofnem-2017-036

7 authors, including:

Heonil Kang
RURAL DEVELOPMENT ADMINISTRATION
22 PUBLICATIONS **26** CITATIONS
SEE PROFILE

Jihye Ha
Pusan National University
7 PUBLICATIONS **17** CITATIONS
SEE PROFILE

Yang Kim
1,168 PUBLICATIONS **15,951** CITATIONS
SEE PROFILE

Donggeun Kim
Pusan National University
23 PUBLICATIONS **43** CITATIONS
SEE PROFILE

Journal of Nematology 48(4):280–289. 2016.

New Cyst Nematode, *Heterodera sojae* n. sp. (Nematoda: Heteroderidae) from Soybean in Korea

Heonil Kang,[1] Geun Eun,[1] Jihye Ha,[1] Yongchul Kim,[1,2] Namsook Park,[2] Donggeun Kim,[2] and Insoo Choi[1,2]

Abstract: A new soybean cyst nematode *Heterodera sojae* n. sp. was found from the roots of soybean plants in Korea. Cysts of *H. sojae* n. sp. appeared more round, shining, and darker than that of *H. glycines*. Morphologically, *H. sojae* n. sp. differed from *H. glycines* by fenestra length (23.5–54.2 μm vs. 30–70 μm), vulval silt length (9.0–24.4 μm vs. 43–60 μm), tail length of J2 (54.3–74.8 μm vs. 40–61 μm), and hyaline part of J2 (32.6–46.3 μm vs. 20–30 μm). It is distinguished from *H. elachista* by larger cyst (513.4–778.3 μm × 343.4–567.1 μm vs. 350–560 μm × 250–450 μm) and longer stylet length of J2 (23.8–25.3 μm vs. 17–19 μm). Molecular analysis of rRNA large subunit (LSU) D2–D3 segments and ITS gene sequence shows that *H. sojae* n. sp. is more close to rice cyst nematode *H. elachista* than *H. glycines*. *Heterodera sojae* n. sp. was widely distributed in Korea. It was found from soybean fields of all three provinces sampled.

Key words: Heterodera sojae n. sp., morphology, phylogenetic, soybean, taxonomy.

Soybean *Glycine max* (L.) Merr is one of the most important crops in the world. It is believed to have originated from Manchuria, north of Korea, and was cultivated in Korea between 200 BC and 300 AD (Hymowitz, 1970). Soybean is cultivated on 102 million hectares and produces 254 million tons per year over the world (USDA, 2010). The United States, Brazil, Argentina, China, and India produce about 92% of world's soybeans (Masuda and Goldsmith, 2009).

Plant-parasitic nematodes cause estimated yield losses of more than $100 billion per year throughout the world (Opperman and Bird, 1998). Among them, cyst-forming nematodes are the most economically important species worldwide, currently encompassing about 80 species including soybean cyst nematode (SCN; *Heterodera glycines*) which is the most harmful species on soybean.

Nematode taxonomy has been traditionally based on morphological characters (De Ley et al., 2005). Scanning electron microscopy (SEM) is required to differentiate critical microscopic features, and in order to make a more robust phylogeny-based identification, the combination of morphological and molecular approaches must be employed (Alvani et al., 2015). To date, analysis of ribosomal RNA gene sequences have commonly been used to study the phylogeny of cyst nematodes (Ferris, 1998; Subbotin et al., 2001, 2006; Sturhan et al., 2007).

During a survey of plant-parasitic nematodes on soybean in Korea, a new species of the cyst nematode was found from soybean root. Comparative morphological, morphometric, and molecular studies of the nematode revealed that it differed from *Heterodera glycines*. The new species is described and illustrated herein as *Heterodera sojae* n. sp.

Materials and Methods

Nematode isolation: Cysts were collected from soybean roots at Miryang (latitude N35°26′, longitude E128°47′), Gyeongsangnam-do, Korea. A typical single cyst was picked, crushed, and inoculated on 10-day old soybean seedlings of cv. Lee74 in 10-cm diam. clay pots containing steam-sterilized sand and placed in a growth chamber at 24°C to establish a pure cyst population. After 60 days, nematodes (cysts, females, J2, and males) were recovered and used throughout in this study. Females and cysts were collected by sieving technique using 20, 60-mesh sieves (Handoo, 2005). For J2s and males, nematodes were separated from soil by the modified Baermann funnel method.

Morphological study: Males 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, then processed according to the Seinhorst method (Seinhorst, 1959; 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). Also, overall shape of cyst, shape of annule, vulva cone, head, and lateral field were observed.

SEM study: For SEM study, nematodes were dehydrated through an ethanol series ranging from 20% through 100%, with specimens left at each step for about 1 d (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.

Received for publication June 28, 2016.
[1]Department of Plant Bioscience, College of Natural Resource and Life Sciences, Pusan National University, Miryang 50463, Korea.
[2]Nematode Research Center, Life and Industry Convergence Research Institute, Pusan National University, Miryang 50463, Korea.
This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agricultural, Forestry and Fisheries (IPET) through Agri-Bioindustry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (315023-3).
E-mail: kimdgkr@naver.com.
This paper was edited by Zafar A. Handoo.

