

Schistonchus fistulosus n. sp. (Nematoda: Aphelenchoididae) associated with *Ficus fistulosa* in China

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Summary – A new nematode species was recovered from syconia of *Ficus fistulosa* from the South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong Province, China, during a survey of nematode biodiversity from 2007 to 2012. It is described herein as *Schistonchus fistulosus* n. sp. and is typologically characterised by possessing the combined characters of a long post-uterine sac, excretory pore located near the metacorpus, amoeboid sperm, three pairs of subventral papillae on the male tail, a mucron on the male tail tip, absence of a gubernaculum and possession of ox-horn-shaped spicules without a terminal cucullus and with a short, broad and bluntly rounded rostrum. *Schistonchus fistulosus* n. sp. was easily differentiated from other sequenced species by the partial small subunit rRNA gene (SSU) and D3 expansion segments of the large subunit rRNA gene (LSU). Phylogenetic analysis with partial SSU sequences suggests that *S. fistulosus* n. sp. has a closer relationship with *S. guangzhouensis* and *S. baculum* in the same highly supported monophyletic clade than to the other sequenced *Schistonchus* species. Based upon inferences using LSU D3 sequence data, it is also in a well-supported monophyletic clade with *S. guangzhouensis* and *S. baculum*.

Keywords – description, fig, large subunit rRNA (LSU), molecular, morphology, morphometrics, new species, phylogeny, small subunit rRNA (SSU), taxonomy.

Ficus fistulosa Reinw. ex Blume, 1825 belongs to the genus *Ficus*, subgenus *Sycomorus*, section *Sycocarpus*, and subsection *Sycocarpus*. It is native to India, South China and Malesia. It naturally grows in forests, along streams or on rocks. It is distributed in Fujian, Guangdong, Guangxi, Hainan, and Yunnan in mainland China and Taiwan, and is also distributed in Bangladesh, north-eastern India, Indonesia, Malaysia, Myanmar, the Philippines, Thailand and Vietnam. This fig is pollinated by the fig wasp, *Ceratosolen hewitti*, in Singapore (Galil, 1973). However, in China, Malaysia, the Philippines, Java and Indonesia, it is pollinated by *C. constrictus* (see http://www.figweb.org/Fig_wasps/Agaonidae/Kradibiinae/Ceratosolen/Ceratosolen_constrictus.htm).

Gasparrini (1864) first described the aphelenchoidid, *Schistonchus caprifici* (Gasparrini, 1864) Cobb, 1927, as an associate of the fig-pollinating wasp, *Blastophaga psenes* L. (Gasparrini, 1864), in *F. carica*. *Schistonchus* spp. have been reported from the sycones of *Ficus* species worldwide (Giblin-Davis *et al.*, 1995, 2003, 2004) with a current total of 20 species described from Central America, North America, Asia, Africa and Australia. A recent survey on diversity of fig nematodes in Guangdong Province, P.R. China, during the period 2007 to 2012, revealed an undescribed species of nematode from *F. fistulosa* in the South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong Province, P.R. China. Using morphological and molecular means, it is described here as *Schistonchus fistulosus* n. sp.

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Materials and methods

NEMATODE MATERIAL

Syconia in phases B-D (Giblin-Davis *et al.*, 1995) were collected from *F. fistulosa* from the South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong Province, P.R. China, on several occasions from September 2007 to November 2012, and were opened with a scalpel and placed in distilled water for 20 min. Nematodes were cleaned and collected from a Baermann funnel. They were then hand-picked alive into water for DNA extraction, amplification and sequencing attempts, or collected, heat-killed at 65°C and placed into FG (formalin:glycerin:dH₂O = 10:5:85) for measurements by light microscopy and then processed into 100% glycerin for permanent mounts (Southey, 1970).

MORPHOLOGICAL OBSERVATIONS

Drawings and measurements of nematodes were done with the aid of a camera lucida and a stage micrometer. Photomicrographs were taken with a Leica video camera (DFC490) attached *via* a C-mount Adapter fitted on a Leica microscope (DM4000B) and edited using Adobe Photoshop CS2.

Male spicule length is the distance between the condylus and the posteriormost point of the lamina measured in a straight line (Zeng *et al.*, 2007). Spicular terminology and measurements used herein are as presented by Ryss *et al.* (2005). The morphometric data was processed using Excel software (Ye, 1996).

MOLECULAR PROFILES

Ten male nematodes were separately picked into distilled water and their identity was confirmed with light microscopy before being placed into 50 µl of worm lysis buffer (WLB) containing Proteinase K for DNA extraction (Williams *et al.*, 1992). DNA samples were stored at -20°C until used as a PCR template.

