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Description of *Xiphinema parachambersi* n. sp. (Nematoda: Longidoridae) from Imported Ornamental Plants in Japan with a Key to *Xiphinema* Species in Group 1

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Abstract

Xiphinema parachambersi n. sp. recovered in Ningbo, China, from the rhizosphere of ornamental plants (*Gardenia jasminoides* and *Euonymus hamiltonianus*) imported from Japan is described. The new species is characterized by a long female body 1,830 to 2,109μm long, odontostyle 105 to 116μm long, reproductive system mono-opisthodelphic, vulva located anteriorly at 25.2 to 27.7% of total body, long ovary 119 to 292μm with simple uterus and Z-organ absent, female tail elongated conoid with a rounded terminus. Only two juvenile stages were available to study and no male was found. The polytomous identification codes for this new species are A1, B4, C2, D23, E1, F2, G2, H2, I2, J2, K?, L1 and it belongs to the morphospecies group 1. Phylogenetic analysis based on the 18S, ITS1 and 28S D2/D3 sequences of the new species showed close relationships with *X. chambersi*. Morphologically, the new species is similar to *X. chambersi*, *X. hangzhouense*, and *X. winotoi* but can be differentiated by morphological characters and DNA sequences. To help identify the species, a diagnostic key to the group 1 species is presented.

Key words

Molecular, morphology, morphometrics, nematode, new species.

Dagger nematodes (*Xiphinema* Cobb, 1913) contain more than 260 species (Palomares-Rius et al., 2017). They are polyphagous and ectoparasites on a variety of cultivated and wild plants. Their feeding behavior causes considerable mechanical damage to plants due to its excessive long stylet. The root symptoms include darkening of tissues, cortical hyperplasia, lateral root proliferation, tip galling, and necrosis (Hunt, 1993). In addition, nine *Xiphinema* species, three species from *Xiphinema* non-*americanum* group including *X. index* (Thorne and Allen, 1950), *X. diversicaudatum* (Micoletzky, 1927) (Thorne, 1939), and *X. italiae* (Meyl, 1953) and six putative species in the *X. americanum* group including *X. americanum s. str., X. californicum* (Lamberti and Bleve-Zacheo, 1979), *X. bricolense* (Ebsary et al., 1989, *X. intermedium* (Lamberti and Bleve-Zacheo, 1979), *X. revesi* (Dalmaso, 1969), and *X. tarjanense* (Lamberti and Bleve-Zacheo, 1979) are known to transmit nepoviruses, which cause additional indirect damages to plants (Hunt, 1993; Decraemer and Robbins, 2007). Because of their economic importance, species in *Xiphinema* have received considerable attention. Virus-transmitting *Xiphinema* species are listed as quarantine pests in many countries including China.

During a routine quarantine inspection, a *Xiphinema* population was detected from the soil samples from imported ornamental plants, *Gardenia jasminoides* J. Ellis and *Euonymus hamiltonianus* Wall. from Japan. The preliminary morphological investigation revealed that the species has a medium size body, opisthodelphic reproductive system, anteriorly located vulva and elongated tail, very similar to North American species *X. chambersi* (Thorne, 1939).

In order to make the final species identification, a detailed morphological and DNA sequencing analysis was conducted which resulted in a new species and was herein described as *X. parachambersi* n. sp. The objectives of the present study were to: (i) provide a morphological description of the new species and compare it with other similar species; (ii) characterize the species molecularly, using three DNA markers, 18S, ITS1, and 28S D2/D3 ribosomal (iii) examine the phylogenetic relationships of the new species with other species in *Xiphinema*.

Materials and methods

Nematode samplings, extraction, and morphological study

Xiphinema specimens were collected from the rhizosphere of *Gardenia jasminoides* (sample number: 2186-1) and *Euonymus hamiltonianus* (sample number: 2186-2) from the same container using modified Baermann funnel method for 24 to 48 hr. Measurements were made on specimens fixed in TAF and processed to glycerin following the method of Seinhorst's (1959). The nematodes were measured using AxioVs40 (v4.6.3.0) of Zeiss company. All the abbreviations used are as defined in Decraemer and Hunt (2006). Light micrographs were made using a Zeiss Imager Z1 microscope equipped with a Zeiss AxioCam MRm CCD camera. Drawings were made with a drawing tube. Juvenile stages were determined by a plot with scattergraph method of the lengths of odontostyles and replacements.

Molecular analyses

For DNA extraction, a single nematode was transferred to worm lysis buffer (WLB: 20 mM Tris-HCl pH 8.0, 100 mM KCl, 3.0 mM Mg2Cl, 2.0 mM DTT, 0.9% Tween) and crushed with a sterilized pipette tip. The crushed nematode was pipetted into 8 µl ddH_2O with 2 µl proteinase K (60 µg/ml) in an Eppendorf tube, which was then briefly spun and stored at –70°C for at least 10 min. Subsequently, the Eppendorf tube was incubated at 65°C for 1 to 2 h and the proteinase K was denatured at 95°C for 10 min. Finally, the DNA suspension was cooled to 4°C and used for conducting PCR (Li et al., 2008). Three sets of primers (synthesized by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the partial 18S, ITS1, and 28S rDNA D2/D3. Primers for amplification of 18S were forward primer K4f and reverse primer K1r (Penas et al., 2006). Primers for amplification of ITS1 were forward primer V1 (Ferris et al., 1993) and reverse primer 5.8S (Cherry et al., 1997). Primers for amplification of 28S D2/D3 were forward primer D2A and reverse primer D3B (De Ley et al., 1999). The 25-µl PCR was performed using Master Mix DNA polymerase (Invitrogen, Shanghai, China) according to the manufacturer's protocol in a thermocycler. The thermal cycler program for 28S was as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 2 min. A final extension was performed at 72 °C for 10 min. The thermal cycler program for 18S and ITS was as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 60 s, annealing at 55 °C for 60 s, and extension at 72 °C for 2 min. A final extension was performed at 72 °C for 5 min as described by Ye et al. (2007) and Li et al. (2008). PCR products were separated and visualized on 1% agarose gels and stained with ethidium bromide. PCR products of sufficiently high quality were sequenced by Invitrogen (Shanghai, China).

Phylogenetic analysis

The sequences were deposited into the GenBank database. DNA sequences were aligned by MEGA7 (Kumar et al., 2016.) using default settings. The DNA sequences were compared with those of the other nematode species available at the GenBank sequence database using the BLAST homology search program. The model of base substitution was evaluated using MODELTEST (Posada and Criandall, 1998; Huelsenbeck and Ronquist, 2001). The Akaike-supported model, the base frequencies, the proportion of invariable sites and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck and Ronquist, 2001) running the chain for 1×10^6 generations and setting the "burnin" at 2,500. We used the Markov Chain Monte Carlo (MCMC) method within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using 50% majority rule.

Results

SYSTEMATICS

Xiphinema parachambersi n. sp.

(Table 1; Figs. 1–4)

Table 1. Morphometrics data for *Xiphinema parachambersi* n. sp. All measurements are in µm and in the form of mean ± s.d. (range).