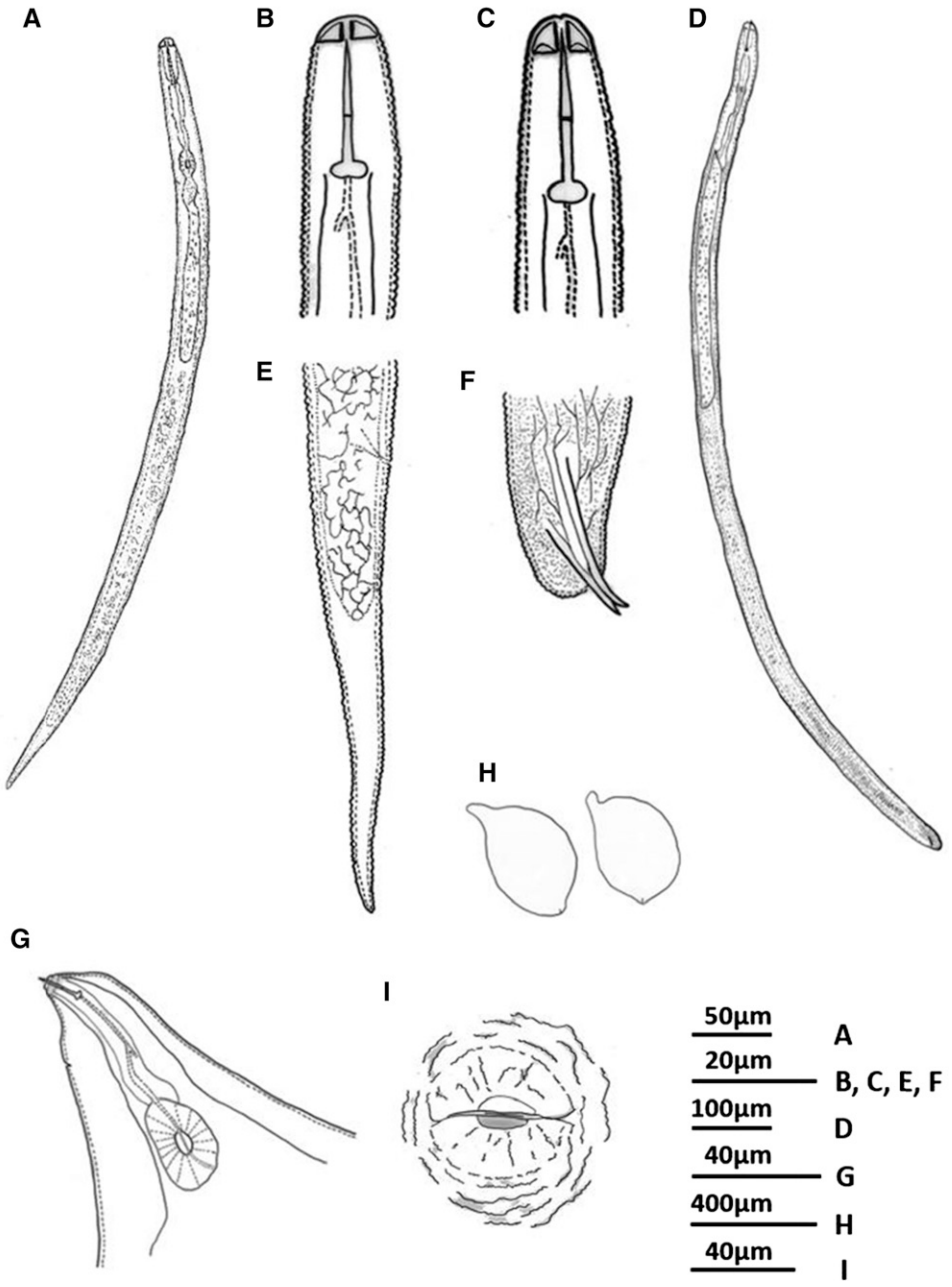

Fig. 1. Drawings of *Heterodera sojae* n. sp. A. Entire body of J2. B. Anterior region of J2. C. Anterior region of male. D. Entire of male. E. Tail region of J2. F. Tail region with spicule of male. G. Anterior region of female. H. Cysts. I. Vulval cone.

Molecular study: The new species *H. sojae* was collected and compared to *H. glycines* for this study. *Heterodera glycines* was collected from Cheongsong, Gyeongsangbuk-do, and *H. sojae* n. sp. was collected from Jangseong, Jeollanam-do, and Miryang, Gyeongsangnam-do in Korea. For pure isolation and DNA extraction, both *Heterodera* species were single cyst cultured as mentioned before. After 60 days, each cyst species was separately removed from soybean roots under a stereo microscope, and total genomic DNA was extracted from the cyst.

Each picked specimen was moved to a 2.0-ml microcentrifuge tube. 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′-ACAAGTACCGT GAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACC AGCTACTA-3′) (Subbotin et al., 2006). Primers for ITS amplification were TW81 (5′-GTTTCCGTAGGT GAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTT CAGCGGGT-3′) (Subbotin et al., 2000). The genomic DNA was used as a template for PCR 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

