Description of *Schistonchus altissimus* n. sp. (Nematoda: Aphelenchoididae), an associate of *Ficus altissima* in China

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**Abstract**

A new nematode species was recovered from the syconia of *Ficus altissima* from the residential area of Huajingxincheng, Guangzhou, Guangdong Province, China during a survey of nematode diversity. *Schistonchus altissimus* n. sp. is characterised by having females with a short post-uterine sac, an ovoid spermatheca and a conoid tail with a mucron in the female, excretory pore located near the lip; and males with amoeboid sperm, a conoid tail without a mucron and three pairs of subventral papillae, no gubernaculum, and hook-shaped spicules with a cucullus and a thorn-shaped rostrum. *Schistonchus altissimus* n. sp. is typologically differentiated from all other described species in this genus, except for *S. microcarpus*, by having a spicule with cucullus on the male tail tip. *Schistonchus altissimus* n. sp. is easily differentiated from other sequenced species by the partial small subunit rRNA gene (SSU), D3 expansion segment of the large subunit rRNA gene (LSU) and mitochondrial DNA subunit I (mtCOI). Phylogenetic analysis with partial SSU sequences suggests that *S. altissimus* n. sp. is in a highly supported monophyletic clade with two Chinese species (*S. microcarpus* and *S. centerae*) and two neotropical species (*S. aureus* and *Schistonchus* sp. ex *Ficus colubrinae* Standl.). Based on inferences using LSU D3 sequence data, *S. altissimus* n. sp. has a closer relationship with four Chinese species (*S. centerae, S. fistulosus, S. guangzhouensis* and *S. microcarpus*) than with *S. hirtus* and *S. superbus*, also from China.

**Key words:** morphology, morphometrics, fig, large subunit rRNA (LSU), mitochondrial DNA subunit I (mtCOI), molecular, new species, phylogeny, small subunit rRNA (SSU), taxonomy

**Introduction**

*Ficus altissima* Blume is a woody tree that grows in mountains and on plains at elevations of 100–2,000 m (Zhang 1998). It is native to the Asian temperate area (China) and the Asian tropics (Bhutan, India, Nepal, Myanmar, Thailand, Vietnam, Indonesia, Malaysia and the Philippines) (Zhou & Gilbert 2003) and distributed in Guangdong, Guangxi, Hainan and Yunnan provinces in China?http://www.invasive.org./weeds/asian/ficus.pdf). It is an important plant species being used as a woody ornamental and a host for the Lac-producing insect, *Kerria lacca* Kerr (). *Ficus altissima* is a member of the monoeocious subgenus *Urostigma* and is pollinated by the fig wasp *Eupristina altissima* Balakrishnan & Abdurahiman (http://www.figweb.org/Ficus/Subgenus_Urostigma/Section_Urostigma/Subsection_Conosycea/Ficus_altissima.html).

*Schistonchus* Cobb, 1927 (Aphelenchoididae) has long been recognised as associated with fig wasps and fig sycones (Gasparrini 1864). So far, 21 species of *Schistonchus* have been described from Ficus species from Central America, North America, Asia, Africa and Australia (Anand, 2002; Bartholomaeus et al. 2012; Cobb 1927; Davies et al. 2010, 2013; DeCrappeo & Giblin-Davis 2001; Kumari & Reddy 1984; Reddy & Rao, 1984; Vovlas et al. 1998; Zeng et al. 2007, 2010, 2011, 2013a, 2013b). A recent survey on the diversity of fig nematodes in Guangdong Province, China revealed an undescribed species of nematode from *F. altissima* in the residential area
of Huajingxincheng, Guangzhou, Guangdong Province, China. Using typological and molecular approaches, it is described as *S. altissimus* n. sp.

**Material and methods**

**Nematode material.** Syconia in phases C–D (Giblin-Davis *et al.* 1995) were collected from a single tree of *F. altissima* from the residential area of Huajingxincheng, Guangzhou, Guangdong Province, China, on November 22, 2012, and opened with a scalpel and placed in distilled water for 20 min. Nematodes were cleaned and collected on a Baermann funnel. Living males were handpicked into water for DNA extraction, amplification, and sequencing attempts. Nematodes for morphological study were collected, heat-killed at 65°C for 2–3 min and placed into FG (formalin: glycerin: dH2O = 10: 5: 85) (designed by the last author) for 2 months and then processed into 100% glycerin for permanent mounts (Southey 1970).