	Holotype	Paratype		
Character/ratios	Female	Female	J2 or J3	J3 or J4
n	1	15	5	11
L	1,918	2,008 ± 78.7 (1,830.0–2,109.0)	1,127.0 ± 52.6 (1,046.0–1,210.0)	1,419.6 ± 38.5 (1,349.0–1,587.0)
a	45.6	47.2 ± 2.3 (44.2–53.1)	42.7 ± 3.6 (36.7–46.3)	48.3 ± 3.5 (41.60–55.5)
b	5.4	5.5 ± 0.2 (5.1–6.0)	3.9 ± 0.5 (3.0–4.4)	4.7 ± 0.3 (4.2–5.0)
c	18.1	17.9 ± 1.0 (16.1–19.6)	11.8 ± 1.0 (10.5–12.8)	13.2 ± 0.6 (12.0–15.1)
c'	4.4	4.9 ± 0.4 (4.2–6.0)	6.4 ± 0.3 (5.9–6.8)	5.9 ± 0.4 (5.2–6.9)
V	27	26.2 ± 0.6 (25.2–27.7)	–	–
Lip diam.	10	10.3 ± 0.6 (8.8–11.2)	8.6 ± 0.3 (8.2–9.0)	8.9 ± 0.5 (8.2–10.1)
Lip height	5	4.1 ± 0.7 (2.9–5.2)	3.6 ± 0.4 (3.0–4.2)	3.8 ± 0.4 (3.1–4.7)
Odontostyle	115	110.4 ± 2.6 (105.0–115.6)	71.0 ± 3.8 (64.4–75.6)	85.5 ± 2.0 (79.5–90.0)
Odontophore	67	65.5 ± 1.6 (61.0–68.1)	46.1 ± 1.4 (44.5–47.8)	53.8 ± 1.0 (52.2–54.8)
Replacement odontostyle	–	–	88.0 ± 2.5 (85.0–92.0)	107.0 ± 7.9 (90.5–116.3)
Total stylet	173	176 ± 2.9 (169.0–181.2)	116.6 ± 4.7 (109.0–120.5)	140.0 ± 2.2 (137.2–142.5)
Flanges width	10	10.4 ± 0.7 (8.8–11.5)	8.1 ± 0.4 (7.7–8.7)	9.0 ± 0.6 (7.6–10.8)
Esophagus	358	366.6 ± 10.6 (348.4–387.2)	289.6 ± 28.2 (272.2–345.6)	311.4 ± 14.7 (275.0–344.2)
Esophageal bulb length	78	81.5 ± 2.1 (77.4–85.1)	63.7 ± 2.2 (60.1–66.1)	70.4 ± 3.0 (64.8–76.5)
Esophageal bulb diam.	24	24.5 ± 1.1 (22.5-26.5)	16.5 ± 1.4 (14.1–17.9)	18.6 ± 0.6 (17.6–23.0)
Body diam.	42	42.7 ± 2.0 (38.8–46.7)	27.2 ± 1.9 (24.3–29.8)	29.6 ± 1.5 (26.4–35.1)
Anterior genital branch length	11	10.6 ± 1.3 (8.4–12.3)	–	–
G1%	0.5	0.5 ± 0.1 (0.4–0.6)	–	–
Posterior genital branch length	240	218.3 ± 40.9 (119.2–292.1)	–	–
G2%	8.9	10.7 ± 1.9 (5.8–13.9)	–	–
Distance from anterior end to vulva	522	525.2 ± 15.9 (493.0–548.3)	–	–
Anal body width.	24	23.1 ± 1.5 (20.1–25.0)	15.2 ± 1.0 (14.0–16.4)	18.7 ± 0.7 (16.8-20.3)
Tail	106	112.2 ± 6.3 (97.9–120.3)	98.0 ± 6.0 (88.0–104.0)	109.0 ± 1.4 (103.5–116.0)
Hyaline tail part	44	43.0 ± 2.2 (39.4–47.2)	19.3 ± 1.2 (18.0–20.7)	26.1 ± 2.7 (20.2–32.0)
H%	41	38.5 ± 2.4 (34.4–43.4)	19.5 ± 2.0 (17.5–23.1))	23.8 ± 2.9 (17.4–28.2)
Rectum	38	35.2 ± 2.7 (30.4–40.8)	20.0 ± 4.5 (16.4–27.6)	28.4 ± 2.3 (23.1–30.8)

Description

Female

Body thin, almost straight in the anterior half, posterior portion bending ventrally, open "C" shaped upon fixation, narrows gradually and evenly in the tail region. Cuticle smooth and rather thick, lateral cords not visible. Lip region flattened, cephalic region rounded and, offset by weak depression. Amphids stirrup-shaped, fovea with wide slit-like aperture, slightly narrower than lip width. Five to six body

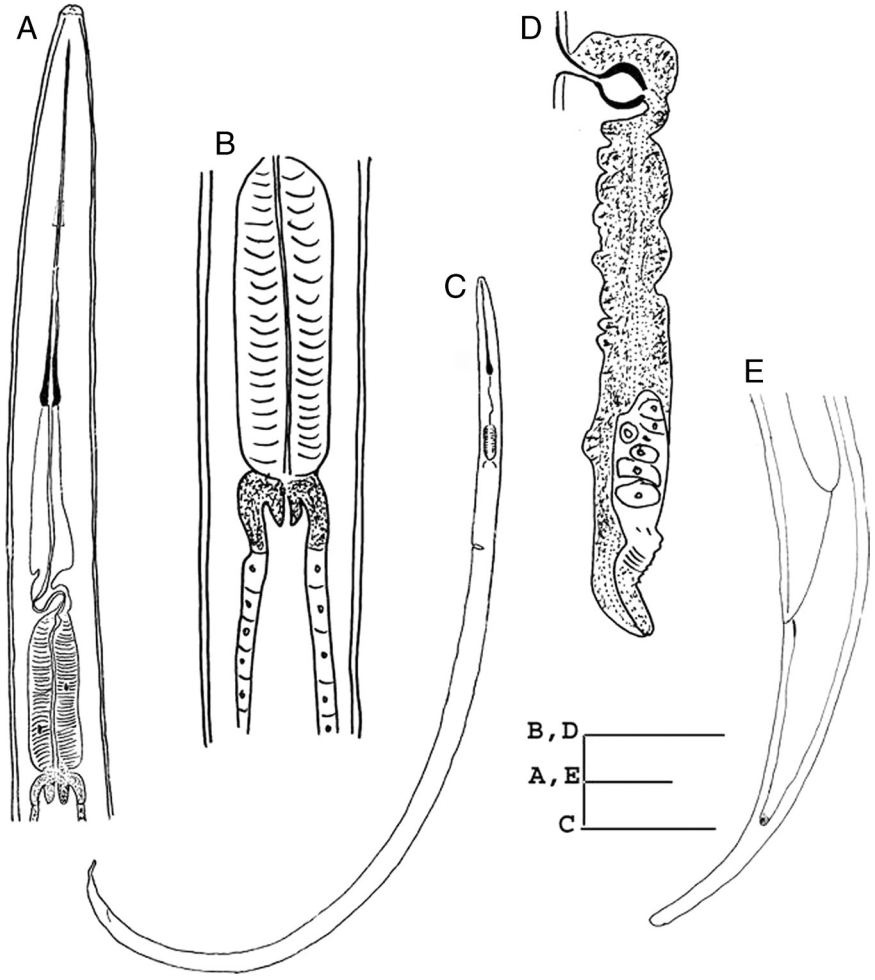

Figure 1: Line drawings of *Xiphinema parachamberi* n. sp. female. A: Esophageal region; B: Esophageal bulb; C: Entire body; D: Genital branch; E: Tail region (Scale bars: A, E = 40; B, C=20, D=100μm).