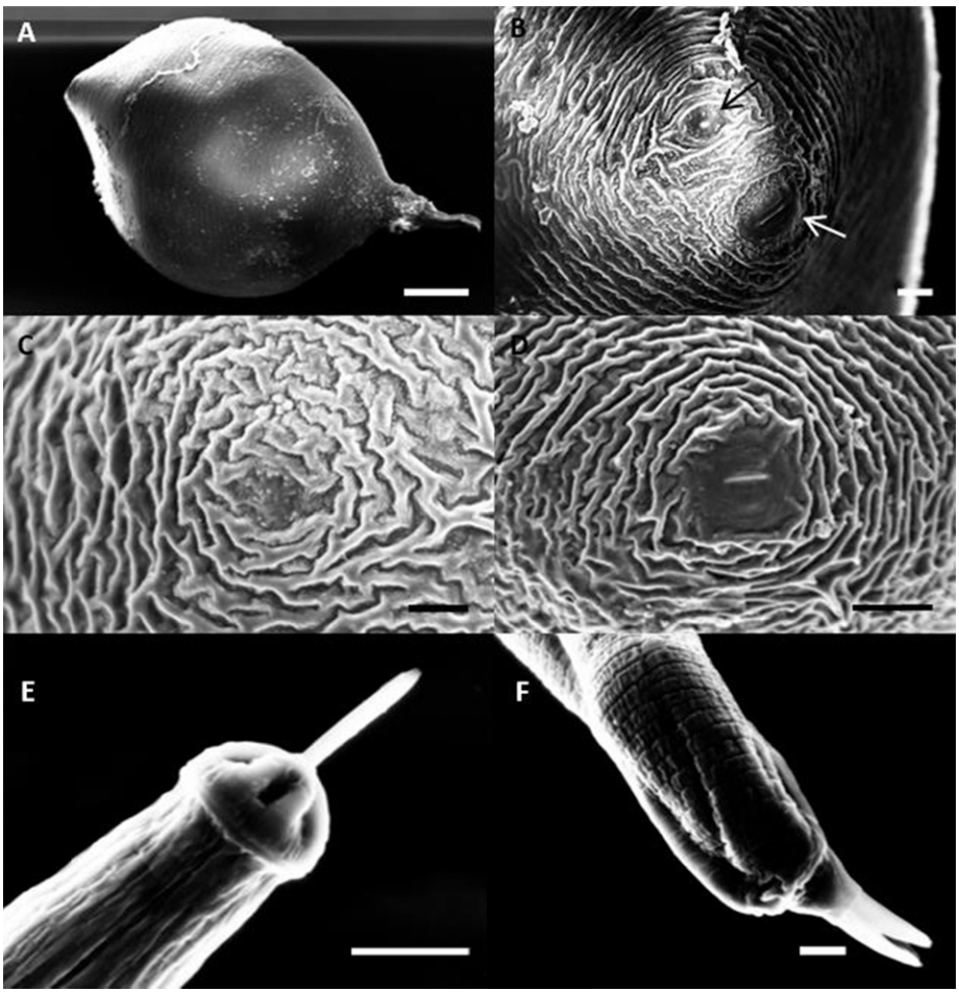

Fig. 2. SEM photos of *Heterodera sojae* n. sp. A. Cysts. B. Vulval cone of a cyst showing anus and vulva (black arrow: anus, white arrow: vulva). C. Cuticles around the anus. D. Fenestra of cyst. E. Head of second-stage juvenile. F. Tail region with spicule of male. (Scale bars: A = 100 μm, B = 10 μm, C = 10 μm, D = 20 μm, E = 5 μm, and F = 3 μm.)

conducted. The final extension step was 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

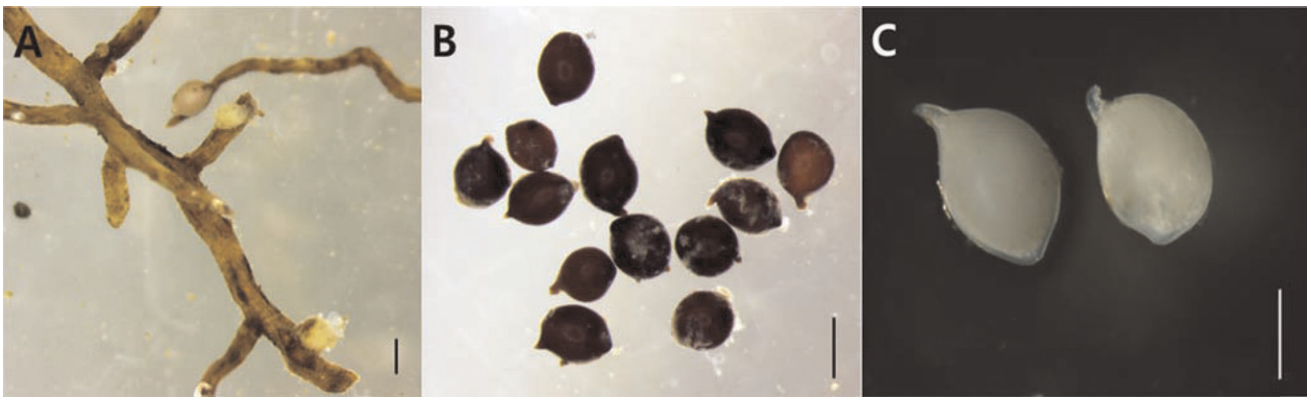

Fig. 3. Stereo microscopic photos of *Heterodera sojae* n. sp. A. Females attached on soybean root. B. Cysts. C. White females. (Scale bars: A = 500 μm, B = 500 μm, and C = 200 μm.)

