Morphological and Molecular Characterization of *Paralongidorus sali* Siddiqi, Hooper, and Khan, 1963 with a Description of the First-Stage Juvenile and Male of *Longidorus jonesi* Siddiqi, 1962 from China

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Abstract

Longidorids are economically important plant-parasitic nematodes because several species are virus vectors. Populations of *Paralongidorus sali* and *Longidorus jonesi*, isolated from woody perennials of Hangzhou, Zhejiang, China, were characterized molecularly and morphologically. The morphometric data of the Chinese populations of both species were compared with other populations. The present study provided a first record of the occurrence of *Paralongidorus* in China coupled with description of the first-stage juvenile and male of *L. jonesi*. Phylogenetic analysis based on 18S and D2–D3 expansion segments of 28S gene indicated that *L. jonesi* clustered with *L. jonesi* reported from Japan and *P. sali* grouped with *P. bikanerensis* from Iran. Considering the pathological and economic importance of this group of nematodes, the study emphasized the need of updated descriptions from accurately identified specimens, isolation of sufficient material for examination, and molecular and phylogenetic analysis for a better understanding and diagnostics of Longidorid nematodes.

Key words

First record, Juvenile stages, Molecular, Morphology, Morphometrics, Phylogeny, SEM, Taxonomy.

The family Longidoridae Thorne, 1935 comprises a group of migratory plant-parasitic species that damage a wide range of wild and cultivated plants through direct feeding on root cells and the transmission of several plant-pathogenic viruses (Decraemer and Robbins, 2007; Decraemer and Chaves, 2012). Members of these genera are known to transmit nepoviruses and are regulated by quarantine inspections in many countries (Gutiérrez-Gutiérrez et al., 2016). *Longidorus* and *Paralongidorus* belong to family Longidoridae, both are globally distributed and have 160 and 90 known species, respectively (Palomares-Rius et al., 2013; Archidona-Yuste et al., 2016; Esmaeili et al., 2016).

Some species of *Paralongidorus* have controversial status due to synonymization of *Longidoroides* (Khan et al., 1978) and *Siddiqia* (Khan et al., 1978), and some *Paralongidorus* species have been wrongly included

and belong to genus *Longidorus* (Decraemer and Coomans, 2007). The major difference used to separate *Longidorus*, *Longidoroides*, and *Paralongidorus* is the shape of amphids (pouch like in *Longidorus* and *Longidoroides* vs. funnel/stirrup shaped in *Paralongidorus*) and the opening of amphidial aperture (pore-like in *Longidorus* vs. slit-like in *Longidoroides* and *Paralongidorus*) (Oliveira and Neilson, 2006). Several new species of *Paralongidorus* have published with complete molecular characterization and scanning electron microscopy (SEM) observations (Palomares-Rius et al., 2008, 2013; Pedram et al., 2012; Gutiérrez-Gutiérrez et al., 2017; Barsi and Luca, 2017) which enables the discrimination between *Longidorus* and *Paralongidorus* species. However, there is no molecular evidence to distinguish *Longidoroides* species which leaves the status of this

genus as junior synonym of *Paralongidorus* as suggested by Decraemer and Coomans (2007).

During a routine nematological survey of Hangzhou, Zhejiang Province, eastern China, two populations of longidorid nematodes were isolated from the rhizosphere of woody perennials. The population isolated from *Cyclobalanopsis glauca* (Thumb.) Oerst, 4 juvenile stages were recovered and identified as *Longidorus jonesi*, the population from *Castanopsis sclerophylla* (Lindl.) Schottky, 3 juvenile stages were recovered and was identified as *Paralongidorus sali.*

Robbins et al. (1995) reported 3 juveniles stages of *L. jonesi* and no first stage juvenile or male were observed additionally this is the first report of genus *Paralongidorus* found in China. Therefore, the objectives of the present study were to: (i) provide updated morphological descriptions of first-stage juvenile *P. sali*, and male of *L. jonesi*, (ii) characterize the molecular data of both species using the D2–D3 expansion segments of 28S rRNA and partial 18S rRNA gene sequences, and (iii) demonstrate the phylogenetic relationships of both species with related species.

Materials and methods

Nematode sampling, extraction and morphological study

Nematodes were extracted from soil samples using modified Baermann funnel method for 24 hr. For morphometric studies, nematodes were killed and fixed with hot formalin (4% with 1% glycerol), and processed in glycerine (Seinhorst, 1959) as modified by De Grisse (1969). The measurements and light micrographs of nematodes were performed using a Nikon eclipse Ni–U 931845 compound microscope. For the SEM examination, the nematodes were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde, (the mixture contained = 25 ml of 8% paraformaldehyde, 10 ml of 25% glutaraldehyde, 50 ml of 0.2 M phosphate buffer, and 15 ml distilled water) washed three times in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in a series of ethanol solutions and critical-point dried with CO_2. After mounting on stubs, the samples were coated with gold at 6 to 10 nanometer thickness and the micrographs were made at 3 to 5 kv operating system (Maria et al., 2018).

Molecular analyses

DNA was extracted from single specimens as described by Zheng et al. (2003). Polymerase chain reaction (PCR) and sequencing were completed in two laboratories: LPN and IB, China. PCR conditions were as described by Ye et al. (2007). Several sets of primers were used for PCR: the forward D2A (5′-ACAAGTACCGTG AGGGAA AGTTG-3′) and the reverse D3B (5′-TCGGAAGGAACCAGCTACTA-3′) primers for amplifying the D2–D3 expansion segments of 28S rRNA gene (De Ley et al., 1999). Nearly full length 18S region was amplified with two sets of primers, the first set was 18s39F (5′-AAAGATTAAGCCATG-CATG-3′) and 18s977R (5′-TTTACGGTTAGAACTAG-GGCGG-3′). The second set was 18s1713R A second set, 18s900F (5′-AAGACGGACTACAGCGAAAG-3′) and 18s1713R (5′-TCACCTACAGCTACCTTGTTACG-3′) (Olson et al., 2017). PCR products were separated on 1% agarose gels and visualised by staining with ethidium bromide. PCR products of sufficiently high quality were purified for cloning and sequencing by Invitrogen, Shanghai, China or Quintara Biosciences CA, USA.

Phylogenetic analysis

D2–D3 28S segments, and partial 18S rRNA sequences of different *Longidorus* and *Paralongidorus* species from GenBank were used for phylogenetic reconstruction. *Rotylenchus paravitis* (JX015422) for D2–D3 of 28S and *Tylencholaimus mirabilis* (EF207253), *Xiphinema rivesi* (HM921344) for 18S tree were selected as outgroup taxa for each dataset following previous published studies (He et al., 2005; Holterman et al., 2006; Gutiérrez-Gutiérrez et al., 2013; Archidona-Yuste et al., 2016). Multiple sequence alignments of the different genes were made using the Q-INS-i algorithm of MAFFT V.7.205 (Katoh and Standley, 2013). Sequence alignments were manually visualized using BioEdit (Hall, 1999) and edited by Gblocks ver. 0.91b (Castresana, 2000) in Castresana Laboratory server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences +1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). Percentage similarity between sequences was calculated using the sequence identity matrix in BioEdit. For that, the score for each pair of sequences was compared directly and all gap or place-holding characters were treated as a gap. When the same position for both sequences had a gap it was not treated as a difference. Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The best-fit model of DNA evolution was obtained using JModelTest V.2.1.7 (Darriba et al., 2012) with the Akaike Information Criterion (AIC). The best-fit
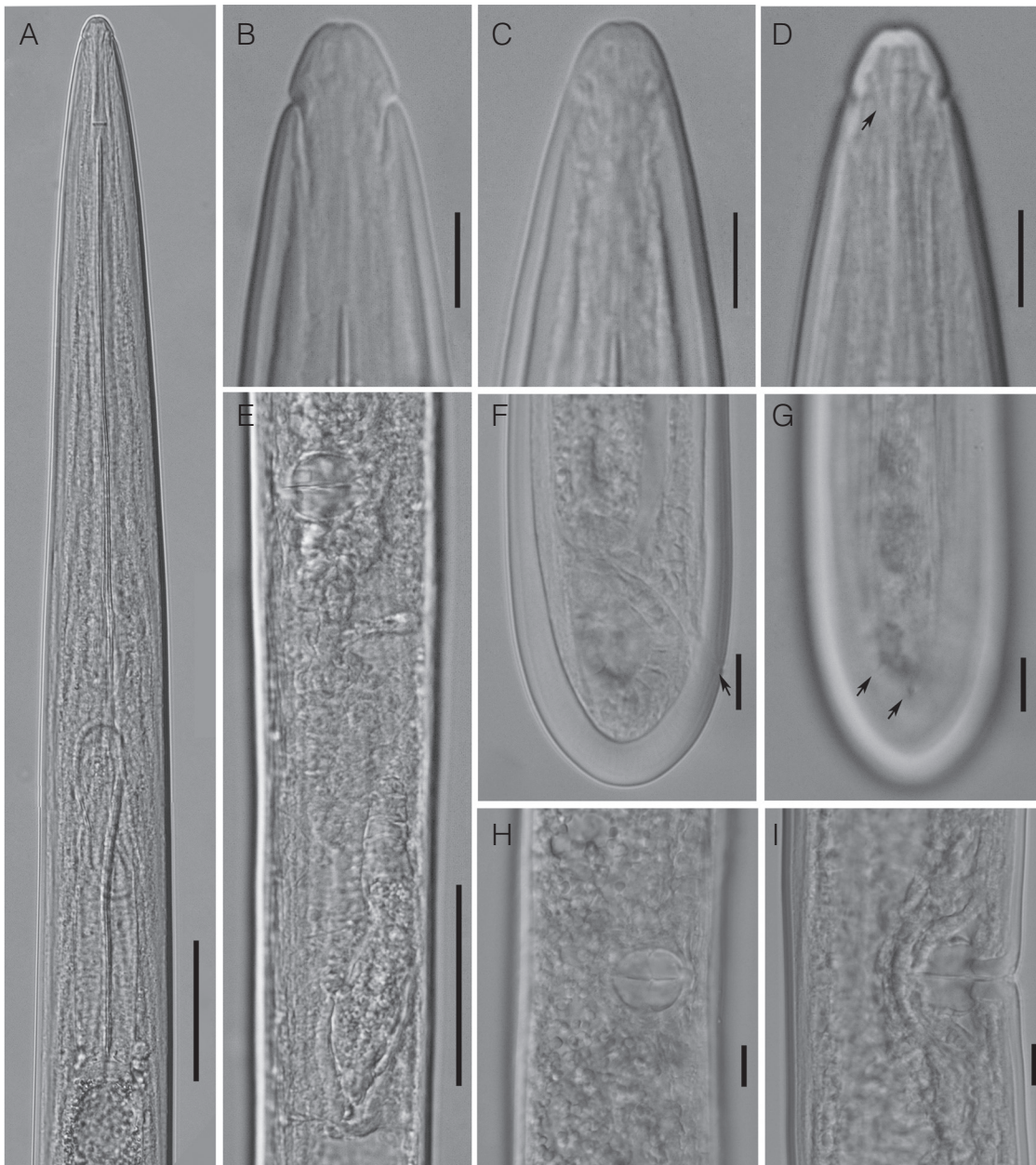
Figure 1: Light micrographs of *Paralongidorus sali* (Siddiqi et al., 1963). Female: A, Pharynx; B–D, Lip region arrow showing amphid; E, Gonad; F, Tail region arrow showing position of anus G, Tail region arrows showing position of caudal pores; H, Ventral view of vulva; I, Vulval region (Scale bars: A = 50 μm; B–D = 10 μm; E = 50 μm; F–I = 10 μm).