Primers for LSU amplification were forward primer SchD2F1 (5'-AAGTTGAAAAGCACTTTGAA-3') (designed by the second author), and reverse primer D3B (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn, 1992). Primers for SSU amplification were forward primer 18S965 (5'-GGCGATCAGATACCGCCCTAGTT-3'), and reverse primer 18S1573R (5'-TACAAAGGGCAGGGACGTAAT-3') (Mullin *et al.*, 2005). Primers for COI amplification were forward primer COI-F1 (5'-CCTACTATGAT

TGGTGGTTTTGGTAATTG-3') and reverse primer COI-R2 (5'-GTAGCAGCAGTAAAATAAGCACG-3') (Kanzaki & Futai, 2002a). The 25 µl PCR was performed using TaqMix DNA polymerase (Guangzhou Dongsheng Biotech) according to the manufacturer's protocol. The thermal cycler programme for PCR 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 (Ye *et al.*, 2007). PCR products were cleaned using an EZ Spin Column DNA Gel Extraction Kit (Bio Basic) according to the manufacturer's protocol. PCR products were sequenced by Shanghai Sangon Biological Engineering Technology & Services using an ABI PRISM 3730 sequencing system.

The nematode sequences from this project were deposited in the GenBank database. For phylogenetic analysis, we used some species from our previous sequencing projects whilst others were from the GenBank database. DNA sequences were aligned using ClustalW (available online at <http://workbench.sdsc.edu>, Bioinformatics and Computational Biology group, Department of Bioengineering, UC San Diego, San Diego, CA, USA). The model of base substitution in the SSU and LSU sets were evaluated using MODELTEST version 3.7 (Posada & Crandall, 1998). The Akaike-supported model, 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 3.1.2 (Huelsenbeck & Ronquist, 2001) running the chain for 10⁶ generations and setting the 'burn in' at 1000. We used MCMC (Markov Chain Monte Carlo) methods within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using the 50% majority-rule.

Results

*Schistonchus fistulosus** n. sp. (Figs 1, 2)

MEASUREMENTS

See Table 1.

* The species epithet is derived from the species name of the type host.