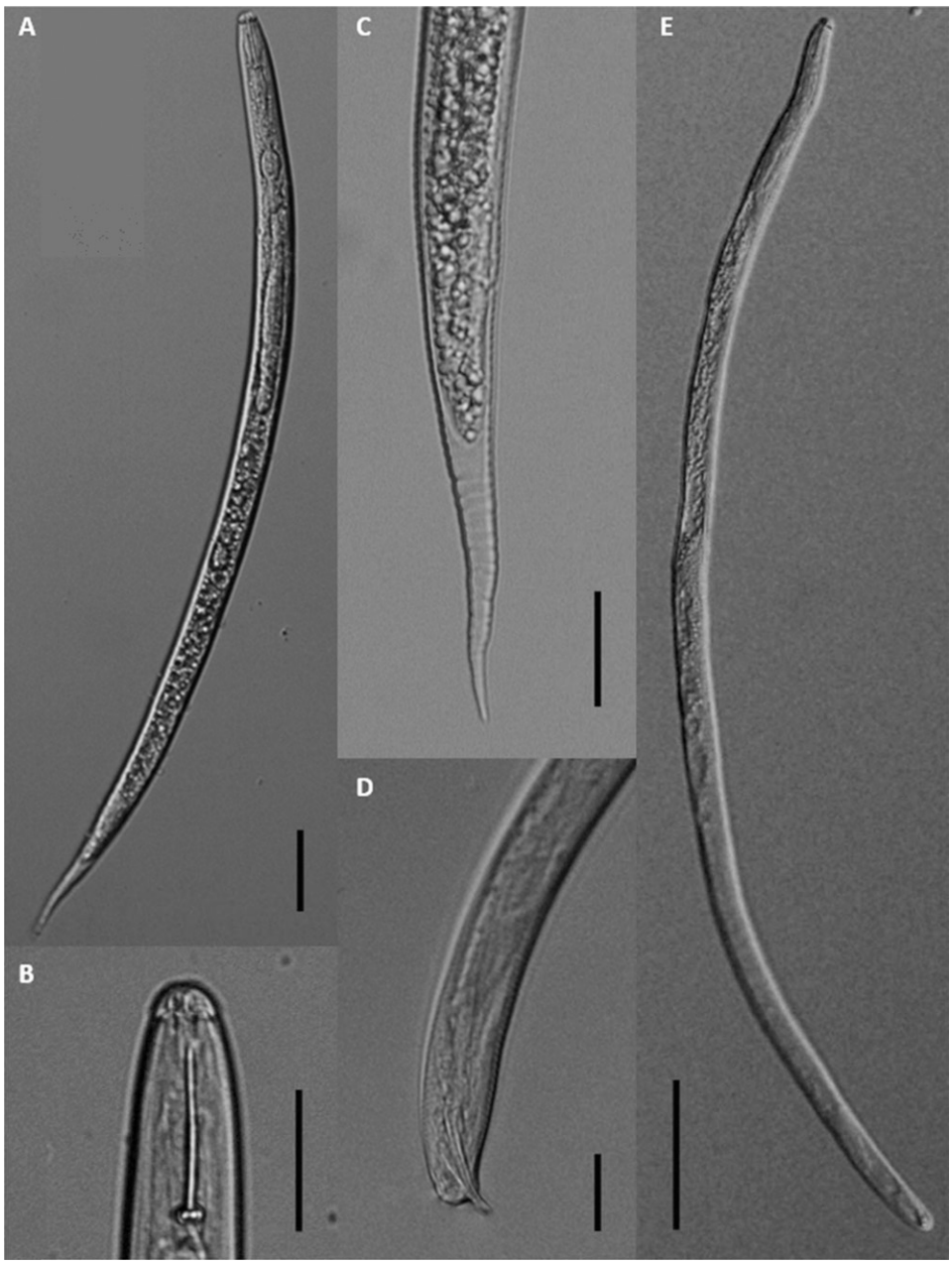

FIG. 4. Light microscope photos of male and juvenile *Heterodera sojae* n. sp. A. Entire body of second-stage juvenile (J2). B. Stylet of J2. C. Tail of J2. D. Tail region with spicule of male. E. Entire body of male. (Scale bars: A = 50 μm, B = 20 μm, C = 20 μm, D = 20 μm, and E = 100 μm.)

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 KU160507, KU160508, KU160509, KU160510, KU160511, and KU160512.

Phylogenetic study: For phylogenetic analyses, the sequences of *H. sojae* 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 dataset were chosen according to previous molecular phylogenetic analyses for cyst-forming nematodes (Subbotin et al., 2001, 2006; Ferris et al., 2004; Mundo-Ocampo et al., 2008; Zhuo et al., 2013; Wang and Peng, 2013), *Cryphodera brinkmani* Karssen & van Aelst, 1999, and *Meloidogyne alni* Turkina & Chizhov, 1986 (Subbotin et al., 2000; De Luca et al., 2013). 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 (Posada and Crandall, 1998; Huelsenbeck and Ronquist, 2001) combined

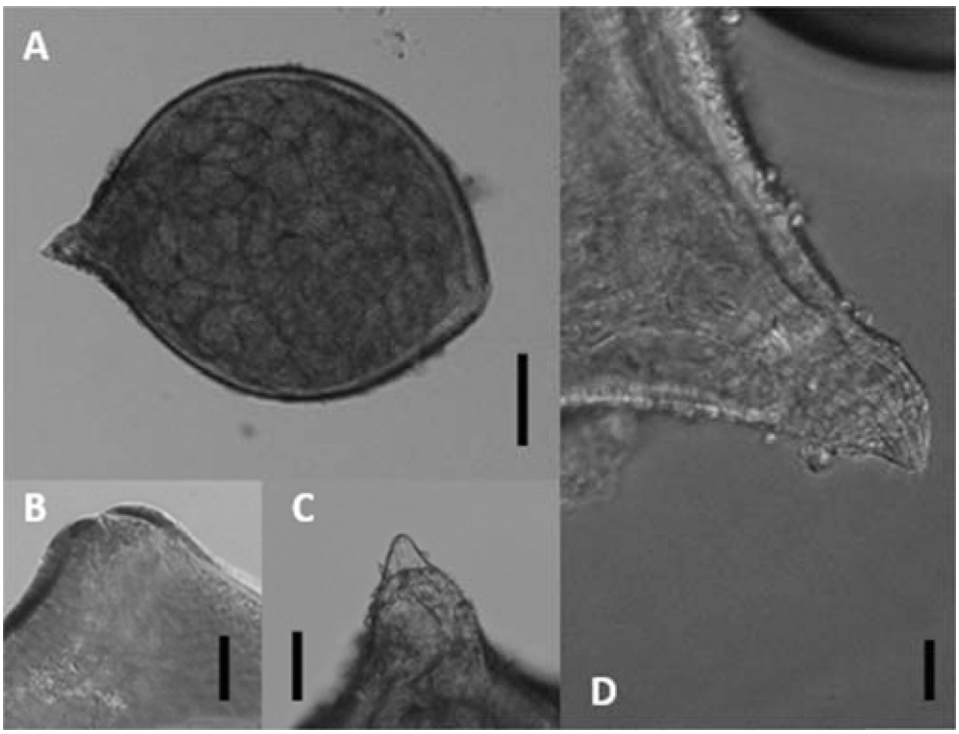

Fig. 5. Light microscope photos of females of *Heterodera sojae* n. sp. A. Female with eggs. B. Vulval cone. C. Stylet of female. D. Neck of female. (Scale bars: A = 100 μm, B = 50 μm, C = 20 μm, D = 20 μm.)

with PAUP 4.0 (Swofford, 1998). 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 3.1.2 (Huelsenbeck and Ronquist, 2001) running the chain for 1×10^6 generations and setting the "burn-in" at 2,500. The MCMC (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

Heterodera sojae n. sp.
(Figs. 1–6; Table 1)

Measurements

See Table 1.

Description

Female cysts: Lemon-shaped variable in size (513.4–778.3 μm). Particularly, tail end appeared rounded compared

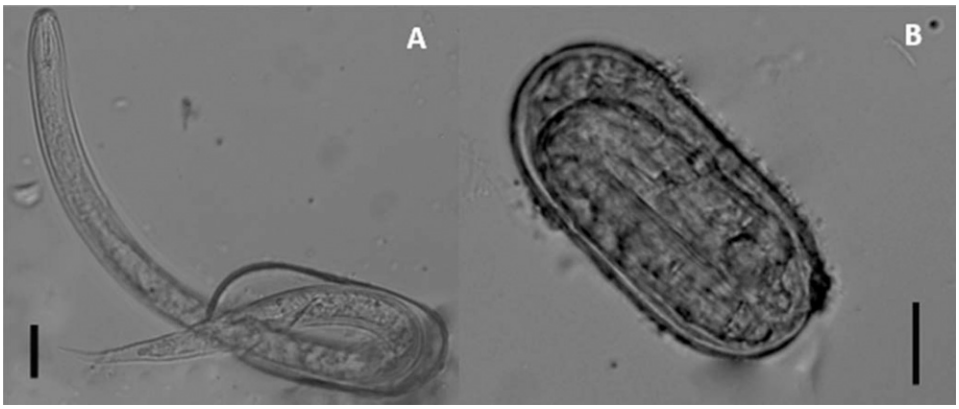

Fig. 6. Light microscope photos of eggs of *Heterodera sojae* n. sp. A. Second-stage juveniles hatching from egg. B. Egg. (Scale bars: A = 20 μm, B = 50 μm.)