model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the AIC were then given to MrBayes for the phylogenetic analyses. General time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) for the D2–D3 segments and a transitional of invariable sites model with invariable sites and a gamma-shaped distribution (TIM2 + I + G) for the 18S rRNA gene.

These BI analyses were run separately per dataset using four chains for 2×10^6 generations for all of molecular markers. A combined analysis of the two genes was not undertaken due to some sequences not being available for all species. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses.
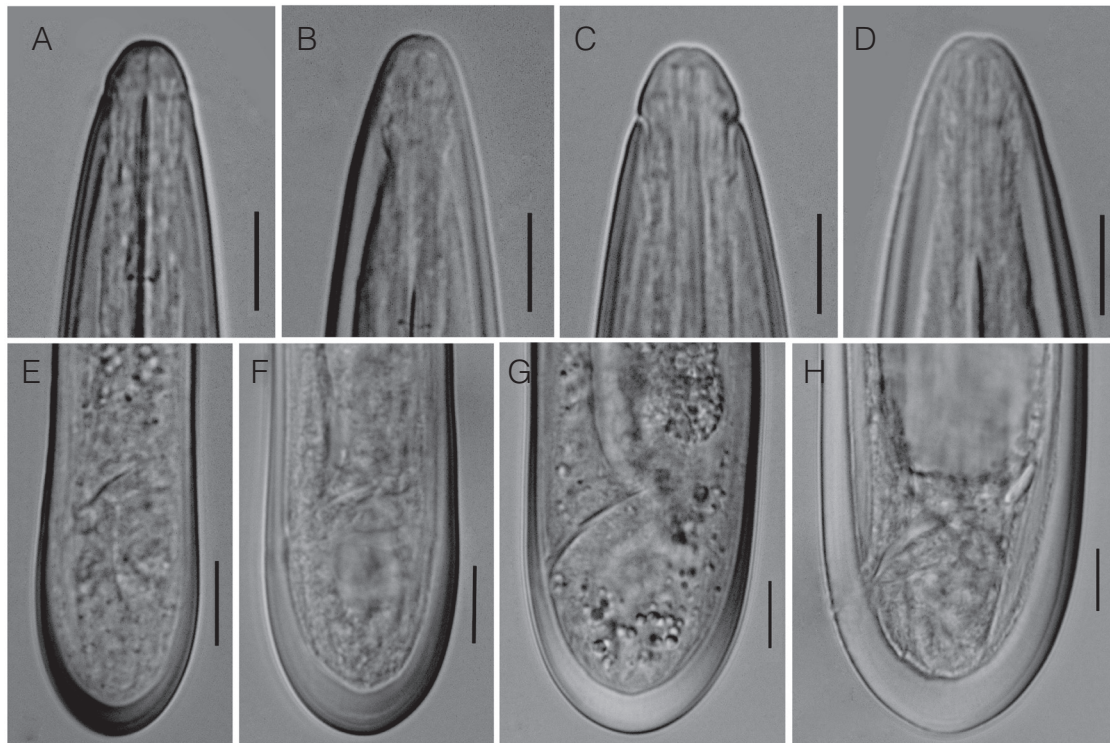
Figure 2: Light micrographs of *Paralongidorus sali* (Siddiqi et al., 1963). A–D, lip region of 1st, 2nd, and 3rd stage juveniles and female; E–H, Tail region of 1st, 2nd, and 3rd stage juveniles and female (Scale bars: A–H = 10 μm).

The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees from all analyses were visualized using FigTree software V.1.42 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

SYSTEMATICS
Paralongidorus sali (Siddiqi et al., 1963)
Description

Female

Body elongated, slender, slightly tapering anteriorly and posteriorly, open C-shape when heat relaxed. Cuticle appearing smooth, 3.9 (3.5–4.0) μm thick near vulva, 8.6 (7.0–10.0) μm thick at tip tail, and marked by very fine, superficial, transverse striae mainly, in tail region. Lip region dome-shaped, continuous with the rest of the body. SEM observations showed a slit-like oral aperture surrounded by six inner and six outer labial papillae in en face view, labial lobe distinct, amphidial fovea stirrup-shaped, with conspicuous, crescent-shaped amphidal aperture having slightly rounded margins. Odontostyle straight or slightly arcuate, twice the length of odontophore, odontophore weakly developed, with slight basal swellings. Guiding ring located anteriorly *ca* more than three times the lip region width distance from anterior end. Nerve ring encircling pharynx, located slightly posterior to middle of pharynx. Oesophagus dorylaimoid, typical of genus. Anterior slender part of oesophagus usually with loop overlapping basal bulb. Basal bulb cylindrical, 108 (104–115) μm long and 24.5 (24.0–25.0) μm in diam. Dorsal oesophageal gland nucleus located 28 to 32 μm from the anterior end of oesophageal bulb, two ventro-sublateral nucleus located (SV1 = 61.2–63.5, SV2 = 59.1–61.3) μm from the anterior end of pharyngeal bulb. Cardia elongated, conoid shaped 9.8 (8.5–11.0) μm long. Reproductive system didelphic with reflexed ovaries, vulva a transverse slit, vagina perpendicular to body axis *ca* less than half of corresponding body diam. Inconspicuous, oocytes arranged in multiple rows, pre-rectum variable in length 403 (359–445) μm and rectum 29.5 (28.5–30.5) μm long, anus a small slit. Tail short, hemispherical (Figs. 1–4, Tables 1, 2).
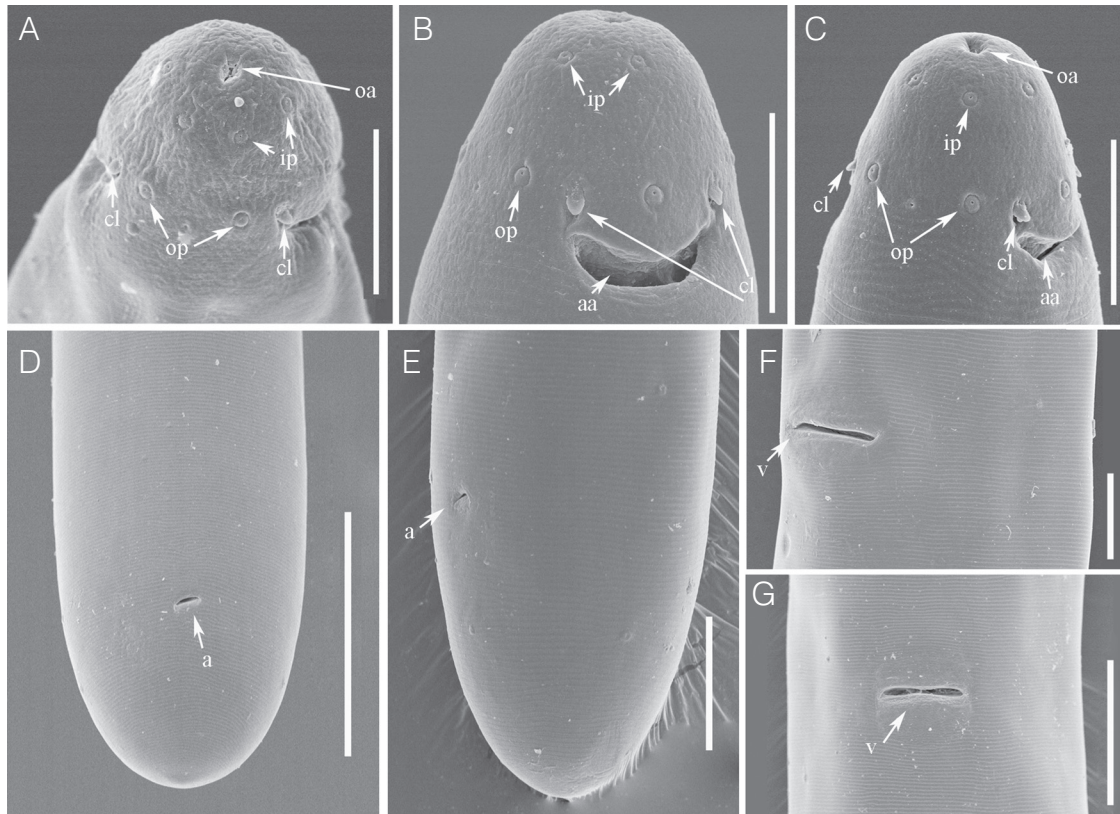
Male