pores present between anterior end and guiding ring on the dorsal and ventral side, respectively. Odontostyle (9–12) times lip region width. Odontophore with well-developed basal flanges (8.8–11.5μm wide). Guiding ring double, guiding sheath 12 to 17μm long depending on degree of protraction/retraction of stylet. Esophagus, extending to a terminal Esophageal bulb with three nuclei, dorsal gland nucleus located at the beginning of bulb i.e. (12–17%) of the esophageal bulb length, two subventral-lateral nuclei located around the middle of bulb (SV1 = (51–53%); SV2 = (52–54%). Esophageal basal bulb (77.4–85.1) μm long and (22.5–26.5)μm wide. Esophageal intestinal valve (cardia) not well developed, 7.6 to 8.8μm long, wide lumen appeared as two separated parts hanging on the base bulb, free in the intestine lumen, in a few specimens conoid-oblonged shaped. Intestine simple, rectum 30.4 to 40.8μm long. Female reproductive system mono-opisthodelphic, undifferentiated uterine sac, 8.4 to 12.3μm long. Posterior genital branch 119 to 292μm long, reflexed, vulva a transverse slit, vagina thick walled (16–19μm long), slightly directed posteriorly, ovijector well developed, 17 to 21μm wide, extending inwards less than half of the corresponding body diameter, uterus short and undifferentiated, Z organ absent. Tail elongated, ventrally curved, tapers gradually and evenly forming a rounded terminus bearing two body pores.

Male

Not found.

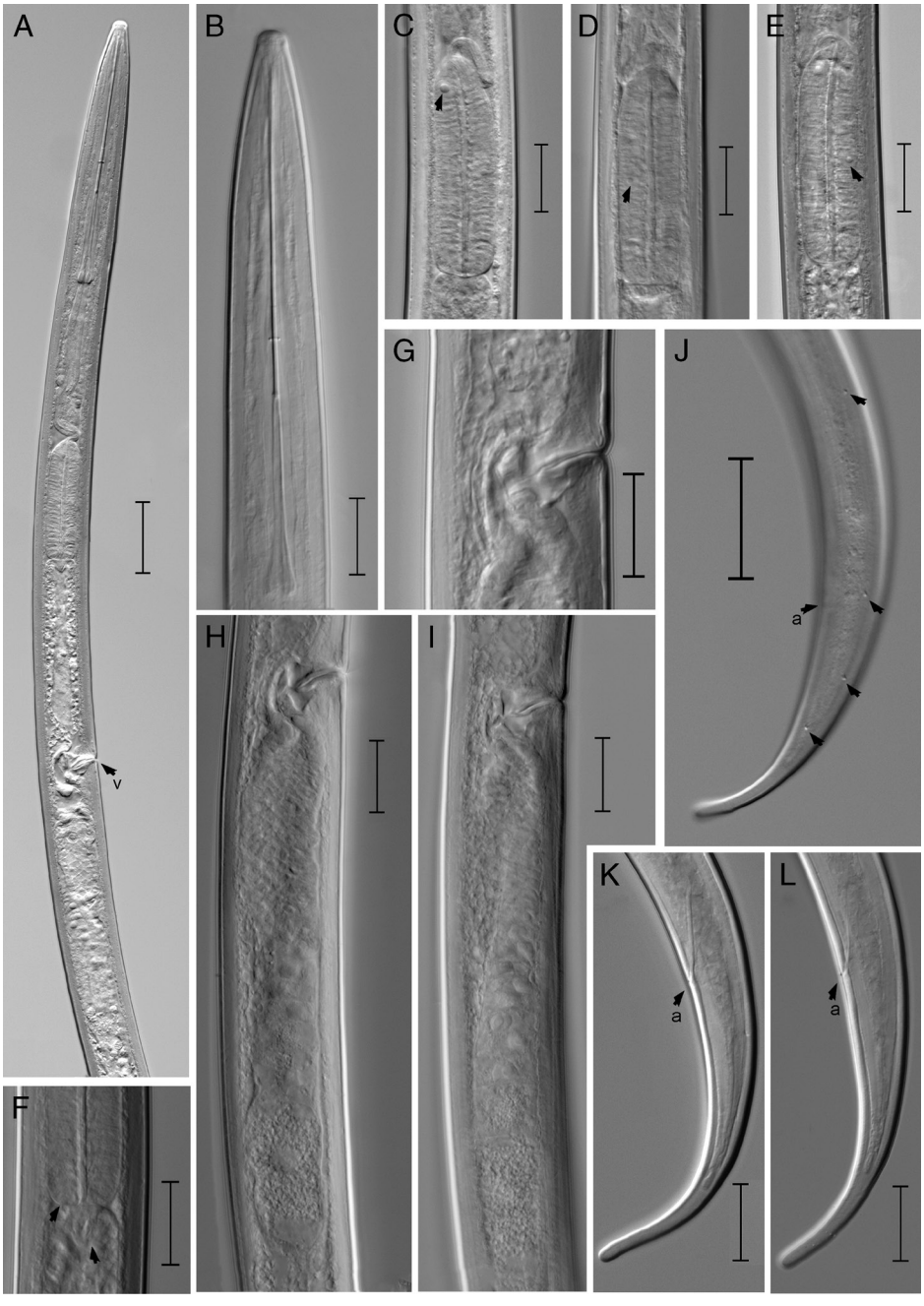

Figure 2: Light photomicrographs of *Xiphinema parachamberi* n. sp. female. A: Anterior region, arrow showing position of vulva (v); B: Lip region; C–E: Esophageal bulb (arrows showing different position of gland nuclei); F: Esophago-intestinal junction arrows pointing the base of pharyngeal bulb and cardia. G: Vulval region; H, I: Gonad; J: Female tail (arrows showing position of (a) anus and caudal pores); K, L: Female tail (arrows showing position of (a) anus) (Scale bars: A, L = 10μm).