TABLE 1. Morphometric measurements of *Heterodera sojae* n. sp.

Stage	Character	Holotype	Paratype	
			Range (minimum–maximum)	Mean ± SD
Cyst				
	n		25	
	L (including neck)	606.8	513.4–778.3	612.2 ± 70.4
	L (excluding neck)	545.6	434.7–663.8	526.7 ± 59.4
	W (width)	499.6	343.4–567.1	448.8 ± 56.2
	Neck	60.3	60.3–117.1	85.5 ± 15.7
	L (including neck)/W ratio	1.2	1.1–1.8	1.4 ± 0.1
Vulval cone				
	n		10	
	Fenestra length	48.6	23.5–54.2	41.9 ± 9.3
	Semifenestra length	16.5	12.3–18.4	15.2 ± 1.9
	Fenestra width	44.6	22.4–51.0	37.9 ± 9.1
	Vulval silt length	20.6	9.0–24.4	18.1 ± 4.5
	Vulval–anal distance	44.9	29.5–65.2	40.9 ± 11.0
Female				
	n		10	
	L (including neck)	550.4	497.3–680.6	565.7 ± 54.5
	L (excluding neck)	454.5	424.2–604.9	485.5 ± 54.8
	W (width)	340.6	313.5–484.5	403.2 ± 68.6
	Neck	95.8	71.7–95.8	80.2 ± 9.6
	L (including neck)/W ratio	1.6	1.1–1.6	1.4 ± 0.2
	Stylet length	22.1	22.1–25.3	24.1 ± 1.3
	Anterior end to median bulb	74.2	50.4–77.1	64.1 ± 11.5
Second stage (J2)				
	n		15	
	L (body length)	462.2	411.7–477.8	451.3 ± 20.2
	a (L/body diam.)	26.8	20.7–26.8	23.6 ± 1.4
	b (L/esophago-intestinal valve)	4.0	3.5–4.4	3.9 ± 0.3
	b′ (L/end of esophageal)	2.7	2.3–2.9	2.5 ± 0.1
	c (L/tail length)	6.7	6.2–7.9	6.9 ± 0.6
	c′ (tail length/tail diam.)	5.2	3.8–6.4	5.0 ± 0.6
	Stylet length	24.2	23.8–25.3	24.5 ± 0.4
	Tail length	68.6	54.3–74.8	65.8 ± 5.9
	Maximum body diam.	17.2	17.1–21.1	19.2 ± 1.3
	Hyaline length	41.6	32.6–46.3	38.7 ± 3.9
Male				
	n		15	
	L	908.3	668.3–967.5	870.0 ± 81.5
	a	41.5	32.8–42.0	38.0 ± 2.9
	b	6.8	5.7–8.3	7.2 ± 0.8
	b′	5.2	3.7–5.6	4.8 ± 0.6
	Stylet length	27.6	25.4–28.7	26.8 ± 0.8
	Maximum body diam.	21.9	20.0–26.0	23.0 ± 1.9
	Spicule length	29.9	23.2–30.2	27.1 ± 2.1
	Gubernaculum length	7.3	6.4–7.3	6.9 ± 0.5
Egg				
	n		10	
	L		94.3–108.3	102.1 ± 4.3
	W		45.9–61.5	51.7 ± 4.8
	L/W ratio		1.8–2.1	2.0 ± 0.1

All measurements are in μm.

to *H. glycines* (Fig. 3C). Female cuticle appeared white to creamy when young (Fig. 3C), then changed to brown-black with shiny appearance when old (Fig. 3B). Neck prominent, stylet, and other pharyngeal structures indistinct. Vulval cone ambifenestrate (Fig. 2D). Mature females contained more than 100 eggs in various stages of development. Gelatinous egg sac not observed. Cyst surface with irregular zigzag pattern (Fig. 2C,D). Remnant subcrystalline layer not observed. Anus small, but distinct (Fig. 2C). Bullae or underbridge not observed so far.

Second-stage juveniles (J2): Body cylindrical, tapering posteriorly, straight or slightly ventrally curved after fixation (Fig. 4A). Lip region offset, a labial disc with six to seven annuli. Body annuli 1.4 to 1.6 μm width at mid-body. Stylet knobs oval shaped (Fig. 4B). Anus and hyaline part of tail distinct (Fig 4C). Hyaline terminal section was 38.7 (32.6-46.3) μm long, taking up 59.1% (47.8%–66.8%) of tail length.

Males: Body vermiform, straight or slightly curved ventrally after relaxation (Fig. 4D). Well-developed stylet