Not found.

Figure 3: Scanning electron microscopy of *Paralongidorus sali* (Siddiqi et al., 1963). A–C, Female head region in lateral and ventrolateral view showing internal (ip) and outer labial papillae (op), cephalic lobe (cl), cephalic papillae (cp), oral aperture (oa), and amphidial aperture (aa). D–E, Female tail in lateral and ventral view (a = anus). F–G: Vulval region (v = vulva). (Scale bars: A–C = 5 μm; D = 30 μm; E, F = 10 μm; G = 20 μm).
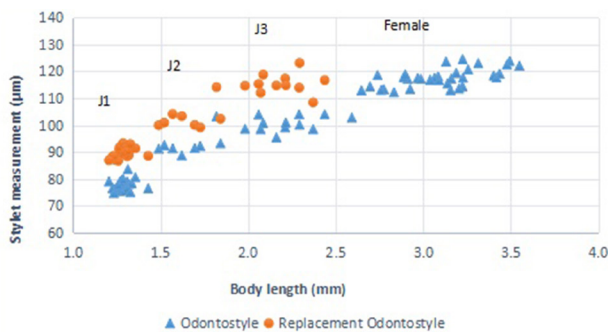

Figure 4: Relationship of body length to length of functional and replacement odontostyle (▲ = Odontostyle and ● = replacement odontostyle); length in three developmental stages and mature females of *Paralongidorus sali*.

Juveniles

Three juvenile stages (J1, J2, and J4) were found and they were basically similar to adults, except for their smaller size, shorter tails, and sexual characteristics (Fig. 4). Tail becomes progressively wider after each moult. Juvenile stages are distinguishable by relative body lengths, functional and replacement odontostyle (Robbins et al., 1995).

Locality and habitat

The population was found in the rhizosphere of *Castanopsis sclerophylla* from botanical garden, Hangzhou, Zhejiang Province, China on July 1, 2017. The geographical position of the sampling site was "30°15′46″N; 120°07″20″E."

Remarks

Paralongidorus sali was originally described from India by Siddiqi et al. (1963), later on it was reported from Korea by Choi and Duan (1998) and this is the first report from China. Males were not described in original or any other report; similarly males

Table 1. Morphometrics of *Paralongidorus sali* (Siddiqi et al., 1963). All measurements are in micrometer and in the form: mean±s.d. (range).

Characters/ratios	J1	J2	J4	Females
n	25	8	13	30
L	1290±46 (1205–1429)	1636±116 (1621–1695)	2199±204 (1819–2592)	3089±243 (2647–3494)
a	52.3±2.4 (46.3–56.8)	51.9±2.5 (48.9–56)	52.4±5.2 (43–57.8)	55.9±4.4 (49.3–64.6)
b	4.4±0.5 (3.8–5.9)	4.8±0.3 (4.6–5.3)	5.7±1 (3.9–7.6)	6.6±0.5 (5.7–7.8)
c	59.4±6.3 (48.5–78.9)	66.4–4.2 (58.8–73.1)	90.2±10.8 (71.5–109.4)	128.7±9.1 (111.9–142)
c'	1.17±0.11 (0.9–1.37)	0.97±0.12 (0.72–1.11)	0.78±0.08 (0.68–0.96)	0.65±0.05 (0.56–0.78)
V	–	–	–	48.6±1.7 (46.2–54.3)
Total stylet	127.8±3.4 (120.0–137.0)	148.2±3.2 (143.0–154.0)	158.2±5.0 (149.0–165.0)	186.8±4.4 (179.0–196.0)
Odontostyle	77.9±2 (75.0–84.0)	90.8±1.6 (89.0–94.0)	100.7±2.7 (95.0–104.0)	117.4±3.3 (112.0–124.5)
Odontophore	50.0±2.5 (44.0–54.0	56.7±2.6 (52.0–61.0)	58±4.2 (51.0–63.0)	68.9±1.9 (66.0–74.0)
Replacement Odontostyle	89.8±1.9 (87.0–93.0)	101.2±1.8 (99.0–104.0)	115.3±3.6 (108.0–123.0)	–
Oral aperture to guide ring	23.9±0.4 (23.0–25.0)	29.6±0.8 (28.0–31.0)	33.7±1.1 (32.0–35.0)	39.2±1.2 (37.0–41.0)
Lip region width	7.7±0.6 (7.0–9.0)	8.1±0.5 (7.0–9.0)	9.3±0.6 (9.0–10.0)	10.4±0.5 (9.0–11.0)
Body width at guide ring	14.9±0.4 (14.0–16.0)	17.3±0.5 (17.0–18.0)	20.4±0.8 (20.0–22.0)	23.4±0.7 (22.0–25.0)
Anal body width	18.8±0.8 (18.0–22.0)	24.5±1.9 (21.0–27.0)	31.2±2.2 (27.0–35.0)	36.7±1.9 (33.5–41.0)
Tail length	21.9±2.2 (17.0–26.5)	23.8±2.1 (20.0–27.0)	24.4±2.7 (22.0–31.0)	24±1.7 (21.0–27.0)
Anterior end to vulva	–	–	–	1497±111 (1287–1663)
Pharynx	298.2±25.8 (217.0–340.06)	341.1±24.6 (295–376.8)	393±40.7 (311–462)	470.7±32 (413.4–528.7)
Body width at vulva	–	–	–	53.8±5.5 (48–67.4)
Hyaline tail	4.8±0.4 (4.0–5.5)	5.8±0.5 (5.0–6.5)	6.6±0.7 (6.0–8.0)	8.5±0.7 (7.0–10.0)
Max body diameter	24.7±1.5 (22.5–28.0)	31.6±2.9 (27.0–36.0)	42.5±7.4 (33.0–60.0)	55.5±5.9 (45.0–69.0)

Table 2. Comparative morphometrics of females of *Paralongidorus sali* (Siddiqi et al., 1963) from different localities. All measurements are in micrometer and in the form: mean±s.d. (range).

	This study	Siddiqi et al. (1963)	Choi and Duan 1988
Locality	Hangzhou, China	India	Korea
Host	*Castanopsis sclerophylla* (Lindl.) Schottky	*Shorea robusta* Gaertn	*Pinus densiflora* L.
n	30	20	9
L	3.09±0.24 (2.65–3.49)	2.58 (2.25–2.85)	3.34±0.21 (3.0–3.63)
a	55.9±4.4 (49.3–64.6)	65 (60–71)	67.3±3.4 (61.7–71.7)
b	6.6±0.5 (5.7–7.8)	6.3 (5.2–7.4)	8.0±0.3 (7.6–8.6)
c	128.7±9.1 (111.9–142)	117 (107–129)	120.9±6.8 (110.9–131)
c′	0.65±0.05 (0.56–0.78)	–	0.7 (0.6–0.8)
V	48.6±1.7 (46.2–54.3)	51 (50–54)	48.1±1.0 (46.0–49.9)
Total stylet	186.8±4.4 (179.0–196.0)	–	–
Odontostyle	117.4±3.3 (112.0–124.5)	102 (98.0–107.0)	112.4±2.5 (109.0–117.0)
Odontophore	68.9±1.9 (66.0–74.0)	59 (52.0–62.0)	66.5±7.3 (54.0–84.0)
Oral aperture to guide ring	39.2±1.2 (37.0–41.0)	31 (30.0–32.0)	37.7±1.5 (34.0–40.0)
Lip region width	10.4±0.5 (9.0–11.0)	–	–
Body width at guide ring	23.4±0.7 (22.0–25.0)	–	–
Anal body width	36.7±1.9 (33.5–41.0)	–	–
Tail length	24±1.7 (21.0–27.0)	–	–
Anterior end to vulva	1497±111 (1287–1663)	–	–
Pharynx	470.7±32 (413.0–529.0)	–	–
Body width at vulva	53.8±5.5 (48.0–67.0)	–	–
Hyaline tail	8.5±0.7 (7.0–10.0)	–	–
Max body diameter	55.5±5.9 (45.0–69.0)	–	–
Presence/absence males	Absence	Absence	Absence

were also not found in this population as well. The Chinese population is slightly longer than the original description and subsequently has slightly longer odontostyle (112–124.5 vs. 98–107) μm and odontophore (66–74 vs. 52–62) μm but all the morphometric variation is in the range of Korean population. The morphology fits well with the original description except, for slight morphometrical values. We consider these small intraspecific differences are due to the geographical variability.