Juveniles

Two juvenile stages, either J2/J3 orJ3/J4 were found and they were morphologically similar to adults except for their smaller size, shorter tails, and sexual characteristics. There is an immediate and quick progression of odontostyle length which makes it difficult to distinguish between juvenile stages. However, the characteristic feature of J1 as having replacement odontostyle being embedded in the base of

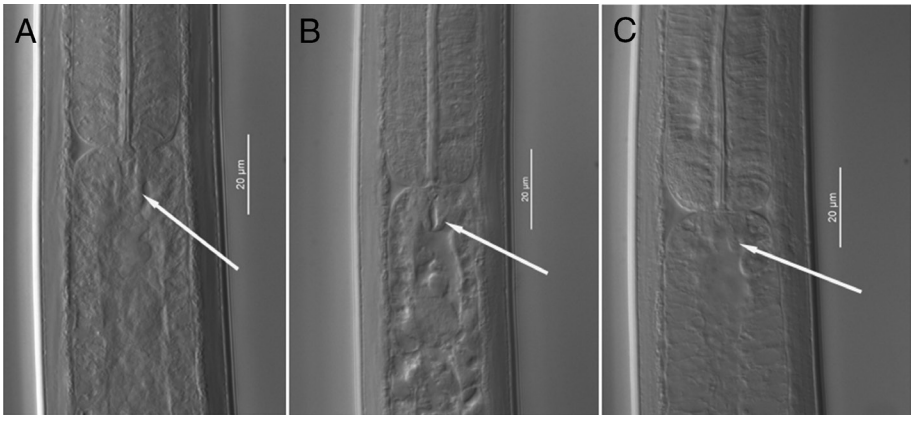

Figure 3: Light photomicrographs of cardia of *Xiphinema parachamberi* n. sp. Scale bars: A–C = 20 µm).

odontophore was not observed in any of the juvenile specimens. Based on the information obtained through scatter graph (Fig. 5), it is not clear whether three or four juvenile stages are present in this species because only two juvenile stages were available to study.

Type host and locality

The type specimens were extracted from the rhizosphere of *Gardenia jasminoides* (2186-1) and *Euonymus hamiltonianus* (2186-2) trees from the same container imported from Japan on June 2016 and intercepted at Ningbo Port, P. R. China.

Type specimens

Holotype female and nine female paratypes (slide numbers 2186-1 to 2186-8) were deposited in the nematode collection of Ningbo Entry-Exit Inspection and Quarantine Bureau, China. Five paratype females (slide numbers T550a-e) were deposited in the Canadian National Collection of Nematodes, Ottawa, Canada.

Differential diagnosis

Xiphinema parachambersi n. sp. is a mono-opisthodelphic species characterized by the female having an elongated tail with round terminus, with a hyaline part over one-third of the tail length, the cardia with wide lumen, total stylet length = 169 to 181 µm, vulva located anteriorly at 25.2 to 27.7% of the total body length. Based on the polytomous key of *Xiphinema* species presented by Loof and Luc (1990) and Loof et al. (1996), the new species belongs to the morphospecies group 1 and has the following specific diagnostic alphanumeric codes: A1, B4, C2, D23, E1, F2, G2, H2, I2, J2, K?, L1.

Morphospecies group 1 represents species having single gonad (monodelphic), all the known species in this group are small and have an anteriorly located vulva, simple or undifferentiated posterior uterus, lacking Z organ (Cohn and Sher, 1972). With the most recent described *X. hanzhouense* (Cai et al., 2018), there are 12 nominal species in this group. The important characters to distinguish these species are the shape and lengths of tail and its hyaline region.

Xiphinema parachambersi n. sp. is morphologically most similar to *X. chambersi*, but can be differentiated by cardia (weakly developed with wide lumen *vs* well developed with narrow lumen), shorter tail (98.0–120.3) *vs* (110.2–177.3) µm, longer hyaline part (39.4–47.2) *vs* (22–43.4) µm, slightly shorter body, (1830–2109) *vs* (2100–2400) µm, more anteriorly located guide ring, (92.1–102) *vs* (105.4–115) µm from anterior end, shorter anterior undifferentiated sac, (8.4–12.3) *vs* (13.9–21) µm and higher H%, (34.4–43.4) *vs* (19.9–34.4).

The new species can be differentiated from *X. hangzhouense* by a pronounced shorter hyaline tail part, (39.4–47.2) *vs* (62.4–81) µm, more anteriorly located vulva, V = (25.2–27.7) *vs* (27.6–31.2), shorter odontostyle, (105.0–115.6) *vs* (117–128) µm, anterior undifferentiated sac (present *vs* absent) and tail terminus (rounded *vs* clavate); from *X. manasiae* Sen et al., 2010 by a longer tail (98.0–120.3) *vs* (61–86) µm, longer hyaline part (39.4–47.2) *vs* 20 µm (based on the drawings), slightly anterior vulva, V = (25.2–27.7) *vs* (30.2–33) %, longer odontostyle, (105.0–115.6) *vs*

Figure 4: Light photomicrographs of *Xiphinema parachamberi* n. sp. juveniles and female. A–D: Anterior regions of J2/J3, J3/J4 and female; E–H: Tail regions of J2/J3, J3/J4 and female (arrows showing position of anus (a)) (Scale bars: A-L = 10μm).

(98–105.3)μm, shorter anterior undifferentiated sac (8.4–12.3) *vs* (27–59)μm; from *X. naturale* (Lamberti et al., 2002) by the elongated tail with c′ (4.2–6.0) *vs* a tail somewhat conoid, c′ (2.5–3.5), shorter body, 2008 (1,830–2,109) *vs* (2,500–3,000)μm, slightly posterior vulva, V=(25.2–27.7) *vs* (22–25)%, shorter odontostyle, (105.0–115.6) *vs* (130–140)μm, longer tail, (98.0–120.3) *vs* (70.6–88.2)μm, longer hyaline tail region, (39.4–47.2) *vs* (19.5–26.5); from *X. orthotenum* (Cohn and Sher, 1972) by shorter tail,

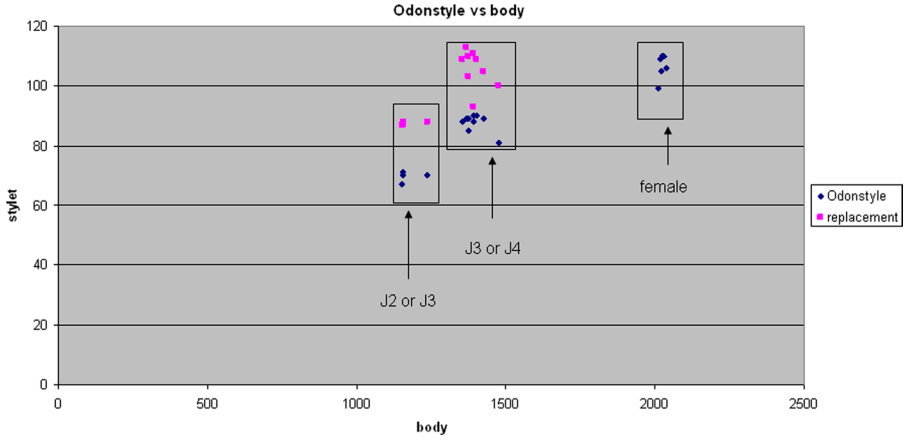

Figure 5: Relationship of body length to length of functional and replacement odontostyle (Ost and rOst, respectively); length in two juvenile developmental stages and mature females of *Xiphinema parachambersi* n. sp.