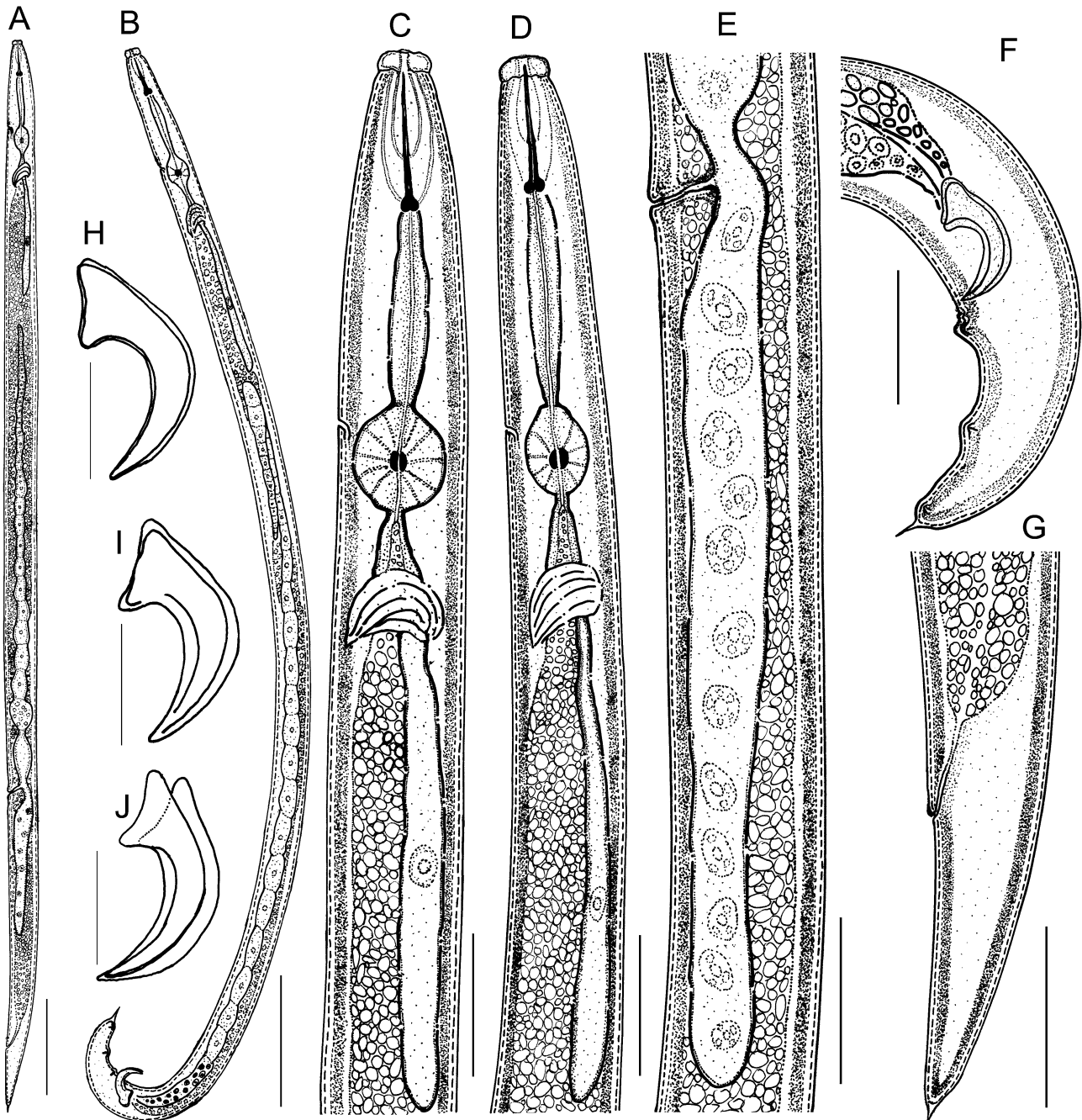


Fig. 1. Adult reproductive female and male of *Schistonchus fistulosus* n. sp. in lateral view. A: Entire reproductive female; B: Entire male; C: Anterior body of male; D: Anterior body of reproductive female; E: Vulva, post-uterine sac of reproductive system; F: Male tail; G: Reproductive female tail; H-J: Spicules from different specimens. (Scale bars: A, B = 50 μ m; C-G = 20 μ m; H-J = 10 μ m.)

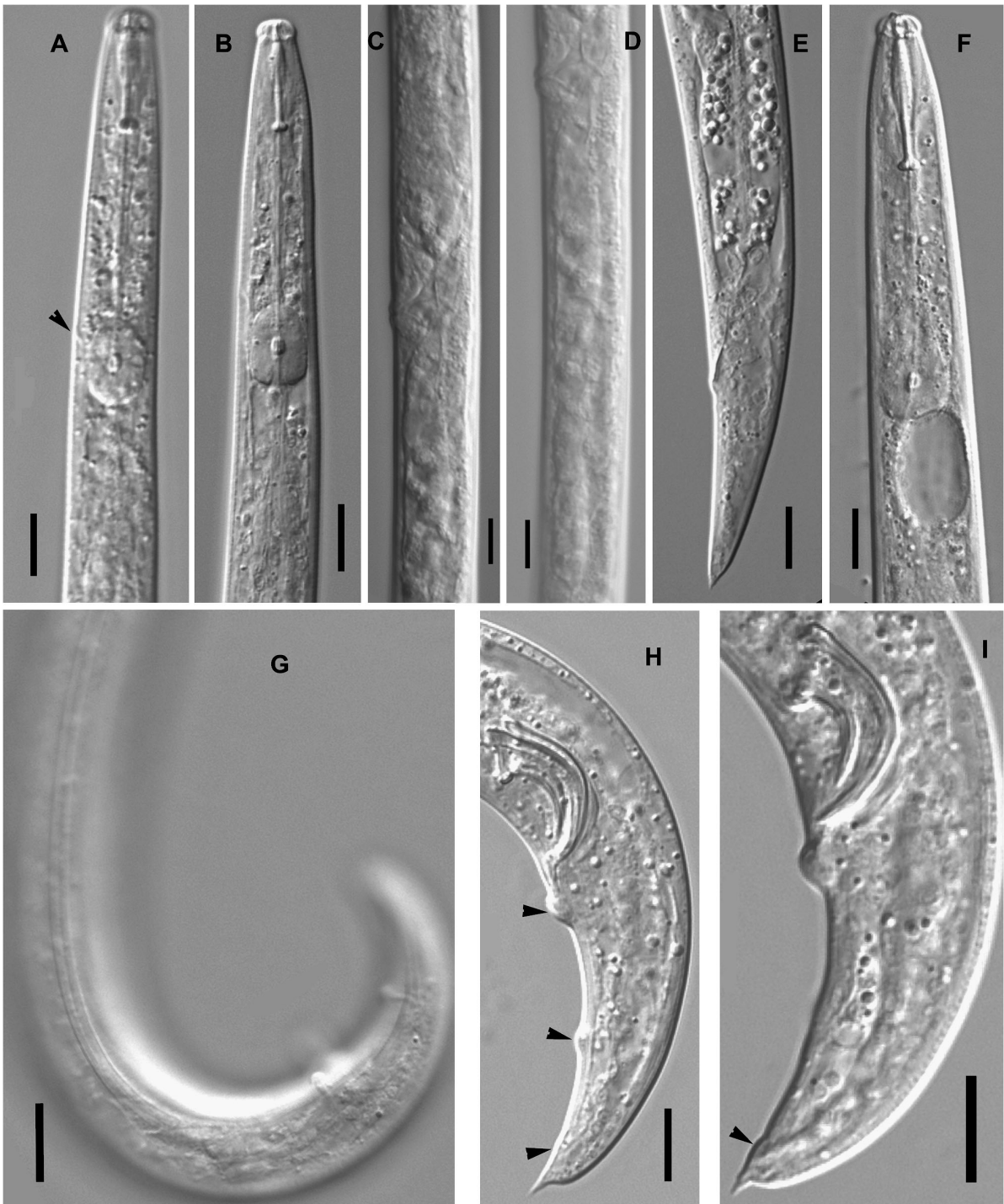


Fig. 2. Adult male and reproductive females of *Schistonchus fistulosus* n. sp. in lateral view. A: Anterior body of reproductive female (arrow = excretory pore); B: Anterior body of reproductive female; C, D: Vulva, post-uterine sac of reproductive female reproductive system; E: Reproductive female tail; F: Anterior body of male; G: Incisures of male in lateral view; H, I: male tails (arrows = papillae). (All scale bars = 10 μ m.)