Longidorus jonesi (Siddiqi, 1962)
Description

Female

Body cylindrical, slightly tapering towards anterior end, open C- to spiral shape when heat relaxed. Cuticle appearing smooth, 3.7 (3.5–4.0) μm thick vulva, 7.9 (7.0–10.0) μm thick at tail tip. Lip region rounded, continuous with the rest of the body. SEM observations showing a slit-like oral aperture surrounded by six inner and six outer labial papillae en face view, amphidial fovea pouch like with pore-like amphidial apertures. Stylet guiding ring single, located 5.2–5.3 times lip region diameter from anterior end. Odontostyle long and narrow,
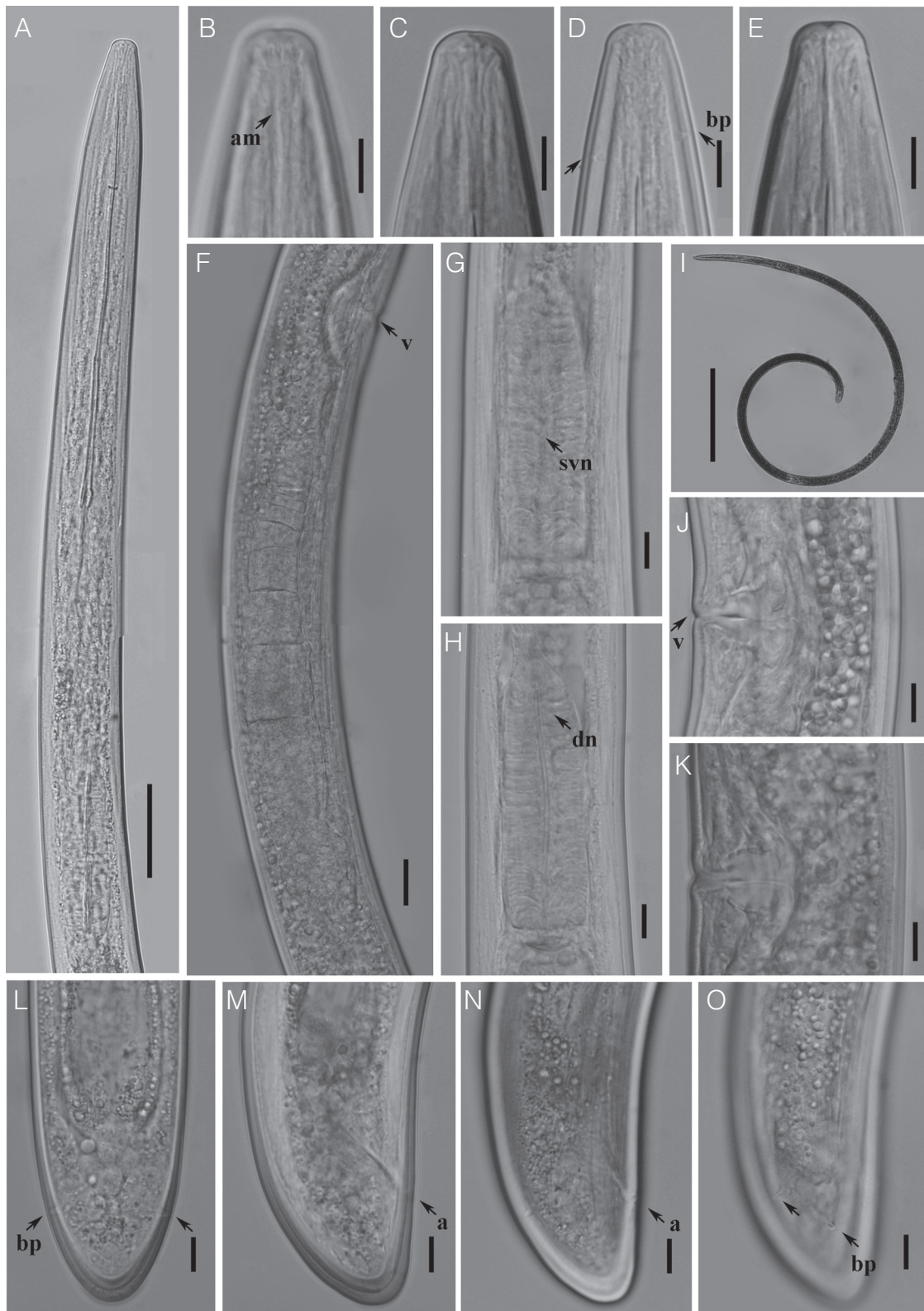
Figure 5: Light micrographs of *Longidorus jonesi* (Siddiqi, 1962). Female: A, Pharynx; B–E, Lip regions; F, Gonad; G–H, Pharyngeal bulb; I, Entire female body; J–K, Vulval regions; L, ventral view of tail; M–O, Female tails (Scale bars: A = 50 μm; B–E, G–H, J–O = 10 μm; F = 20 μm; I = 500 μm) am = amphid; bp = body pores; v = vulva; svn = subventrolateral nuclei; dn = dorsal nuclei; a = anus).

1.7 (1.6–1.9) times as long as odontophore, straight or slightly arcuate, odontophore moderately developed, with slightly swollen base, not set-off from esophageal contour. Oesophagus extending to a terminal oesophageal bulb with three nuclei, with one dorsal gland nucleus located at the beginning of bulb, i.e. (22%–23%) of the oesophageal bulb length, while the other two sub-ventro-lateral nuclei located around the middle of bulb. Oesophageal basal bulb (91.4–112) long and (22–27) μm wide. Oesophageal intestinal valve conoid-oblong, 9.6 (9.0–10.5) μm long. Reproductive system with both genital branches equally developed, each 519 (439–577) μm long, with reflexed ovaries variable in length. Vulva in form of a transverse slit, located about mid-body, vagina perpendicular to body axis, less than half of corresponding body width, surrounded by well-developed muscles. Ovaries paired, roughly symmetrical, with oocytes arranged in a single file. Both sets of reproductive organs lying on left side of intestine. Pre-rectum distinct.

Rectum about less than one anal body width long, anus a transverse slit. Tail conoid, obtusely rounded, with two pairs of caudal pores (Figs. 5–9; Tables 3, 4).

Male

Very rare, morphologically similar to female except for genital system. Male genital tract diorchic with testes opposed, containing multiple rows of spermatogonia. Tail conoid, rounded, with 7 to 8 adcloacal supplements.

Juveniles

Four juvenile stages (J1, J2, J3, and J4) were found and they were basically similar to adults, except for their smaller size, shorter tails, and sexual characteristics (Fig. 9). The first-stage juvenile of *L. jonesi* was
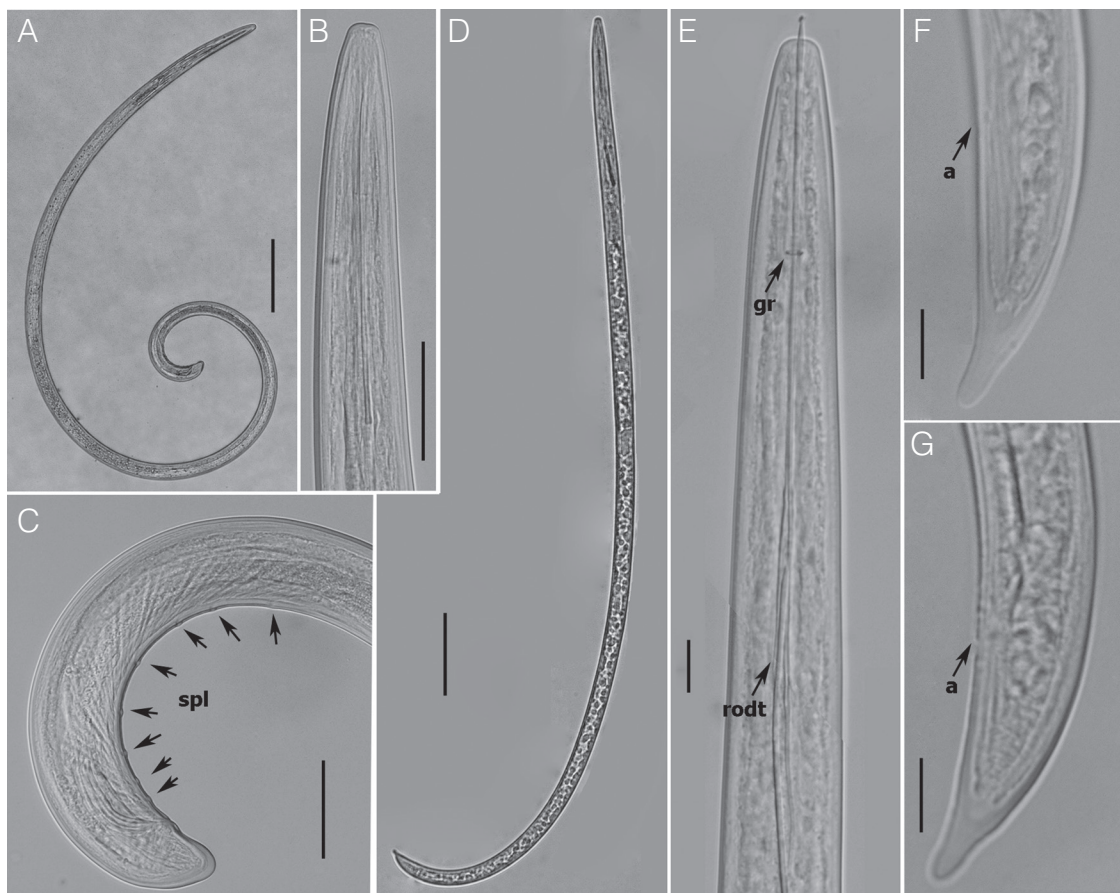