(98.0–120.3) *vs* (136–197)*µ*m, tail elongated *vs* somewhat filiform, c′ = (4.2–6.0) *vs* (7.0–10.5), and shorter odontostyle, (105.0–115.6) *vs* (110–127)*µ*m; from *X. winotoi* (Rahman–Razak and Loof, 1998) by tail curved ventrally *vs* straight or bent slightly dorsally, shorter hyaline tail region, (39.4–47.2) *vs* (62–74)*µ*m, shorter odontostyle, (105.0–115.6) *vs* (125–148)*µ*m; from *X. monohysterum* (Brown, 1968), *X. hunaniense* (Wang and Wu, 1992) and *X. radicicola* (Goodey, 1936) by an elongated tail with c′ value more than 4 *vs* a shorter and conoid shaped tail, c′ value less than 4; from *X. brasiliense* (Lordello, 1951), *X. fagesi* (Germani, 1990), and *X. ensiculiferum* (Cobb, 1893) (Thorne, 1937) by an elongated tail with c′ value more than 4 *vs* a short round to hemisphere tail with c′ value less than 1.

To help identifying *Xiphinema* species in group 1, a key was proposed as below:

1. Tail short round to almost hemisphere with c′ < 1 ……..2
   Tail conoid shaped with 1 < c′ < 4……..
   ……3
   Tail elongated with c′ > 4……..
   ……..6
2. Tail ends with a digit……..
   …..……X. *brasiliense*
   Tail ends round and hemispherical……..
   ……..X. *ensiculiferum*
   Tail ends swollen……..
   ……...……..*X. fagesi*
3. Tail conoid shaped tapers off evenly with c′ = 2.6
   ……..…… *X. monohysterum*
   Tail with c′ < 2……..……..
   ……...……..…….4
4. Cardia visible……..
   ……..*X. naturela*
   Cardia not visible……..……..
   ...…...……..…….5
5. Tail tapers off evenly and with hyaline part 20 *µ*m long…...……..…*X. radicicola*
   Tail tapers off unevenly and without hyaline part……...……..*X. hunaniense*
6. Tail very long almost filiform c′ > 7……..
   ……...…..*X. orthotenum*
   Tail elongated with 4 < c′ < 7……..
   ……..……...…...……7

7. Tail straight or bent dorsally……...…..
   ……..… *X. winotoi*
   Tail bent ventrally……..……..
   ……..8
8. Tail hyaline part > 50 *µ*m long…..……..
   …..……..*X. hangzhouense*
   Tail hyaline part < 50 *µ*m long……..
   ……...……...……...9
9. Tail hyaline part < 30 *µ*m long……...……..
   ……..……..*X. manasiae*
   Tail hyaline part > 30 and < 50 *µ*m long……..
   ……...…...10
10. Cardia weak with wide lumen……..…..
    ……...…*X. parachamberi* n. sp.
    Cardia strong with narrow lumen……..
    ……*X. chambersi*

Etymology

The species epithet is formed from the Latin word para = beside or near, and *chambersi*, thereby reflecting its close similarity to *X. chambersi.*

Molecular profiles and phylogenetic status

The sequenced fragments of near-full-length 18S, 28S D2/D3 and ITS are of ca 1,700bp, 800 bp and 1,000 bp, respectively. DNA sequences of *X. parachambersi* n. sp. have the highest match with the *Xiphinema* sequences deposited in GenBank, but with distinct differences, most close to *X. chambersi*. The near-full-length 18S rRNA (MG786444) from *X. parachambersi* n. sp. showed a 97 to 98% similarity (differ in 7–34 bps) with *X. chambersi*, *X. ifacolum*, *X. paritaliae*, and *X. turcicum*. 28S D2/D3 rRNA sequences of *X. parachambersi* n. sp. (MG786445) showed 80 to 95% similarity (differ in 36–103 bps) with *X. chambersi*, *X. insigne*, *X. elongatum*, *X. savanicola*, *X. setariae*, *X. vulgare*, and *X. hangzhouense*. The ITS1 rRNA sequence (MG786441–MG786443) of *X. parachambersi* n. sp. showed 80 to 84% similarity (9–192 bps) and 6 to 9% gaps with *X. chambersi*.

Phylogenetic relationships among *Xiphinema* species inferred from analyses of 18S, 28S D2/D3, and ITS1 gene sequences using BI are given in Figures 6, 7, and 8, respectively (Table S1). Near-full-length 18S rRNA gene (Fig. 6), tree was constructed from multiple sequence alignments of 66 sequences. In this tree, the *X. parachambersi* n. sp. is grouped with *X. chambersi* with 100% support, with a sister clade formed by morphospecies which belongs to group 4 and 5, i.e. *X. ifacolum*, *X. paritaliae* and *X. turcicum*. Two other species in group 1, i.e. *X. brasiliense* and *X. ensiculiferum* are not close to *X. parachambersi* n. sp.

The 28S D2/D3 gene tree (Fig. 7), based on a multiple alignment of 85 sequences, revealed two major clades consisting of *X. americanum* and *X.* non-*americanum* group species. *X. parachambersi* n. sp. is grouped with *X. chambersi* and *X. naturale* in group 1 with 100% support. This clade is in a 100% supported monophyletic clade with some other species in morphospecies group 1 and 7, i.e., *X. hangzhouense*, *X. elongatum*, *X. insigne*, *X. savanicola*, *X. setariae*, and *X. vulgare*. Another species in group 1, i.e. *X. brasiliense*, are not close to *X. parachambersi* n. sp.

The ITS1 tree (Fig. 8), was constructed from multiple sequence alignments of 68 sequences. In this tree, *X. parachambersi* n. sp. is in a highly supported monophyletic clade with 12 populations of *X. chambersi* with 95% support. This clade is sister to some species in morphospecies group 1, i.e., *X. hangzhouense*, and group 7, i.e., *X. hunaniense*, *X. elongatum*, *X. setariae*, and *X. insigne*.

Discussion

Mono-opisthodelphic dagger nematodes in the genus *Xiphinema* belongs to morphospecies group 1. This morphospecies group comprises species with anterior genital branch completely absent to a very small post uterine sac. Among 12 species in this group, i.e., *X. brasiliense*, *X. chambersi*, *X. ensiculiferum*, *X. fagesi*, *X. hunaniense*, *X. hangzhouense*, *X. monasiae*, *X. monohysterum*, *X. naturale*, *X. orthotenum*, *X. radicicola* and *X. winotoi* (Loof and Luc, 1993; Loof et al., 1996; Cai et al., 2018), only a few species were described with molecular characterizations. This study added another species with complete morphological and molecular characterization, which will help to enhance the knowledge and understanding to the classification of this group of nematodes. The diagnostic value of the apparently unique cardia in this new species is still unclear as few species were studied with this structure. The function of cardia is proposed to prevent the regurgitation of the food, however, at this point, we can only assume that sheath-less cardia could be primitive to cardia with sheath.