Table 1. Morphometrics of *Schistonchus fistulosus* n. sp. mounted in formalin-glycerin. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Male		Entomophilic female	Reproductive female
	Holotype	Paratypes	Paratypes	Paratypes
n	–	15	15	14
L	610.0	566.0 \pm 59.9 (515-640)	457.0 \pm 40.3 (400-490)	542.0 \pm 36.9 (500-600)
a	40.7	36.2 \pm 5.8 (30.3-42.7)	29.2 \pm 4.1 (25.0-34.3)	28.3 \pm 1.1 (27.3-29.6)
b'	10.2	9.3 \pm 1.0 (8.5-10.7)	7.8 \pm 0.7 (6.9-8.4)	9.2 \pm 0.5 (8.8-10.0)
c	13.9	13.8 \pm 1.5 (12.3-15.5)	15.5 \pm 2.1 (13.9-18.5)	17.2 \pm 1.8 (16.1-20.4)
c'	3.4	3.2 \pm 0.3 (3.0-3.7)	3.4 \pm 0.1 (3.3-3.6)	3.1 \pm 0.4 (2.6-3.6)
V	–	–	72.2 \pm 1.2 (70.8-73.5)	69.2 \pm 2.9 (65.5-73.0)
Body diam. (males = GBD; females = VBD)	15.0	15.8 \pm 1.0 (15-17)	15.8 \pm 1.3 (14-17)	19.2 \pm 1.8 (18-22)
Stylet length	16.0	16.3 \pm 1.0 (15-17)	18.3 \pm 0.5 (18-19)	17.8 \pm 0.4 (17-18)
Stylet shaft length	5.0	5.6 \pm 0.5 (5-6)	6.8 \pm 1.0 (6-8)	6.8 \pm 0.8 (6-8)
Pharynx length (head to metacarpus base)	60.0	61 \pm 2.2 (59-64)	59 \pm 3.4 (55-63)	59 \pm 1.8 (56-60)
Spicule length	16.0	17.0 \pm 0.8 (16-18)	–	–
Vulva to anus distance (VA)	–	–	97.0 \pm 6.6 (89-105)	136.0 \pm 22.6 (105-159)
Post-uterine sac length (PUS)	–	–	61.0 \pm 7.5 (53-70)	69.0 \pm 12.4 (51-80)
Anal/cloacal body diam.	13.0	12.8 \pm 1.0 (12-14)	8.8 \pm 1.0 (8-10)	10.4 \pm 0.9 (10-12)
Tail length	44.0	41.0 \pm 2.6 (38-44)	29.8 \pm 3.3 (26-33)	31.8 \pm 3.9 (26-36)
Excretory pore from anterior end	52.0	53.0 \pm 3.5 (50-58)	48.0 \pm 3.1 (44-51)	48.0 \pm 2.5 (45-50)
Lip diam.	6.0	6.8 \pm 0.5 (6-7)	8.1 \pm 0.6 (7.5-9.0)	7.3 \pm 1.0 (6-8)
Lip height	3.0	3.5 \pm 0.6 (3-4)	3.8 \pm 0.5 (3-4)	4.0 \pm 0.0 (4-4)
Metacarpus length	13.0	13.3 \pm 1.0 (12-14)	14.0 \pm 1.4 (13-16)	13.6 \pm 1.3 (12-15)
Metacarpus diam.	10.0	9.5 \pm 1.0 (8-10)	10.8 \pm 1.5 (10-13)	9.8 \pm 1.6 (8-12)
PUS/VBD	–	–	3.9 \pm 0.7 (3.3-5.0)	3.7 \pm 0.8 (2.3-4.3)
PUS/VA	–	–	0.6 \pm 0.1 (0.5-0.7)	0.5 \pm 0.1 (0.3-0.6)
Metacarpus L/D	1.3	1.4 \pm 0.1 (1.3-1.5)	1.3 \pm 0.1 (1.2-1.4)	1.4 \pm 0.1 (1.3-1.5)