Figure 6: Light micrographs of *Longidorus jonesi* (Siddiqi, 1962). Male and first-stage juvenile. A, Entire body of male; B, Anterior region of male; C, Tail region of male arrows showing position of supplements (spl); D, Entire body of J1; E, ANterior region of J1 arrows showing position of guiding ring (gr) and replacement odontostyle (rodt); F–G, Tail regions of J1. (Scale bars: A = 200 μm; B–C = 40 μm; D = 100 μm; E–G = 10 μm).
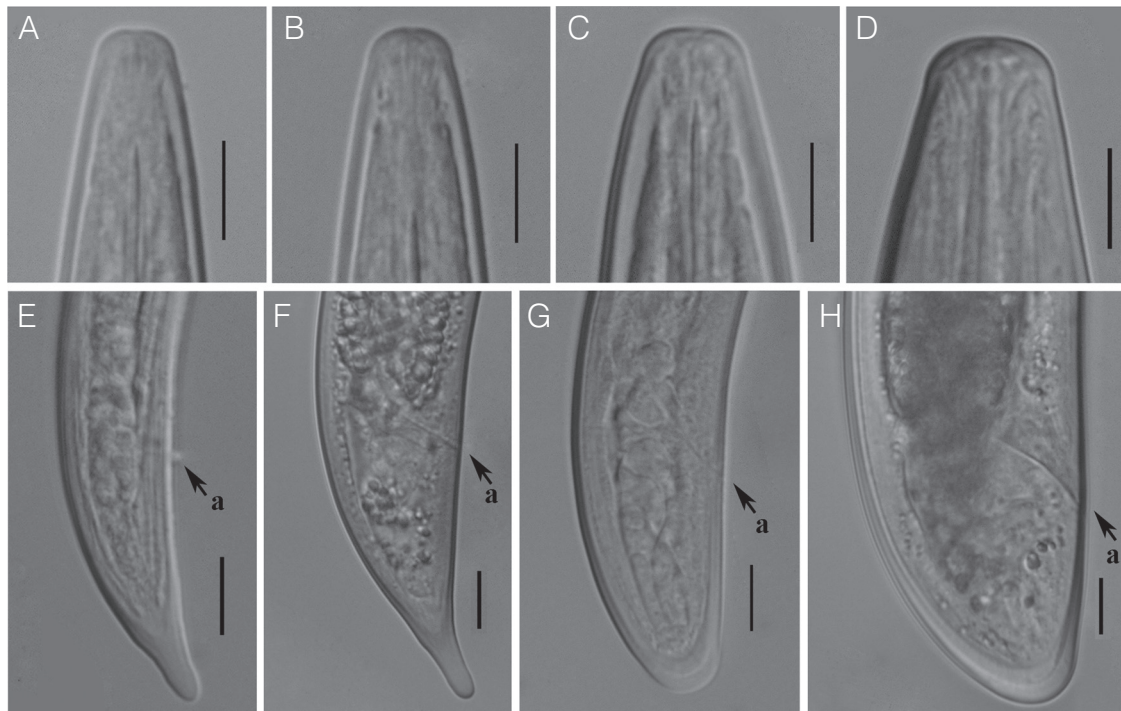
Figure 7: Light micrographs of *Longidorus jonesi* (Siddiqi, 1962). A–D, lip region of 1st, 2nd, 3rd, and 4th stage juveniles; E–H, Tail region of 1st, 2nd, 3rd, and 4th stage juveniles (Scale bars: A–H = 10 μm).

characterized by the position of replacement odontostyle into odontophore, just posterior to base of functional odontostyle. Siddiqi (1962) mentioned three groups of juveniles where the first group have tails with elongated conoid and rounded terminus whereas the other groups have tails similar to the female. The first-stage and second-stage juveniles of the Hangzhou population fits well with this description as they both have a long conoid, rounded peg, except the J1 has a slimmer and shorter tail as compared with the J2. The tails of other stages becomes progressively wider after each moult. All of the stages are distinguishable by relative body lengths, functional, and replacement odontostyle (Robbins et al., 1995).

Locality and habitat

The population was found in the rhizosphere of *Cyclobalanopsis glauca* from botanical garden, Hangzhou, Zhejiang Province, China on July 1st, 2017. The geographical position of the sampling site was "30°15′18″N; 120°06′60″E."

Remarks

Longidorus jonesi was originally described from India by Siddiqi (1962), later on it was reported from Jiangsu Province of China by Xu and Hooper (1990). They have found *L. jonesi* from two localities, i.e., Nanjing and Suzhou. Palomares-Rius et al. (2014) reported another population from Japan. The original description and none of the reported population described the first-stage juvenile or male. In the population found in Hangzhou, Zhejiang Province, the first-stage juvenile and male were detected and described for the first time. The females of Hangzhou population showed slightly smaller *V* value (44–51.8 vs. 50–52.4) as compared with original description, odontostyle (138–143 vs. 107–120) μm and odontophore (71–87 vs. 66–73) μm were slightly longer, but these morphometrics correspond well with the Japanese population. The morphology fits well with the original description, except for slight morphometrical values. We consider, these small intraspecific differences are due to the geographical variability.

Phylogenetic relationships of Paralongidorus sali *and* Longidorus jonesi

Amplification of D2–D3 expansion segment of 28S rRNA and the partial 18S rRNA from *P. sali* and *L. jonesi* yielded a single fragment of *ca* 800 and 1,700 bp, respectively. Four new D2–D3 of 28S rRNA and four partial 18S rRNA gene sequences were obtained
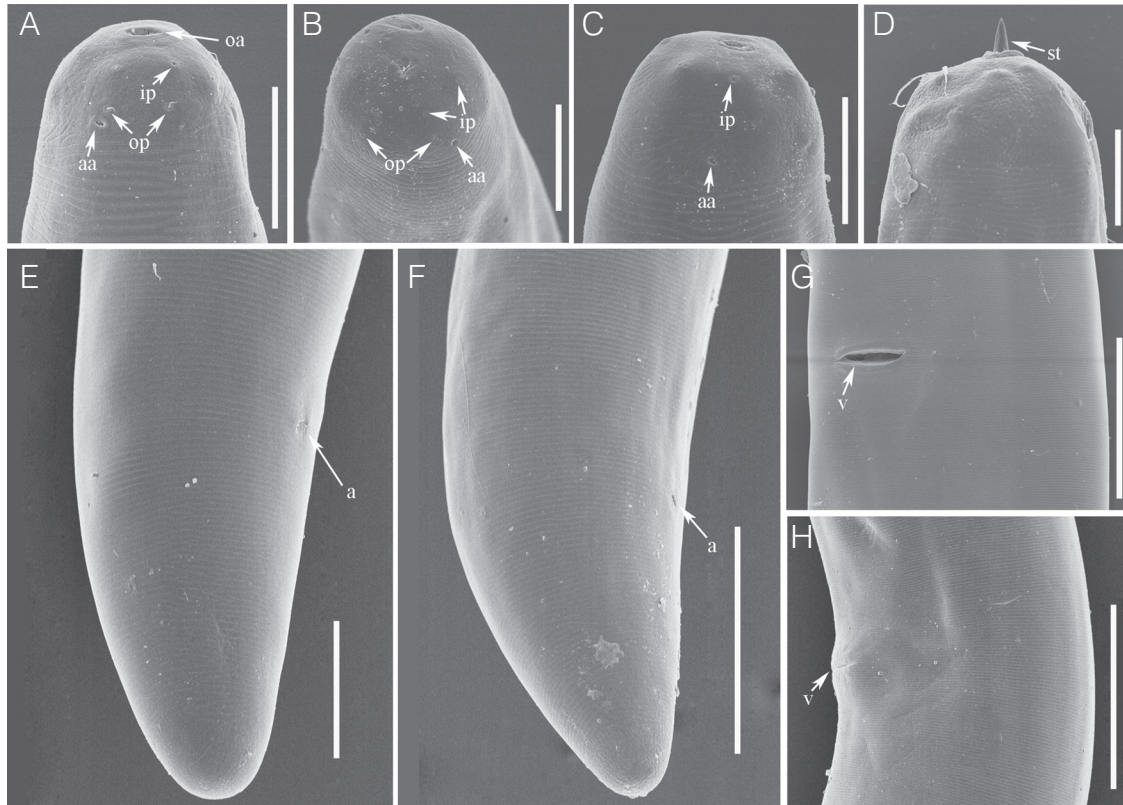
Figure 8: Scanning electron microscopy of *Longidorus jonesi* (Siddiqi, 1962). A–D, Female head region in lateral and ventrolateral view showing internal (ip) and outer labial papillae (op), oral aperture (oa), stylet (st), and amphidial aperture (aa); E–F, Female tail in lateral view (a = anus); G–H, Vulval region in lateral and ventral view (v = vulva). (Scale bars: A–D = 5 μm; E = 10 μm; F = 20 μm; G–H = 30 μm).