Our phylogenetic studies suggested that species in the morphospecies group 1 are not monophyletic, and morphospecies grouping was only established for the convenience of identification and do not always reflect the evolutionary history, which is consistent with other studies (Handoo et al., 2016; Cai et al., 2018). Considering the high variability in the morphological characters in *Xiphinema* species, it is necessary to use integrated morphological and molecular approaches in species diagnosis, especially in making a regulatory decision regarding the movement of soil and plant material.

Morphospecies group 1 species are mainly distributed in Brazil (Oliveira et al., 2003; Silva et al., 2008; De Jesus et al., 2015), China (Zeng et al., 2016), USA, Canada (Ye, 2002; Yu et al., 2010), Malaysia (Rahman-Razak and Loof, 1998), France (Luc and Coomans, 1992) and India (Loof et al., 2001). This new species is described from the ornamental plants imported from Japan, which represents the first group 1 species from this country.

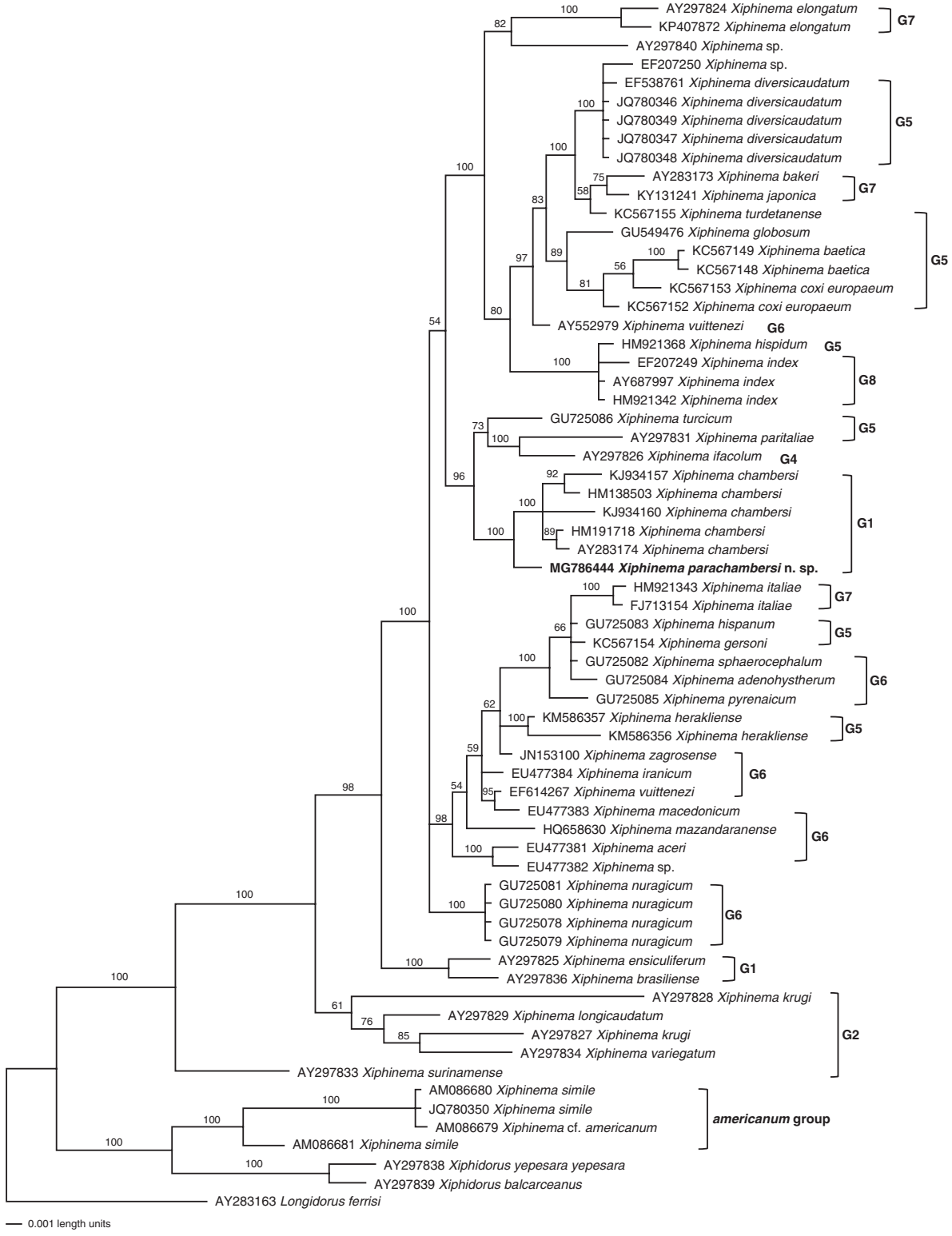

Figure 6: Bayesian consensus tree of *Xiphinema parachamberi* n. sp. inferred from 18S under GTR+I+G model (-lnL = 3332.0762; AIC = 6682.1523; freqA = 0.1821; freqC = 0.209; freqG = 0.3285; freqT = 0.2804; R(a) = 1.4406; R(b) = 5.2581; R(c) = 2.6656; R(d) = 0.7382; R(e) = 5.2581; R(f) = 1; Pinva = 0.2316; Shape = 0.4544). Posterior probability values exceeding 50% are given on appropriate clades.