DESCRIPTION

Male from figs

Body cylindrical, posterior end curved ventrally, usually umbrella-handle-shaped or J-shaped when heat-killed. Cuticle with annulation, annules *ca* 1.0 μm wide on tail. Three incisures visible in lateral field under light microscopy. Head offset. Stylet 15-17 μm long, robust with strong rounded knobs, comprising two parts, conus 62.5-66.7% of total stylet length. Excretory pore (EP) near or at level of metacarpus. Procorpus subcylindroid, 1.4-2.8 times as long as stylet, usually with a narrow and conspicuous tube in middle which is surrounded by softer tissue, and with clear constriction at junction of procorpus and metacarpus. Metacarpus broadly ovoid, muscular, well developed with valve posterior to centre. Postcorpus glandular, well developed, dorsal lobe overlapping intestine. Pharyngo-intestinal junction with small and vague valve apparatus just posterior to metacarpus. Dorsal pharyngeal gland orifice indistinct under light microscope. Deirids and hemizonid not visible. Testis usually reflexed to left of intestine, *vas deferens* usually not clearly differentiated from seminal vesicle in specimens which have less developed reproductive systems under light microscope. Sperm amoeboid. Spicules paired, separate, ox-horn-shaped with a short, broad and bluntly rounded rostrum, 16-18 μm long, arcuate, angle for ventral intersection of line drawn along capitulum and line extending spicule tips = $63.2 \pm 5.3^\circ$ (59-72°), ratio of spicule length along its arc to its width measured posterior to rostrum = 4.5 ± 0.5 (4.0-5.0). Capitulum flattened, ratio of depth of capitulum depression/capitulum width = 0.1 ± 0.0 (0.1-0.1) in lateral view. Condylus short, rounded, lamina dorsal line smoothly and symmetrically curved, junction of rostrum and calomus slightly angular to smoothly rounded. Spicule tip finely rounded, without cucullus. Gubernaculum absent. Three pairs of subventral caudal papillae: one pair adcloacal, one pair postcloacal, halfway between cloacal aperture and tail terminus at 10-15 μm from tail terminus, and one pair near tail tip. Tail conoid, 3.0-3.7 cloacal body diam. (CBD) long, tail tip rounded, with sharp mucron (2-4 μm). Bursa or bursal flap absent.

Entomophilic female from figs (presumed to be associated with fig wasp pollinators for dispersal)

Body straight or slightly ventrally arcuate when heat-relaxed. Cuticle, lateral field, head, pharynx and intestine as in male. Stylet 18-19 μm long with rounded knobs. EP located near metacarpus. Deirids, hemizonid and phasmids not visible. Vulva posteriorly situated at

70.8-73.5% of total body length. Vagina strongly anteriorly directed. Uterus short. Ovary situated on left of intestine, monodelphic, prodelphic, without flexure, contour smooth, crustaformeria apparently absent, oval spermatheca present. Oocytes in single file for entire ovary. Postuterine sac (PUS) long, 3.3-5.0 vulval body diam. (VBD) long. Tail conoid, 3.3-3.6 anal body diam. (ABD) long, tail tip rounded, with mucron.

Reproductive female from figs

Body cylindrical, straight or slightly arcuate ventrally but not C-shaped when heat-relaxed. Body longer and broader than that of entomophilic female. Features of cuticle, lateral field, head, stylet, pharynx, EP and tail as described for entomophilic female. Vulva posteriorly located at 65.5-73.0% of total body length, vulval lips slightly protruding. Ovary situated on left of intestine, monodelphic, prodelphic, well developed, outstretched, crustaformeria absent, oval spermatheca present. Oocytes in single file in entire ovary. PUS *ca* 2.3-4.3 VBD long, containing some sperm.

TYPE HOST AND LOCALITY

Phases B-D syconia from a single *Ficus fistulosa* tree in the South China Botanical Garden, Chinese Academy of Sciences, Longdong, Guangzhou, Guangdong Province, P.R. China (23°11'N, 113°21'E), collected on 2 November 2012.

TYPE MATERIAL

Holotype male, one paratype of each of a male, entomophilic female and reproductive female deposited in the Department of Nematology, University of California, Riverside, CA, USA. One paratype of each of a male, entomophilic female and reproductive female deposited at each of the Department of Nematology, University of California, Davis, CA, USA; the Nematology Laboratory, USDA, ARS, Beltsville, MD, USA. The remaining type materials are deposited in the Plant Pathology Laboratory, Department of Plant Protection, Zhongkai University of Agriculture and Engineering, Guangzhou, P.R. China.

DIAGNOSIS AND RELATIONSHIPS

Schistonchus fistulosus n. sp. is typologically characterised by possessing the combined characters of a medium to long PUS (3.3-5.0 VBD long in entomophilic female and 2.3-4.3 VBD long in reproductive female), EP