in the present study. D2–D3 expansion segments of 28S rRNA sequences of *P. sali* (MG729700– MG729701) showed 88% similarity with *P. bikane-*
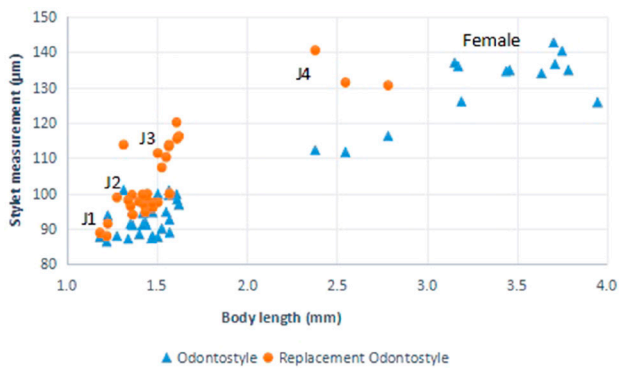

Figure 9: Relationship of body length to length of functional and replacement odontostyle (▲ = Odontostyle and ● = replacement odontostyle); length in three developmental stages and mature females of *Longidorus jonesi*.

rensis (Lal and Mathur, 1987; Siddiqi et al., 1993, JN032584) (differing in 94 nucleotides and 32 indels), 81% similarity with *P. lusitanicus* (Gutiérrez-Gutiérrez et al., 2017, KY750562) (differing in 156 bp and 71 indels); and 84-83% similarity with some *Longidorus* species such as *L. macrosoma* (Hooper, 1961, AY580055, AY601565) or *L. helveticus* (Lamberti et al., 2001, JN627415), differing in 130 to 132 bp and 52 indels. The partial 18S rRNA sequence for *P. sali* (MG729696–MG729697) showed high similarity (99– 98% similar) with several *Paralongidorus* spp. such as *P. bikanerensis* (JN032586), *P. maximus* (Bütschli, 1874, AJ875152), and *P. litoralis* (Palomares-Rius et al., 2008, EU026158), differing in a range from 13 to 29 nucleotides and 7 to 8 indels. Intraspecific sequence diversity (uncorrected p-distance) of ribosomal markers among the studied specimens for *P. sali* was small (8–22 bp, 2–8 indels).

Finally, the Chinese population of *L. jonesi* (MG729702–MG729703) showed high similarity (99%) for D2–D3, 6 to 8 nucleotides and 1 to 4 indels with *L. jonesi* from Japan (KF552069). In addition, the

Table 3. Morphometrics of *Longidorus jonesi* (Siddiqi, 1962). All measurements are in *µ*m and in the form: mean ± s.d. (range).

Characters/ratios	J1	J2	J3	J4	Females	Males
n	4	20	7	3	9	2
L	1227 ± 39 (1183–1278)	1440 ± 62 (1340–1571)	1603 ± 79 (1505–1756)	2570 ± 203 (2379–2784)	3534 ± 242 (3153–3785)	2619 ± 243 (2447–2791)
a	54.5 ± 4.2 (48.7–58.4)	54.4 ± 2.4 (50.7–59.2)	51.8 ± 4.0 (44.5–57.6)	52.6 ± 3.8 (48.3–55.6)	58.8 ± 3.2 (54.6–64)	50.1 ± 7 (45.1–55)
b	4.5 ± 0.1 (4.4–4.6)	5.4 ± 0.6 (4.6–6.8)	4.8 ± 0.6 (4.2–5.4)	5.8 ± 0.5 (5.4–6.4)	7.7 ± 0.4 (7.2–8.2)	5.9 ± 0.8 (5.3–6.5)
c	31.9 ± 2.9 (29.3–35.7)	34.3 ± 2.6 (31.3–42.5)	49.4 ± 5.8 (44.8–61.3)	72.5 ± 7.9 (67.9–81.6)	104 ± 9 (89.1–116.3)	70 ± 4.6 (66.8–73.3)
c′	2.4 ± 0.1 (2.3–2.5)	2.2 ± 0.2 (1.8–2.4)	1.35 ± 0.15 (1.1–1.6)	1 ± 0.1 (0.87–1.07)	0.84 ± 0.07 (0.73–0.92)	0.98 ± 0.07 (0.93–1.03)
V/T	–	–	–	–	48.3 ± 3.3 (44–51.8)	84.7 ± 4.7 (81.4–88.0)
Total stylet	133.2 ± 2.3 (130.0–135.0)	138.1 ± 3.3 (130.0–143.0)	152.7 ± 5.8 (145.0–160.0)	183 ± 5.4 (179–189.0)	216.2 ± 8 (204.0–229.0)	198.8 ± 7 (193.8–203.7)
Odontostyle	89 ± 3.3 (86.0–94.0)	91.3 ± 2.6 (87.0–95.5)	98.7 ± 2.2 (95.0–101.0)	113.4 ± 2.5 (112–116.0)	136.8 ± 2.9 (138.0–143.0)	129.3 ± 12.1 (120.7–137.8)
Odontophore	44.8 ± 2.6 (42.0–47.0)	47 ± 2 (43.0–50.0)	53.3 ± 5.7 (46.0–60.0)	69.7 ± 6.7 (65.0–77.0)	80.3 ± 5.9 (71.0–87.0)	73.2 ± 3.7 (70.6–75.8)
Replacement Odontostyle	91.8 ± 4.9 (88.0–99.0)	98 ± 1.8 (94.0–101.0)	115 ± 4.1 (110.0–121.0)	134.2 ± 5.5 (131–140.5)	–	–
Oral aperture to guide ring	40 ± 2.1 (37.0–42.0)	39.1 ± 1.6 (36.0–42.0)	51.8 ± 3 (45.0–54.0)	61.3 ± 2.7 (58.0–63.0)	75.9 ± 3.7 (71.0–82.0)	71.6 ± 0.2 (71.5–71.8)
Lip region width	7.9 ± 0.3 (7.5–8.0)	8 ± 0.2 (7.5–9.0)	10 ± 0.4 (9.5–10.5)	12.5 ± 0.4 (12.0–13.0)	14.5 ± 0.7 (14.0–15.5)	14.9 ± 0.28 (14.7–15.1)
Body width at guide ring	16.8 ± 0.5 (16.0–17.5)	17.3 ± 0.7 (16.0–19.0)	21.6 ± 1.3 (20.0–24.0)	29.3 ± 1.8 (28.0–31.0)	35 ± 2.1 (32.5–38.0)	33.9 ± 1.8 (32.6–35.1)
Anal body width	16 ± 1.7 (15.0–16.5)	19 ± 1.7 (17.0–25.0)	24.3 ± 1.5 (22.0–26.0)	36.1 ± 3.2 (33.0–39.0)	40.1 ± 3.6 (35.0–46.0)	38.2 ± 3.4 (35.8–40.6)
Tail length	38.7 ± 3 (34.0–41.0)	42.2 ± 3 (35.0–47.0)	32.7 ± 3.1 (26.0–36.0)	35.5 ± 1.8 (34.0–37.5)	33.1 ± 3.6 (27.0–38.0)	37.6 ± 5.9 (33.4–41.8)
Anterior end to vulva	–	–	–	–	1726.5 ± 92.6 (1610–1903)	–
Pharynx	275.6 ± 13.1 (265.0–290.0)	267.9 ± 30.1 (199.0–299.0)	341 ± 44.2 (303.0–397.0)	442.2 ± 26.8 (421–472.5)	451.7 ± 36 (404.0–504.0)	443.7 ± 19.7 (429.7–457.6)
Body width at vulva	–	–	–	–	58.5 ± 5.5 (53.0–66.0)	–
Hyaline tail	11.2 ± 1.2 (10.0–13.0)	11.6 ± 1.3 (9.0–14.5)	6.3 ± 0.4 (6.0–7.0)	7.3 ± 0.7 (6.5–8.0)	7.9 ± 1.1 (7.0–10.0)	6.5 ± 1.3 (5.6–7.4)
Max body diameter	22.6 ± 1.9 (21.0–25.0)	26.9 ± 2.9 (24.0–37.0)	31.3 ± 3 (27.0–36.0)	49.3 ± 7.6 (43.0–58.0)	60.3 ± 5.4 (54.5–67.0)	52.5 ± 2.5 (50.7–54.2)
Supplements	–	–	–	–	–	7–8
Spicule	–	–	–	–	–	63.4 ± 2.6 (61.5–65.2)

Table 4. Comparative morphometrics of female of *Longidorus jonesi* (Siddiqi, 1962) from different localities. All measurements are in micrometer and in the form: mean ± s.d. (range).