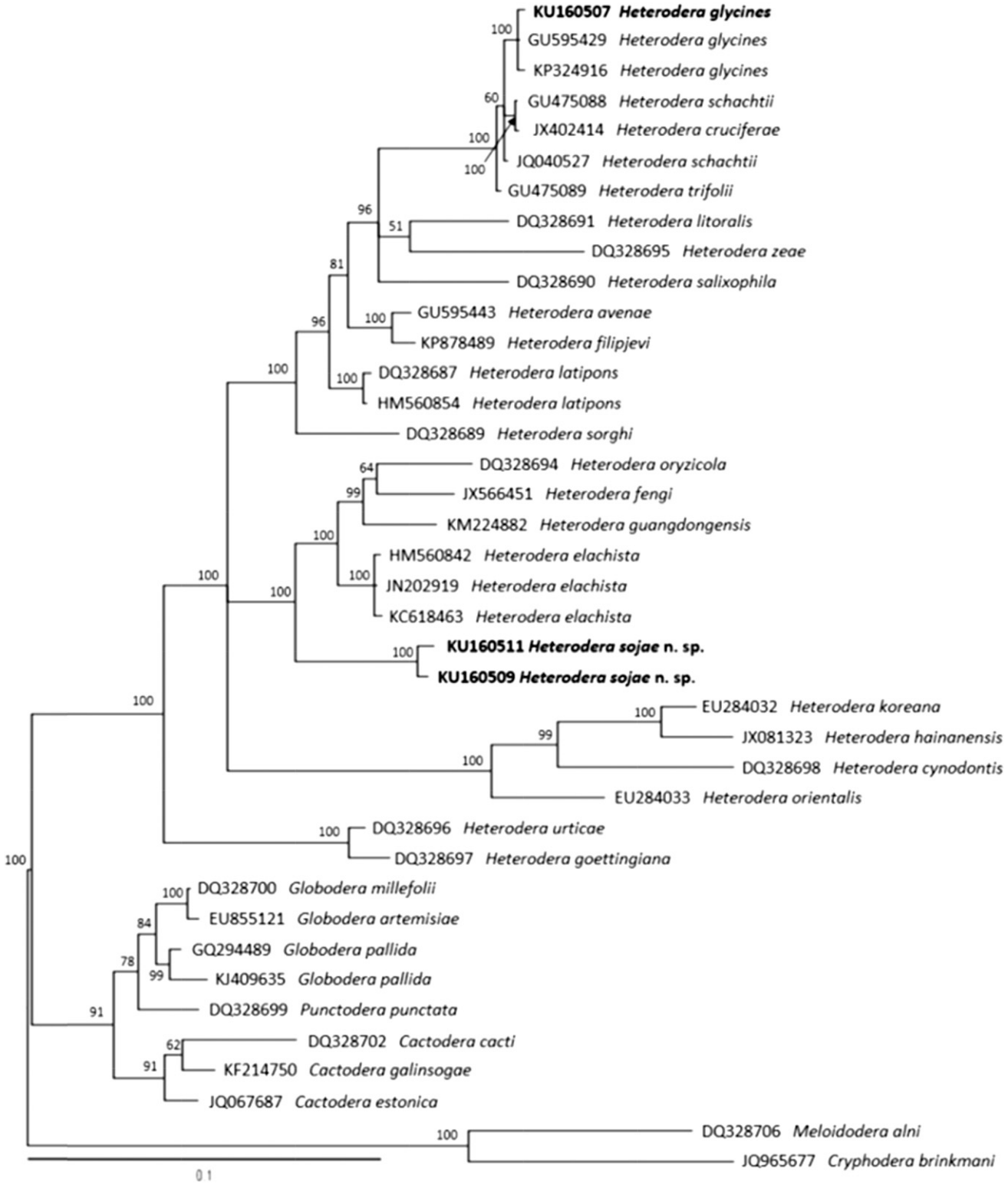

FIG. 7. Phylogenetic relationships within population and species of *Heterodera*. Bayesian 50% majority rule consensus tree from two runs as inferred from the analysis of the D2D3 of 28S rDNA gene sequences under the GTR + I + G model (1 nL = 4688.70; AIC = 9547.40; freqA = 0.1858; freqC = 0.2223; freqG = 0.3394; freqT = 0.2525; R(a) = 0.2855; R(b) = 2.5482; R(c) = 1.1880; R(d) = 0.3908; R(e) = 6.0737; R(f) = 1; Pinva = 0.4326; Shape = 0.7623). Posterior probability values more than 50% are given in appropriate clades. Newly sequenced samples are indicated by bold font.