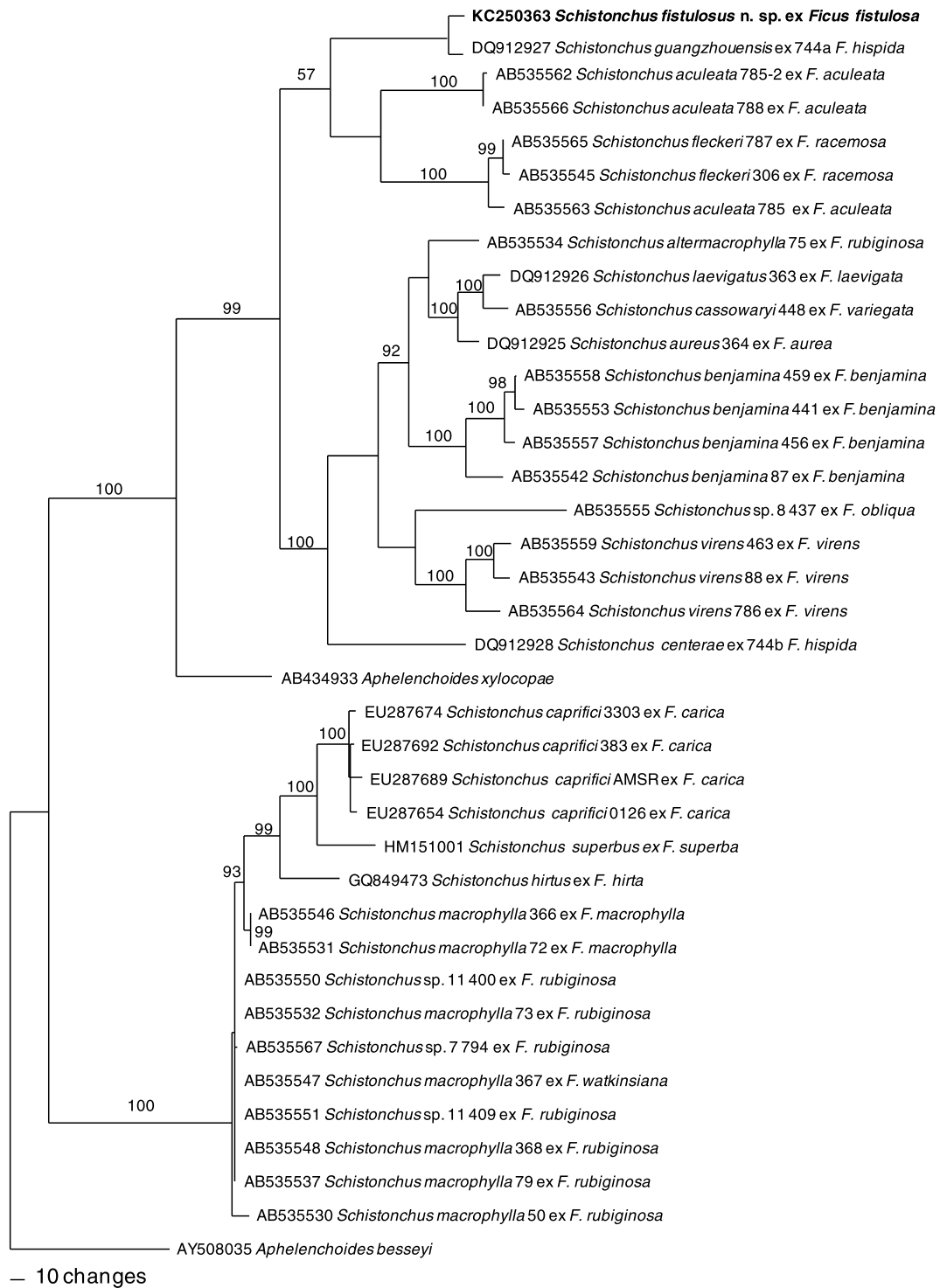


Fig. 4. The 10001st Bayesian tree inferred from LSU D3 under GTR + I + G model (lnL = 7768.7065; freqA = 0.2393; freqC = 0.1791; freqG = 0.2984; freqT = 0.2832; R(a) = 0.6643; R(b) = 3.9509; R(c) = 1.6089; R(d) = 1.1727; R(e) = 6.083; R(f) = 1; Pinva = 0.3108; Shape = 1.2642). Posterior probability values exceeding 50% are given on appropriate clades.

Kanzaki, Ye & Giblin-Davis, 2013 and *S. macrophylla* Lloyd & Davies, 1997, although the character state is not visible and/or not reported in *S. racemosa* Reddy & Rao, 1986 or *S. osmani* Anand, 2002 (both from *F. racemosa* from India). However, *S. fistulosus* n. sp. differs from *S. guangzhouensis* by possessing more papillae (three pairs vs two pairs) on the male tail, a shorter male stylet (15-17 vs 20-29 μm), a higher ratio c' (3.0-3.7 vs 2.0-3.1) in males, a longer stylet (18.0-19.0 vs 16.5-17.5 μm) in entomophilic females and a shorter PUS (53-70 vs 69-99 μm in entomophilic females, 2.3-4.3 vs 5.2-7.9 VBD in reproductive females); from *S. hirtus*, by a longer PUS (2.3-5.0 vs 0.5 VBD) in females, male tail tip with mucron vs without mucron, a larger spicule (16-18 vs 11-14 μm) and a higher ratio c' (3.0-3.7 vs 2.3-2.8) in males; from *S. baculum*, by a shorter PUS (2.3-5.0 vs 6.0 VBD), three pairs vs two pairs of papillae on the male tail, fewer incisures in the lateral field (3 vs 4) in males, tail tip with mucron vs without mucron in reproductive females; from *S. superbus*, by a shorter stylet (15-17 vs 21-27 μm), more papillae (three pairs vs two pairs) on the male tail, male tail tip with mucron vs variable, with or without mucron, spicule tip not offset vs offset, male tail conoid vs wedge-shaped and a higher ratio a (30.3-42.7 vs 16.7-28.7), higher ratio c' (3.0-3.7 vs 1.4-1.9) in males, a longer PUS (2.3-5.0 vs 0.7-1.0 VBD) and a shorter stylet (17-19 vs 24-28 μm) in females; and from *S. macrophylla*, by tail tip (with mucron vs without mucron), positions of the papillae on the male tail (one pair located near tip vs at rostrum level), rostrum bluntly rounded vs conical, and a shorter male stylet (15-17 vs 22-28 μm).