		Xu and Hooper 1990	Xu and Hooper 1990	Siddiqi et al. (1962)	Palomares-Rius et al. (2014)
Locality	This study	Nanjing (China)	Suzhou (China)	India	Japan
Host	*Cyclobalanopsis glauca* (Thumb.) Oerst.	*Prunus persica* (L.) Bastch & *Curpressus funebris* Endl.	*Prunus mune* Sleb.)	*Prunus armeniaca* L.	*Prunus sp.*
n	9	15	3	20	22
L (mm)	3.53 ± 0.24 (3.15–3.79)	3.92 ± 0.32 (3.57–4.86)	4.37 (3.97–4.81)	3.43 (3.17–3.48)	3.6 ± 0.3 (2.9–3.9)
a	58.8 ± 3.2 (54.6–64)	66 ± 4.7 (58–77)	64 (61–66)	66 (61–75)	67.1 ± 7.7 (55.2–84.8)
b	7.7 ± 0.4 (7.2–8.2)	9.6 ± 1.7 (7.9–14.4)	12.0 (10.9–14.3)	8.6 (8–9.3)	8.1 ± 1.4 (6.5–12.4)
c	104 ± 9 (89.1–116.3)	131 ± 14.3 (116–170)	122 (113–128)	167 (140–185)	113.3 ± 11.7 (95.9–131.5)
c′	0.84 ± 0.07 (0.73–0.92)	0.8 ± 0.07 (0.7–1.0)	0.8 (0.8–0.9)	0.75 (0.6–0.87)	0.8 ± 0.1 (0.7–0.9)
V	48.3 ± 3.3 (44–51.8)	49 ± 1.6 (47–52)	47 (46–48)	50.8 (50–52.4)	49.7 ± 1.3 (47.2–51.4)
Total stylet	216.2 ± 8 (204.0–229.0)	190 ± 5.6 (182.0–206.0)	194 (192.0–195.0)	182.7 (174.0–192.0)	–
Odontostyle	136.8 ± 2.9 (138.0–143.0)	118 ± 4.6 (109.0–131.0)	119 (117.0–120.0)	113 (107.0–120.0)	132.9 ± 5.6 (123.0–142.5)
Odontophore	80.3 ± 5.9 (71.0–87.0)	72 ± 3.4 (66.0–77.0)	75 (74.0–76.0)	68.5 (66.0–73.0)	70.5 ± 4.7 (63.0–82.5)
Oral aperture to guide ring	75.9 ± 3.7 (71.0–82.0)	68.7 ± 2.7 (64.0–72.0)	71.6 (68.0–74.0)	61.5 (57.0–66.0)	71.3 ± 3.3 (64.5–76.5)
Lip region width	14.5 ± 0.7 (14.0–15.5)	13.8 ± 0.5 (13.0–15.0)	13.8 (13.5–14.0)	–	12.9 ± 0.7 (12.0–14.0)
Body width at guide ring	35 ± 2.1 (32.5–38.4)	33.8 ± 1.4 (31.8–36.4)	33.7 (32.8–34.6)	–	–
Anal body width	40.1 ± 3.6 (35.4–45.6)	36.8 ± 2.1 (34.1–41.9)	40.0 (37.3–42.8)	–	–
Tail length	33.1 ± 3.6 (27.0–38.0)	30.1 ± 2.5 (25.0–34.0)	33.8 (31.0–35.5)	–	32.0 ± 2.8 (27.0–38.0)
Pharynx	451.7 ± 36 (404.0–504.0)	–	–	–	–
Anterior end to vulva	1726.5 ± 92.6 (1610–1903)	–	–	–	–

Hyaline tail	7.9 ± 1.1 (7.0–10.0)	–	–	–	–
Max body diameter	60.3 ± 5.4 (54.5–67.0)	59.4 ± 5.0 (52.0–68.0)	68.7 (65.5–72.8)	–	–
Presence/ absence males	Absence	Absence	Absence	Absence	Absence

two new partial 18S rRNA sequences of *L. jonesi* (MG729698–MG729699) showed high similarity (99%–98%, respectively) with *L. litchi* (Xu and Cheng, 1992, AY687996) and *L. diadecturus* (Eveleigh and Allen, 1982,AY283167, AY283166), differing in 25 to 33 nucleotides and 6 indels.

Phylogenetic trees reconstructed by the BI method for the two rRNA markers (D2–D3 expansion regions of 28S rRNA gene and the partial 18S rRNA) are presented in Figs. 10, 11. The D2–D3 segments of 28S rRNA gene tree based on a multiple edited alignment (100 sequences) of 749 total characters revealed a major clade for the majority of the *Paralongidorus* species, excluding *P. bikanerensis* and *P. sali* (Fig. 10). This tree is similar to the most recent phylogenetic analysis showed by Gutiérrez-Gutiérrez et al. (2017), Barsi and Luca (2017), and Palomares-Rius et al. (2013) showing *P. bikanerensis* in a position outside of the main clade for *Paralongidorus*. *Longidorus jonesi* is well-related phylogenetically to accession described from Japan (KF552069) (Palomares-Rius et al., 2013).

Similarly, the 50% majority rule consensus BI tree of a multiple alignment including 96 18S rRNA sequences and 1,679 bp alignment length (Fig. 11) showed a clear phylogenetic relationship of *P. sali* with *P. bikanerensis* in both datasets and also outside of the main clade for *Paralongidorus*.

Discussion

Longidorus species are widely distributed in China. Until now, fifteen *Longidorus* species (viz. *L. camelliae* (Zheng et al., 2003) *L. fangi* (Xu and Cheng, 1991), *L. fursti* (Heyns et al., 1987), *L. hangzhouensis* (Zheng et al., 2003), *L. henanus* (Xu and Cheng, 1992), *L. jiangsuensis* (Xu and Hooper, 1990), *L. jonesi*, *L. juglans* (Xu et al., 2017), *L. litchii* (Xu and Cheng, 1992), *L. macromucronatus* (Siddiqi, 1962), *L. martini* (Merny, 1966), *L. moniloides* (Heyns, 1966), *L. pawneensis* (Luc and Coomans, 1988), *L. pisi* (Edward et al., 1964) and *L. intermedius* (Kozlowska and Seinhorst, 1979)) have been reported from 13 provinces, i.e., Anhui, Beijing, Fujian, Gansu, Hebei, Henan, Hunan, Jiangsu, Liaoning, Shandong, Shanxi, Yunnan and Zhejiang (Guo et al., 2011; Xu et al., 2017).

To date, there are no reports of *Paralongidorus* species from China, this genus is known to have global distribution with maximum diversity found from Asia and Africa (Palomares-Rius et al., 2008). Similarly, *P. sali* was originally described from India and later on it was reported from Korea and now it has also been found in China. In the same way, the other longidorid species under investigation, i.e., *L. jonesi*, was originally described from India and later on it was only reported from China and Japan, suggesting the possible prevalence of both species is localized in Asian countries.