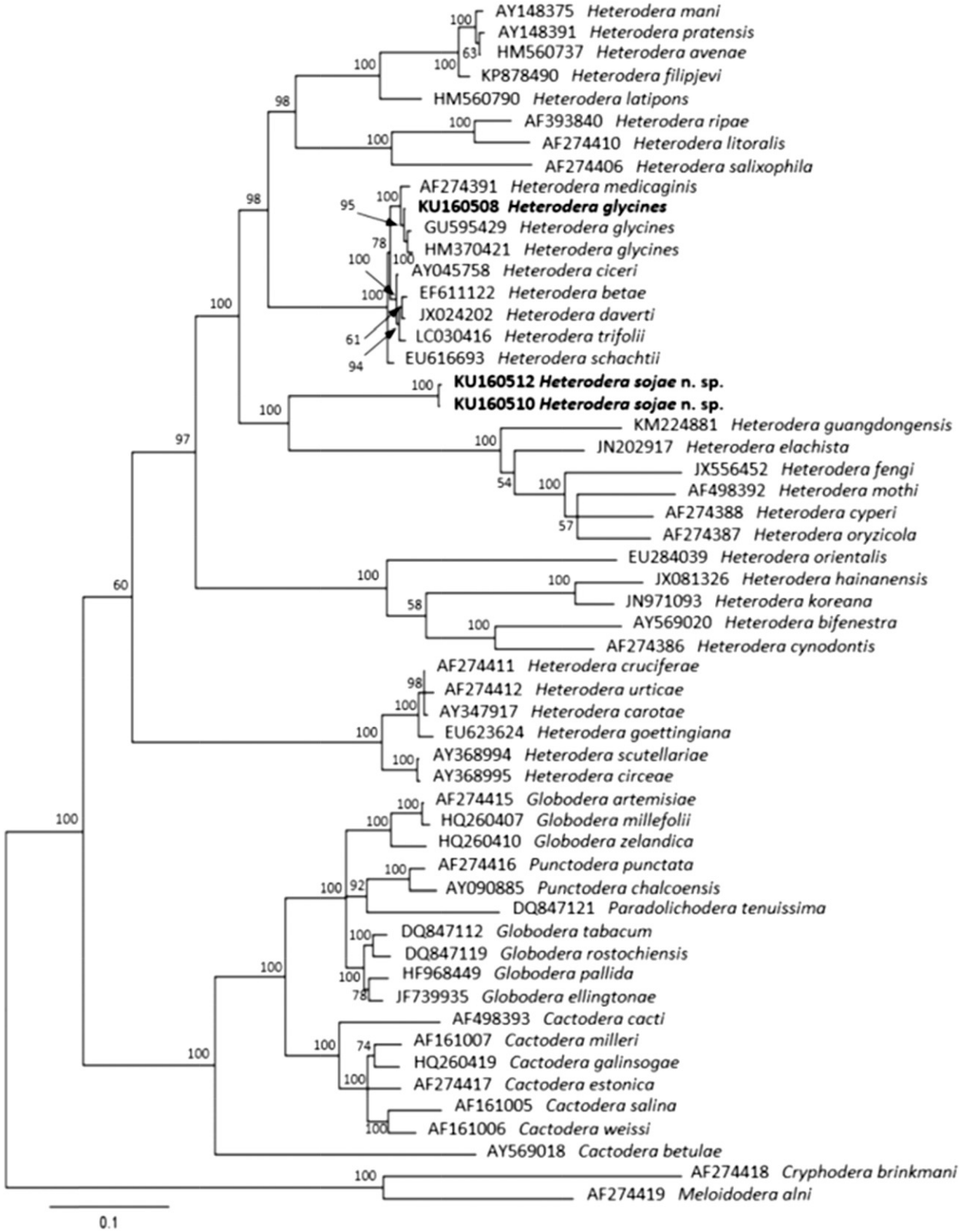

FIG. 8. Phylogenetic relationships within population and species of *Heterodera*. Bayesian 50% majority rule consensus tree from two runs as inferred from the analysis of the ITS rRNA gene sequences under the GTR + I + G model (1 nL = 16405.89; AIC = 33045.77; freqA = 0.1956; freqC = 0.2192; freqG = 0.2871; freqT = 0.2981; R(a) = 1.0361; R(b) = 3.9790; R(c) = 1.6385; R(d) = 0.4961; R(e) = 3.7762; R(f) = 1; Pinva = 0.2170; Shape = 1.5657). Posterior probability values more than 50% are given in appropriate clades. Newly sequenced samples are indicated by bold font.

(25.4–28.7 μm) with rounded basal knobs, sloping posteriorly. Lip region hemispherical. Median bulb ellipsoidal equipped with strong valve plates. Spicules slightly arcuate, with rounded end. Tail very short and bluntly rounded (Fig. 4D). Gubernaculum straight and simple.

Diagnosis and relationships: Cysts of *Heterodera sojae* n. sp. are characterized by vulval cone with ambifenestrate, a short vulval slit 18.1 μm (9.0–24.4 μm) and reduced terminal cone. Second-stage juveniles are characterized by a stylet length of 24.5 μm (23.8–25.3 μm), a tail with bluntly rounded terminus 65.8 μm (54.3–74.8 μm) and hyaline portion forming of the tail length 59.1% (47.8%–66.8%). Males are characterized by a stylet with rounded knobs sloping posteriorly 26.8 μm (25.4–28.7 μm), a spicule length of 27.1 μm (23.2–30.2 μm).

The new species is closely related to *H. glycines* that has the same host plant. However, morphological observations of the cyst, female, male, and J2 indicated that this species is different from *Heterodera glycines*. It can be distinguished from *H. glycines* by the overall cyst shape (reduced and rounded terminal cone vs. substantial terminal cone), and *H. glycines* appeared yellow when young and changed to dull brown when old. The new species also differed in fenestra length of cyst (23.5–54.2 μm vs. 30–70 μm), vulval slit length of cyst (9.0–24.4 μm vs. 43–60 μm), the J2 tail length (54.3–74.8 μm vs. 40–61 μm), and hyaline part of J2 (32.6–46.3 μm vs. 20–30 μm).

Cysts terminal and cone structure of the new species were morphologically similar to *H. avenae* (Mulvey, 1972). *Heterodera sojae* n. sp. differs from *H. avenae* by vulval silt length in cone structure (9.0–24.4 μm vs. 10–13 μm), the stylet length of J2 (23.8–25.3 μm vs. 24.0–28.0 μm), and fenestral region shape (ambifenestrate vs. bifenestrate). Also, the new species differs from *H. mani* by body length of male (668.3–967.5 μm vs. 1,100–1,750 μm), the spicule length (23.2–30.2 μm vs. 35–43 μm), fenestral region shape (ambifenestrate vs. bifenestrate), the stylet knobs of J2 (rounded vs. deeply concave), and cyst color (shiny brown-black vs. brown).

Molecular profiles and phylogenetic status: The sequenced LSU D2–D3 segments and ITS region are 783 and 1,034 bp, respectively. A BLASTn search of *H. sojae* n. sp. on the LSU D2–D3 segments revealed high-scoring matches with some *Heterodera* species, the most similar to *Heterodera elachista* (GenBank accession number HM560842), which is the species isolated from the rice in Ningxia Province in China. The identities of these two sequences were 95.0% (747/783), with one insertions/deletion (0.1%), as compared with *H. glycines* (KP324916); *H. sojae* n. sp. has 91% identity (715/784) and five insertions/deletions (0.6%). A BLASTn search of *H. sojae* n. sp. on the ITS region also revealed similarities with some *Heterodera* species, but the identities were about 80%. The highest match was *H. latipons* (HM560790), with only 83% identity (879/1,065) and 56 insertions/deletions (5%). Compared with *Heterodera glycines* (GenBank accession number HM560780), *H. sojae* n. sp. has 81% identity (850/1,055) and 47 insertions/deletions (4%).

The molecular phylogenetic relationships of the new species are shown in Figures 7 and 8. Figure 7 represents a phylogenetic tree based on LSU D2–D3 segments. The average nucleotide composition is as follows: 18.58% A, 22.23% C, 33.94% G, and 25.25% T. Using *C. brinkmani* and *M. alni* as the outgroup taxa, the molecular phylogeny strongly supports monophyly of *Heterodera* (100% posterior probability). Figure 8 represents a phylogenetic tree based on ITS region. The average nucleotide composition is as follows: 19.56% A, 21.92% C, 28.71% G, and 29.81% T. Also, using *C. brinkmani* and *M. alni* as the outgroup taxa, *H. sojae* n. sp. is close to *H. guangdongensis* and *H. elachista*. *Heterodera sojae* n. sp. is close to the Cyperi group, but morphological characters differ from Cyperi group members *H. elachista* and *H. oryzicola*. Especially of note, the vulval silt of *H. sojae* n. sp. is smaller and the fenestra length is bigger than other Cyperi group species (Nobbs et al., 1992). In the phylogeny of cyst nematodes as inferred from the ITS-rRNA gene sequences, the new species clade is in separate position, between the Cyperi group and the Schachtii group (Perry and Moens, 2006).