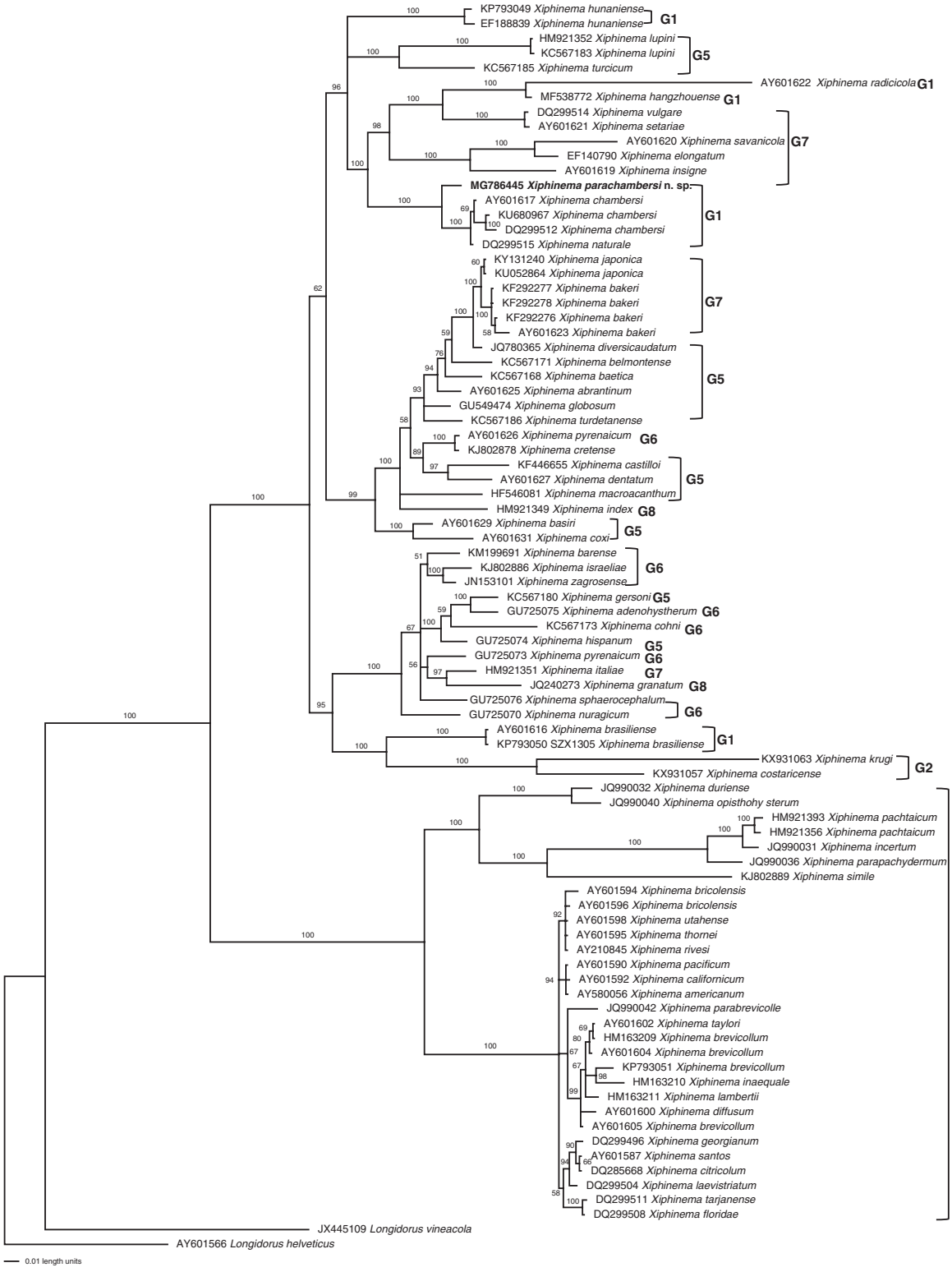

Figure 7: Bayesian consensus tree of *Xiphinema parachamberi* n. sp. inferred from 28S D2/D3 under GTR+I+G model (-lnL = 10912.0693; AIC = 21844.1387; freqA = 0.2463; freqC = 0.2277; freqG = 0.2969; freqT = 0.2291; R(a) = 0.9039; R(b) = 2.4909; R(c) = 2.4092; R(d) = 0.4557; R(e) = 3.9274; R(f) = 1; Pinva = 0.3185; Shape = 0.782). Posterior probability values exceeding 50% are given on appropriate clades.

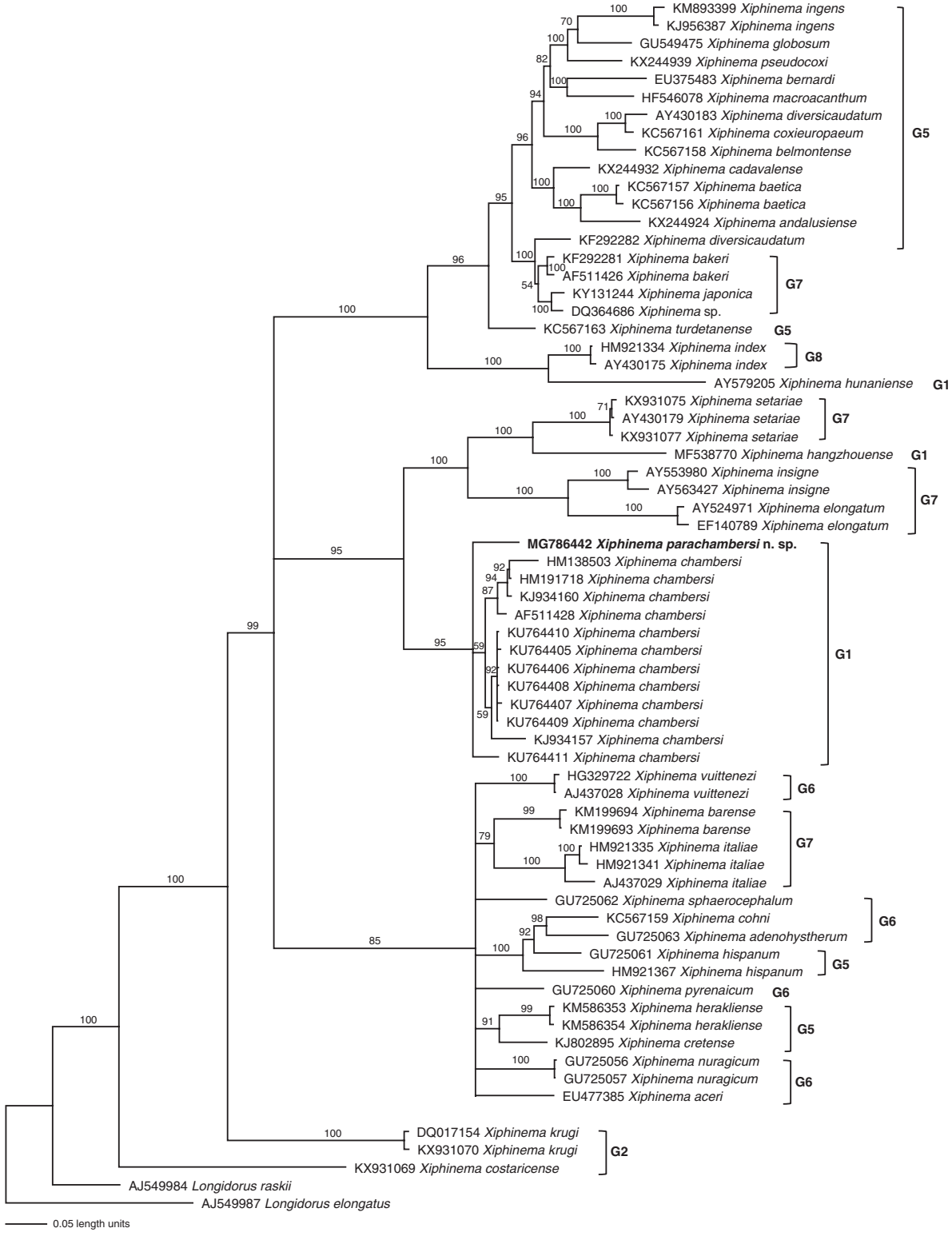

Figure 8: Bayesian consensus tree of *Xiphinema parachamberi* n. sp. inferred from ITS1 under GTR+I+G model (-lnL = 19846.0566; AIC = 39712.1133; freqA = 0.2723; freqC = 0.2059; freqG = 0.2672; freqT = 0.2547; R(a) = 0.851; R(b) = 3.6772; R(c) = 1.3471; R(d) = 0.6487; R(e) = 5.209; R(f) = 1; Pinva = 0.0808; Shape = 1.2786). Posterior probability values exceeding 50% are given on appropriate clades.