Schistonchus fistulosus n. sp. has a long PUS (2.3-5.0 VBD long) in females, differing from *S. aculeata* Davies, Bartholomaeus, Ye, Kanzaki & Giblin-Davis, 2010, *S. africanus* Vovlas, Troccoli, Van Noort & Van Den Berg, 1998, *S. altermacrophylla* Lloyd & Davies, 1997, *S. aureus* DeCrappeo & Giblin-Davis, 2001, *S. benjamina* Bartholomaeus, Davies, Ye & Giblin-Davis, 2012, *S. caprifici*, *S. cassowaryi* Davies, Bartholomaeus, Kanzaki, Ye & Giblin-Davis, 2013, *S. centerae* Zeng, Giblin-Davis & Ye, 2007, *S. fleckeri* Davies, Bartholomaeus, Kanzaki, Ye & Giblin-Davis, 2013, *S. hirtus*, *S. laevigatus* DeCrappeo & Giblin-Davis, 2001, *S. microcarpus* Zeng, Ye, Giblin-Davis, Li, Zhang & Du, 2011, *S. racemosa*, *S. superbus*, and *S. virens* Bartholomaeus, Davies, Ye, Kanzaki & Giblin-Davis, 2009, all of which have a PUS of ≤ 1.0 VBD long.

Schistonchus fistulosus n. sp. possesses amoeboid sperm similar to *S. aculeata*, *S. africanus*, *altermacro-*

phylla, *S. aureus*, *S. baculum*, *S. benjamina*, *S. cassowaryi*, *S. centerae*, *S. fleckeri*, *S. guangzhouensis*, *S. hirtus*, *S. laevigatus*, *S. microcarpus*, *S. superbus* and *S. virens*, but differing from the 'flagellate' sperm of *S. caprifici*, *S. macrophylla*, *S. osmani* and *S. racemosa*.

Males of *S. fistulosus* n. sp. have three pairs of papillae (one pair adcloacal, one pair halfway between cloacal aperture and tail terminus, and one pair near the tail tip), very similar to the arrangement in *S. aculeata*, *S. aureus*, *S. benjamina*, *S. fleckeri*, *S. hirtus*, *S. laevigatus*, *S. microcarpus* and *S. virens*, where the relative positions of the papillae are the same, but differing from *S. africanus*, *S. altermacrophylla*, *S. caprifici*, *S. centerae* and *S. macrophylla* in the relative positions of the adcloacal pair and the two pairs of postcloacal papillae, and from *S. cassowaryi* in which one pair is pre-cloacal, one almost lateral at mid-tail and one subventral near the tail tip. Males of *S. baculum*, *S. guangzhouensis*, *S. hispida* Kumari & Reddy, 1984, *S. racemosa*, *S. superbus*, and possibly *S. osmani* (original description unclear) have two pairs of papillae.

MOLECULAR PHYLOGENETIC RELATIONSHIPS

We sequenced partial SSU, LSU D2/D3 expansion segments and a fragment of mtCOI for molecular phylogenetic inferences (Bayesian analysis) to examine the relative placement of *S. fistulosus* n. sp. among other sequenced *Schistonchus* species available for these loci. The tree inferred from SSU (Fig. 3) using *Ditylenchus halictus* as an outgroup suggested that: i) all the selected Aphelenchoididae are in a monophyletic clade in relation to *Paraphelenchus* Micoletzky, 1922; ii) none of the species in *Aphelenchoides* Fischer, 1894, *Laimaphelenchus* Fuchs, 1937 and *Schistonchus* is monophyletic; iii) *S. fistulosus* n. sp. is sister to *S. guangzhouensis* and is polyphyletic to other *Schistonchus* species sequenced for SSU; iv) *S. fistulosus* n. sp. is in a highly supported monophyletic clade with the *Aphelenchoides*, three Chinese species (*S. guangzhouensis*, *S. microcarpus* and *S. centerae*) and three neotropical species (*S. aureus*, *S. laevigatus* and *Schistonchus* sp. ex. *Ficus colubrinae* Standl.); and v) *Schistonchus* shares a more recent common ancestor with *Aphelenchoides* and *Laimaphelenchus* (*Aphelenchoididae* according to Hunt, 1993) than with *Bursaphelenchus* (*Parasitaphelenchidae* according to Hunt, 1993) or *Seinura* Fuchs, 1931 (*Seinuridae* according to Hunt, 1993).