Type locality and habitat: Heterodera sojae n. sp. was collected from roots of soybean (*Glycine max* (L.) Merr), Miryang (latitude N35°26′, longitude E128°47′), Gyeongsangnam-do, Korea in 2011. The soil type is sandy loam, and the local climate is marine west coast of Köppen climate classification.

Etymology: The species name is given for Korean native legume, *Glycine soja*, which is known as the origin of soybean.

Literature Cited

Alvani, S., Moghaddam, E. M., Rouhani, H., and Mohammadi, A. 2015. Morphological, molecular and phylogenetic study of *Filenchus aquilonius* as a new species for Iranian nematofauna and some other known nematodes from Iran based on D2D3 segments of 28S rRNA gene. Journal of Plant Pathology & Microbiology S3 001.

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.

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 42:201–210.

Cobb, N. A. 1917. Notes on nemas. Contribution to a Science of Nematology 5:117–128.

De Ley, P., De Ley, I., Morris, K., Abebe, E., Mundo, M., Yoder, M., Heras, J., Waumann, D., Rocha-Olivares, A., Burr, J., Baldwin, J., and Thomas, W. 2005. An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. Philosophical Transaction of the Royal Society B: Biological Science 360:1945–1958.

De Luca, F., Volvas, N., Lucarelli, G., Troccoli, A., Radicci, V., Fanelli, E., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., and

Castillo, P. 2013. *Heterodera elachista* the Japanese cyst nematode parasitizing corn in northern Italy: Integrative diagnosis and bionomics. European Journal of Plant Pathology 136:857–872.

Ferris, V. R. 1998. Evolution, phylogeny and systematics. Pp. 57–82 *in* S. Sharma, ed. The cyst nematodes. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Ferris, V. R., Sabo, A., Baldwin, J. G., Mundo-Ocampo, M., Inserra, R. N., and Sharma, S. 2004. Phylogenetic relationships among selected Heteroderoidea based on 18S and ITS ribosomal DNA. Journal of Nematology 36:202–206.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. Nucleic Acids Symposium Serial 41:95–98.

Handoo, Z. A. 2005. Nematode extraction procedures. http://www.ars.usda.gov/Main/docs.htm?docid=9942.

Huelsenbeck, J. P., and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Hymowitz, T. 1970. On the domestication of the soybean. Economic Botany 24:408–421.

Larget, B., and Simon, D. L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16:750–759.

Masuda, T., and Goldsmith, P. D. 2009. World soybean production: Area harvest, yield, and long-term projections. International Food and Agribusiness Management Review 12:143–162.

Mulvey, R. H. 1972. Identification of *Heterodera* cysts by terminal and cone top structures. Canadian Journal of Zoology 50:1277–1292.

Mundo-Ocampo, M., Troccoli, A., Subbotin, S. A., Cid, J., Baldwin, J. G., and Inserra, R. N. 2008. Synonymy of *Afenestrata* with *Heterodera* supported by phylogenetics with molecular and morphological characterisation of *H. koreana* comb. n. and *H. orientalis* comb. n. (Tylenchida: Heteroderidae). Nematology 10:611–632.

Nobbs, J. M., Ibrahim, S. K., and Rowe, J. 1992. A morphological and biochemical comparison of the four cyst nematode species, *Heterodera elachista, H. oryzicola, H. oryzae* and *H. sacchari* (Nematoda: Heteroderidae) known to attack rice (Oryza sativa). Fundamental and applied nematology 15:551–562.

Opperman, C. H., and Bird, D. M. 1998. The soybean cyst nematode, *Heterodera glycines*: A genetic model system for the study of plant-parasitic nematodes. Current Opinion Plant Biology 1:342–346.

Page, R. D. 1996. TreeView: An application to display phylogenetic trees on personal computers. Computer Applications in the Bioscience 12:357–358.

Perry, R. N., and Moens, M. 2006. Molecular taxonomy and phylogeny. P. 56 *in* S. A. Subbotin and M. Moens, eds. Plant nematology, London: British library.

Posada, D., and Crandall, K. A. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–818.

Seinhorst, J. W. 1959. A rapid method for transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4:67–69.

Southey, J. F. 1986. Laboratory methods for work with plant and soil nematodes. London: Her Majesty's Stationery Office.

Sturhan, D., Wouts, W. M., and Subbotin, S. A. 2007. An unusual cyst nematode from New Zealand, *Paradolichodera tenuissima* gen. n., sp. n. (Tylenchida, Heteroderidae). Nematology 9:561–571.

Subbotin, S. A., Sturhan, D., Chizhov, V. N., Vovlas, N., and Baldwin, J. G. 2006. Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. Nematology 8:455–474.

Subbotin, S. A., Vierstraete, A., De Ley, P., Rowe, J., Waeyenberge, L., Moens, M., and Vanfleteren, J. R. 2001. Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS regions of ribosomal DNA. Molecular Phylogenetics and Evolution 21:1–16.

Subbotin, S. A., Waeyenberge, L., and Moens, M. 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLPs. Nematology 2:153–164.

Swofford, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (and other methods) 4.0 beta. Sunderland, MA: Sinauer.

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 22:4673–4680.

U.S. Department of Agriculture. 2010. Soybean area, yield, and production. https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads; updated September 2010.

Wang, H. H., and Peng, D. 2013. *Heterodera fengi* n. sp. (Nematoda: Heteroderinae) from bamboo in Guangdong Province, China—a new cyst nematode in the Cyperi group. Zootaxa 3652:179–192.

Zhuo, K., Wang, H. H., Ye, W. M., Peng, D. L., and Liao, J. L. 2013. *Heterodera hainanensis* n. sp. (Nematoda: Heteroderinae) from bamboo in Hainan Province, China—a new cyst nematode in the *Afenestrata* group. Nematology 15:303–314.