Our phylogenetic analysis based on 18S and D2–D3 expansion segments of 28S sequences *L. jonesi* clustered well with *L. jonesi* from Japan and other *Longidorus* species while in both trees *P. sali* clustered with *P. bikanerensis*. In 18S tree, *P. sali* and *P. bikanerensis* form a separate clade with species of *Longidorus* and *Paralongidorus* (Fig. 11) while in 28S tree both species forms a separate clade with *Longidorus* species (Fig. 10). These phylogenetic results are congruent with recently published data by Pedram et al. (2012), Palomares-Rius et al. (2013), Gutiérrez-Gutiérrez et al. (2017), or Barsi and Luca (2017). Based on morphological and SEM observations, both species belongs to the genus *Paralongidorus* and interestingly both species originally were described from India. Considering the separate position of *P. sali* and *P. bikanerensis* in phylogenetic trees we assume these two species are molecularly intermediate between *Longidorus* and *Paralongidorus*. However, we strongly emphasized the need of further study to support this assumption. In the past, longidorid species identification was mainly based on morphological characters and hierarchical cluster analysis (Ye and Robbins, 2003, 2004, 2005), however, with the advent of molecular sequencing and phylogenetic studies, the species identification is more reliable and equitable. In conclusion, this study provided a first record of the occurrence of *Paralongidorus* species from China coupled with detail morphological and molecular characterisation of *P. sali*, description of the first-stage juvenile of *L. jonesi*, additionally provided the SEM observations of both species in order to
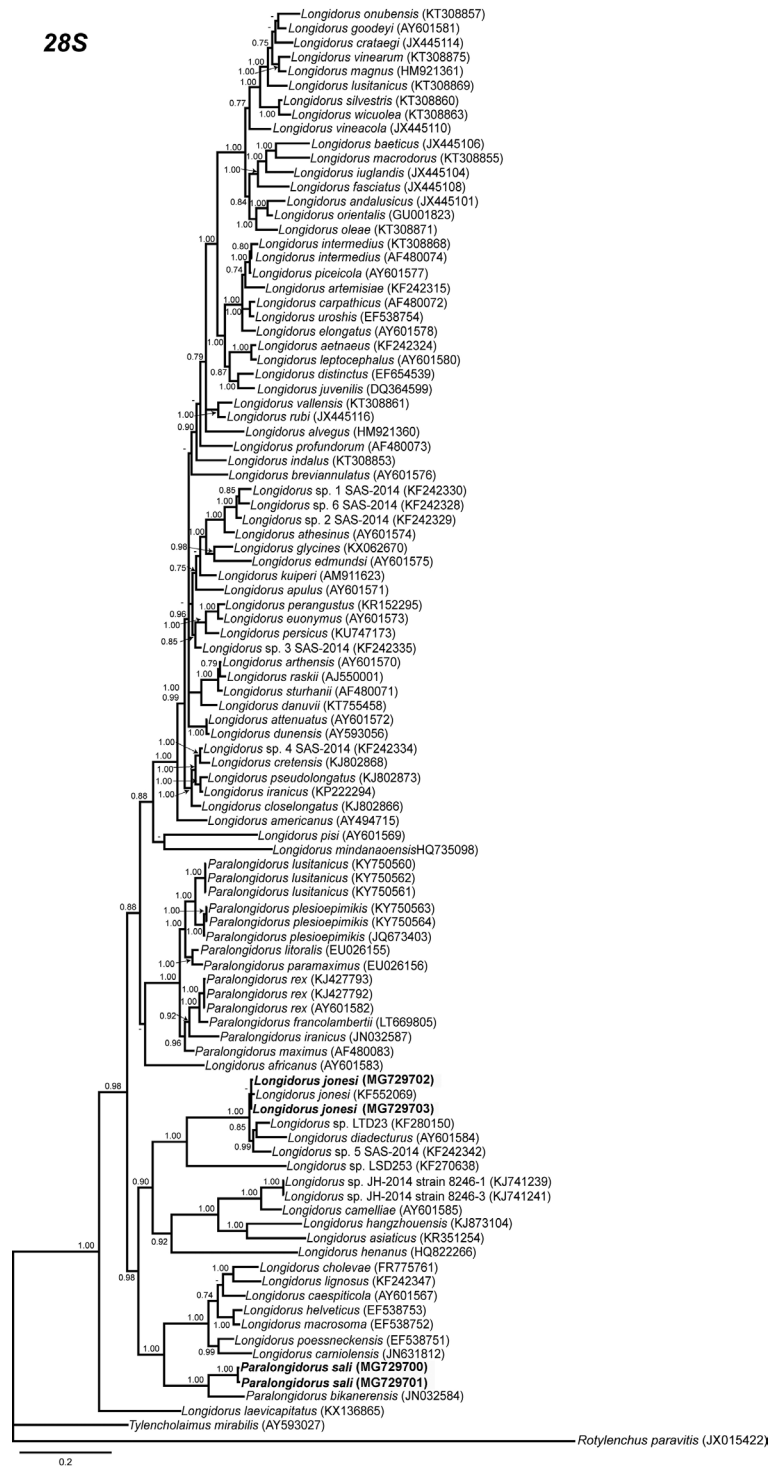
Figure 10: Phylogenetic relationships within *Longidorus* and *Paralongidorus*. Bayesian 50% majority rule consensus tree as inferred from D2 and D3 expansion segments of 28S rRNA sequence alignment under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR+I+G: –lnL = 14601.9935; AIC = 29619.9870; freqA = 0.2204; freqC = 0.2274; freqG = 0.2934; freqT = 0.2588; R(a) = 0.7487; R(b) = 2.4740; R(c) = 1.4407; R(d) = 0.3992; R(e) = 4.6932; R(f) = 1.0000; Pinva = 0.2290; and Shape = 0.6290). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. Scale bar = expected changes per site.
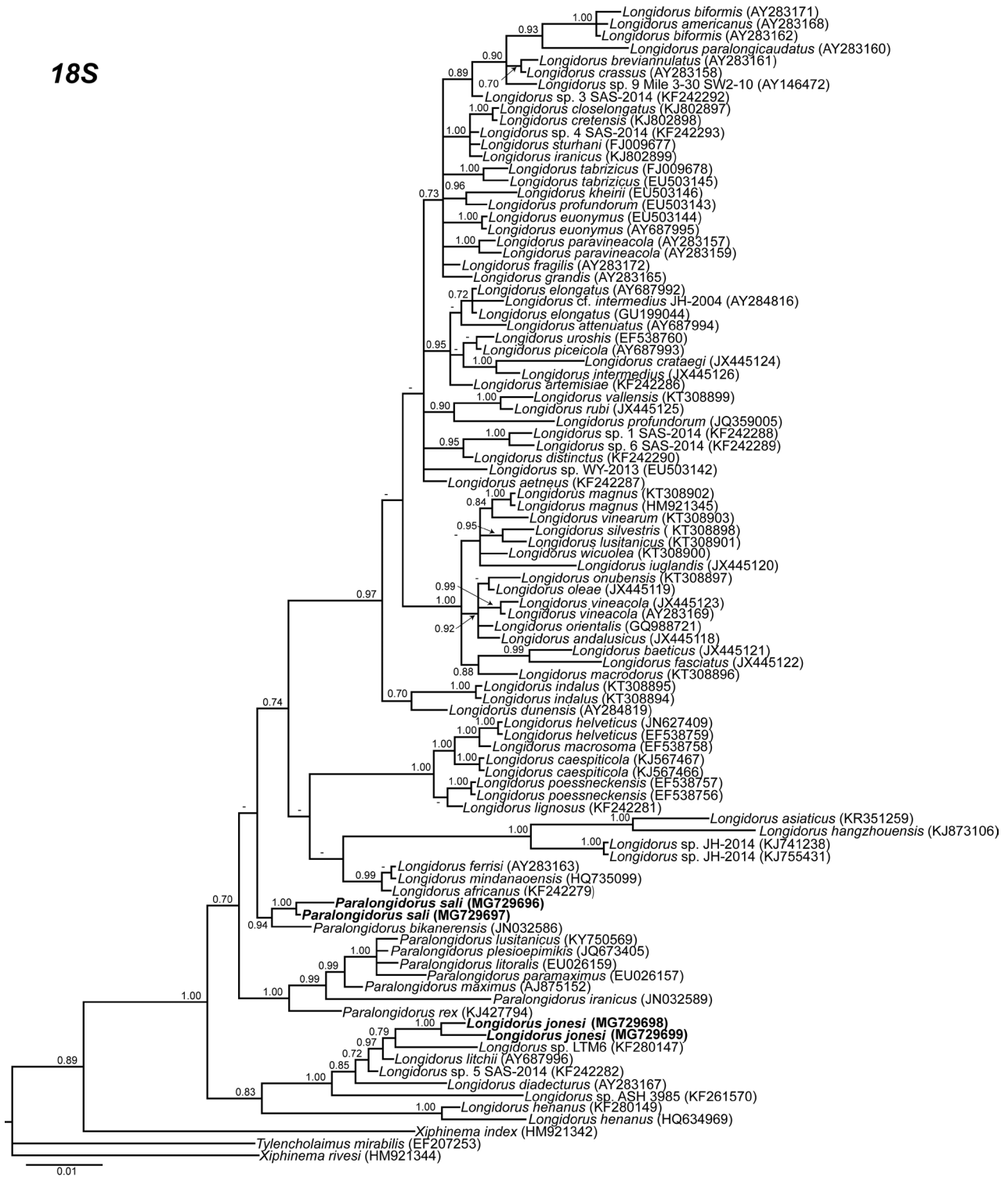
18S

Figure 11: Phylogenetic relationships within *Longidorus* and *Paralongidorus*. Bayesian 50% majority rule consensus tree as inferred from 18S rRNA gene sequence alignment under a transitional of invariable sites model with invariable sites and a gamma-shaped distribution (TIM2 +I+G: −lnL = 6866.9821; AIC = 14129.9643; freqA = 0.2626; freqC = 0.2109; freqG = 0.2668; freqT = 0.2597; R(a) = 1.8892; R(b) = 3.9662; R(c) = 1.8892; R(d) = 1.0000; R(e) = 7.1009; R(f) = 1.0000; Pinva = 0.7060; and Shape = 0.6020). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. Scale bar = expected changes per site.

elucidate the lip morphology and amphidial aperture of both species in detail. The systematics and diagnostics of Longidorid nematodes are important because of regulatory and management issues attributed to this group of nematodes as virus vectors. Thus, we suggest updated descriptions from accurately identified specimens, collection of sufficient materials for examination and close observations based on our present knowledge and molecular analysis are necessary for better understanding of the current distribution and host association of longidorid nematodes.

Acknowledgments

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