The tree inferred from D3 of LSU (Fig. 4), using *Aphelenchoides besseyi* as an outgroup, suggested that:

i) all the sequenced *Schistonchus* species are divided into two well supported monophyletic clades; ii) *S. fistulosus* n. sp. is in a well supported monophyletic clade with two species from *F. hispida*, namely *S. guangzhouensis* from China (2 bp differences among 716 bp aligned D2/D3 region) and *S. baculum* from Australia (3 bp differences among 322 bp aligned D3 region); iii) *S. fistulosus* n. sp. appears to be closer to *S. guangzhouensis* and *S. baculum* than to the other four described Chinese species (*S. centerae*, *S. hirtus*, *S. microcarpus* and *S. superbus*); iv) one Chinese species (*S. centerae*), two American species (*S. aureus* and *S. laevigatus*) and four Australian species (*S. altermacrophylla*, *S. benjamina*, *S. cassowaryi* and *S. virens*) are monophyletic; and v) *S. caprifici*, one Australian species (*S. macrophylla*) and two Chinese species (*S. hirtus* and *S. superbus*) are in a monophyletic clade.

Currently, there are too few mtCOI sequences available for *Schistonchus* for meaningful phylogenetic analysis. However, based upon the available sequences of the 655 bp fragment of mtCOI, *S. fistulosus* n. sp. (GenBank accession no. KC250364) was closest to *S. guangzhouensis* with 91% similarity vs decreasingly respective values of 82, 80, 77 and 75% for *S. superbus*, *S. hirtus*, *S. microcarpus* and *S. caprifici*.

Discussion

In overall morphological appearance, *S. fistulosus* n. sp. is closest to *S. baculum* and *S. guangzhouensis*. Phylogenetically, *S. fistulosus* n. sp., *S. guangzhouensis* and *S. baculum* are putative sisters in a single clade (Figs 3, 4) but its sequences are unique. The collection sites of *S. fistulosus* n. sp. and *S. baculum* were from fig hosts that are geographically widely disparate (from China and Australia, respectively), their hosts are from different taxonomic sections and subsections of the subgenus *Sycomorus* (*Sycocarpus Sycocarpus* vs *Sycomorus Sycomorus*), and they are putatively associated with different species of pollinator wasps (*C. hewitti* or *C. constrictus* vs *C. fusciceps*). Although *S. fistulosus* n. sp. and *S. guangzhouensis* were collected at the same location (Guangzhou, P.R. China), they are from different hosts (*F. fistulosa* vs *F. hispida* which are both from the same *Ficus* subsection of *Sycocarpus*), and are putatively associated with different species of pollinator wasps (*C. hewitti* or *C. constrictus* vs *C. solmsi marchali*). The 2-bp difference among 716 bp from the aligned D2/D3 region and a 9% difference in 655 bp of the mtCOI fragment between

S. fistulosus n. sp. and *S. guangzhouensis* and a 3-bp differences among the 322 bp from the aligned D3 region between *S. fistulosus* n. sp. and *S. baculum* helped to confirm that *S. fistulosus* n. sp. is indeed a separate species from *S. guangzhouensis* and *S. baculum*. These results also suggest that DNA sequences are evolving at different rates, i.e., the LSU is very conserved but mtCOI is evolving rapidly between closely related species, which is in agreement with previous observations in *Bursaphelenchus* (Kanzaki & Futai 2002b; Ye et al., 2007; Kanzaki et al., 2008).

The relationship of the clade represented by *S. guangzhouensis*, *S. baculum* and *S. fistulosus* n. sp. with other described species in *Schistonchus* is not well resolved, but probably represents another ‘*Schistonchus*’ lineage (Zeng et al., 2010). Davies et al. (2013) inferred that *S. baculum* and *S. guangzhouensis* are putative sisters in a single clade based on phylogenetic analysis (Davies et al., 2010). In the present study, we suggest that *S. fistulosus* n. sp. is in a highly supported monophyletic clade with *S. guangzhouensis* and *S. baculum* based on phylogenetic analysis of partial SSU sequences and LSU D3 (Figs 3, 4). Thus, the inferred placement of *S. fistulosus* n. sp. further corroborates the hypothesis that three distantly related lineages probably comprise the current genus *Schistonchus* (Kanzaki et al., 2009a, b; Zeng et al., 2012). Also, despite this being preliminary data that will require extensive biogeographical and fig and fig-wasp host sampling for confirmation, it currently supports the hypothesis of a Laurasian lineage of ‘*Schistonchus*-like’ aphelenchoidids radiating with the subgenus of *Sycomorus* and its pollinating fig-wasps.

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