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Appendix

Supplementary Table S1

Table S1. Sequences of nematode species used for the phylogenetic analyses.

Species	18S	28S D2-D3	ITS
Xiphinema abrantinum	–	AY601625	-
Xiphinema andalusiense	–	–	KX244924
Xiphinema aceri	EU477381	–	EU477385
Xiphinema adenohytherum	GU725084	GU725075	GU725063
Xiphinema baetica	KC567149	KC567168	KC567157
	KC567148	-	KC567156
Xiphinema bakeri	AY283173	KF292277	KF292281
	–	KF292278	AF511426
	–	KF292276	-
	–	AY601623	-
Xiphinema barense	–	KM199691	KM199694
	–	–	KM199693
Xiphinema basiri	–	AY601629	–
Xiphinema belmontense	–	KC567171	KC567158
Xiphinema bernardi	–	–	EU375483
Xiphinema brasiliense	AY297836	AY601616	–
	–	KP793050	–
Xiphinema cadavalense	–	–	KX244932
Xiphinema castilloi	–	KF446655	–
Xiphinema chambersi	AY283174	AY601617	HM138503
	HM138503	KU680967	HM191718
	HM191718	DQ299512	KJ934160
	KJ934157	–	KJ934157
	KJ934160	–	AF511428
	–	–	KU764410
	–	–	KU764405
	–	–	KU764406
	–	–	KU764407
	–	–	KU764408
	–	–	KU764409
	–	–	KU764411
Xiphinema cohni	–	KC567173	KC567159
Xiphinema costaricense	–	KX931057	KX931069
Xiphinema coxi	–	AY601631	–

Xiphinema coxi europaeum	KC567152	–	KC567161
	KC567153	–	–
Xiphinema cretense	–	KJ802878	KJ802895
Xiphinema dentatum	–	AY601627	–
Xiphinema diversicaudatum	EF538761	JQ780365	KF292282
	JQ780349	–	–
	JQ780348	–	–
	JQ780347	–	–
	JQ780346	–	–
Xiphinema ensiculiferum	AY297825	–	–
Xiphinema elongatum	AY297824	EF140790	AY524971
	KP407872	–	EF140789
Xiphinema gersoni	KC567154	KC567180	–
Xiphinema globosum	GU549476	GU549474	GU549475
Xiphinema granatum	–	JQ240273	–
Xiphinema hangzhouense	–	MF538772	MF538770
Xiphinema herakliense	KM586356	–	KM586353
	KM586357	–	KM586354
Xiphinema hispidum	HM921368	–	–
Xiphinema hispanum	GU725083	GU725074	GU725061
	–	–	HM821367
Xiphinema hunaniense	-	KP793049	AY579205
	–	EF188839	–
Xiphinema ifacolum	AY297826	–	–
Xiphinema index	EF207249	HM921349	HM921334
	AY687997	–	AY430175
	HM921342	–	–
Xiphinema ingens	–	–	KM893399
	–	–	KJ9566387
Xiphinema insigne	–	AY601619	AY553980
	–	–	AY563427
Xiphinema iranicum	EU477384	–	–
Xiphinema israeliae	–	KJ802886	
Xiphinema italiae	HM921343	HM921351	HM921335
	FJ713154	–	HM921341
	–	–	AJ437029
Xiphinema japonica	KY131241	KY131240	KY131244
	–	KU052864	–
Xiphinema krugi	AY297827	KX931063	DQ017154
	AY297828	–	KX931070
Xiphinema longicaudatum	AY297829	–	–
Xiphinema lupini	–	HM921352	–
	–	KC567183	–

Xiphinema mazandaranense	HQ658630	–	–
Xiphinema macedonicum	EU477383	–	–
Xiphinema macroacanthum	–	HF546081	HF546078
Xiphinema naturale	–	DQ299515	–
Xiphinema nuragicum	GU725078	GU725070	GU725056
	GU725079	–	GU725057
	GU725080	–	–
	GU725081	–	–
***Xiphinema parachambersi* n. sp.**	**MG786444**	**MG7866445**	**MG786442**
Xiphinema paritaliae	AY297831	–	–
Xiphinema pseudocoxi	–	–	KX244939
Xiphinema pyrenaicum	GU725085	AY601626	GU725060
	–	GU725073	–
Xiphinema radicicola	–	AY601622	–
Xiphinema savanicola	–	AY601620	–
Xiphinema setariae	–	AY601621	KX931075
	–	–	AY430179
	–	–	KX931077
Xiphinema sphaerocephalum	GU725082	GU725076	GU725062
Xiphinema surinamense	AY297833	–	–
Xiphinema turcicum	GU725086	KC567185	–
Xiphinema turdetanense	KC567155	KC567186	KC567163
Xiphinema variegatum	AY297834	–	–
Xiphinema vulgare	–	DQ299514	–
Xiphinema vuittenezi	AY552979	–	HG329722
	EF614267	–	AJ437028
Xiphinema zagrosense	JN153100	JN153101	–
Xiphinema sp.	AY297840	–	DQ364686
	EF207250	–	–
	EU477382	–	–
Xiphinema amercanum group			
Xiphinema cf. *americanum*	AM086679	–	–
Xiphinema americanum	–	AY580056	–
Xiphinema brevicollum	–	HM163209	–
	–	AY601604	–
	–	KP793051	–
	–	AY601605	–
Xiphinema bricolensis	–	AY601594	–
	–	AY601596	–
Xiphinema californicum	–	AY601592	–
Xiphinema citricolum	–	DQ285668	–
Xiphinema diffusum	–	AY601600	–
Xiphinema duriense	–	JQ990032	–

Xiphinema floridae	–	DQ299508	–
Xiphinema georgianum	–	DQ299496	–
Xiphinema incertum	–	JQ990031	–
Xiphinema inaequale	–	HM163210	–
Xiphinema laevistriatum	–	DQ299504	–
Xiphinema lambertii	–	HM163211	–
Xiphinema opisthohysterum	–	JQ990040	–
Xiphinema pachtaicum	–	HM921393	–
	–	HM921356	–
Xiphinema pacificum	–	AY601590	–
Xiphinema parabrevicolle	–	JQ990042	–
Xiphinema parapachydemum	–	JQ990036	–
Xiphinema rivesi	–	AY210845	–
Xiphinema santos	–	AY601587	–
Xiphinema simile	AM086680	KJ802889	–
	AM086681	–	–
	JQ780350	–	–
Xiphinema tarjanense	–	DQ299511	–
Xiphinema taylori	–	AY601602	–
Xiphinema thornei	–	AY601595	–
Xiphinema utahense	–	AY601598	–
Xiphidorus yepesara yepesara	AY297838	–	–
Xiphidorus balcarceanus	AY297839	–	–
Longidorus ferrisi	AY283163	–	–
Longidorus raskii	–	–	AJ549984
Longidorus elongatus	–	–	AJ549987