Drawings and measurements of nematodes were performed 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 onto a Leica microscope (DM4000B), and edited using Adobe Photoshop CS2.

Male spicule length is the distance between the condylus and the posterior-most 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 were processed using Excel software (Ye 1996).

**Molecular techniques.** Ten males were separately picked into distilled water and their identity and morphotype 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’-TGCAGGAAACCAGCTACTA-3’) (Nunn 1992). Primers for SSU amplification were forward primer 18S965 (5’ GGCGATCAGATACCGCCCTAGTT 3’) and reverse primer 18S1573R (5’-TCAAAAGGCAGGACGTAAT-3’) (Mullin *et al.* 2005). Primers for mtCOI amplification were forward primer COI-F1 (5’ CCTACTATGATTGTTGTTTTGGTAATG3’) and reverse primer COI-R2 (5’ GTAGCAGCAGTAAAAAAGCAGC 3’) (Kanzaki & Futai 2002). The 25 µl PCR was performed using TaqMix DNA polymerase (Guangzhou Dongsheng Biotech Ltd., Guangzhou, China) according to the manufacturer’s protocol. The thermal cycler program 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 Inc., Markham, Ontario, Canada) according to the manufacturer’s protocol. PCR products were sequenced by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd, Shanghai, China using an ABI PRISM 37300 sequencing system.

The nematode sequences from this project were deposited into the GenBank database. For phylogenetic analysis, some species from our previous sequencing projects were used while others were from the GenBank database. DNA sequences were aligned using ClustalW (http://workbench.sdsc.edu, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). The models of base substitution in the SSU and LSU sets were evaluated using MODELTEST version 3.07 (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 1,000,000 generations and setting the ‘burn in’ at 1,000. 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.
FIGURE 1. The 10001st Bayesian tree inferred from 18S under GTR+I+G model (-lnL=11975.4033; freqA=0.2441; freqC=0.2019; freqG=0.2693; freqT=0.2847; R(a)=1.2631; R(b)=2.7232; R(c)=1.4524; R(d)=0.8901; R(e)=4.1822; R(f)=1; Pinva=0.2115; Shape=0.6746). Posterior probability values exceeding 50% are given on appropriate clades.
FIGURE 2. The 10001st Bayesian tree inferred from D3 under GTR+I+G model (-lnL=2302.1172; freqA=0.2692; freqC=0.1707; freqG=0.2793; freqT=0.2809; R(a)=1.2318; R(b)=4.7041; R(c)=2.0052; R(d)=0.951; R(e)=7.4182; R(f)=1; Pinva=0.3515; Shape=0.568). Posterior probability values exceeding 50% are given on appropriate clades.
Results

Molecular phylogenetic relationships. For molecular phylogenetic inferences (Bayesian analysis) of the relative placement of S. altissimus n. sp. among other sequenced Schistonchus species, we sequenced partial SSU, the LSU D2/D3 expansion segment and a fragment of mtCOI. The tree inferred from SSU (Fig. 1), using Ditylenchus halictus Giblin-Davis, Erteld, Kanzaki, Ye, Zeng & Center 2010 as an outgroup, suggested that: i) all the selected Aphenelchoideidae are in a monophyletic clade in relation to Paraphelenchus Micoletzky 1922 with 100% posterior probability; ii) none of the species in Aphenelchoides Fischer 1894, Bursaphelenchus Fuchs 1937, Laimaphelenchus Fuchs 1937 or Schistonchus are monophyletic; iii) S. altissimus n. sp. is in a highly supported clade with two Chinese species (S. microcarpus Zeng, Ye, Giblin-Davis, Li, Zhang & Du 2011 and S. centerae Zeng, Giblin-Davis & Ye 2007) and two neotropical species (S. aureus DeCrappeo & Giblin-Davis 2001 and Schistonchus sp. ex Ficus colubrinae Standl.) which share a common ancestor with Aphenelchoides blastophthorus Franklin 1952; iv) S. altissimus n. sp. appears to be closer to two Chinese species (S. guangzhouensis Zeng, Giblin-Davis & Ye 2007 and S. fistulosus Zeng, Ye, Wang, Du & Giblin-Davis 2013b) than the other two Chinese species (S. hirtus Zeng, Ye, Giblin-Davis, Li, Du & Zhao 2010 and S. superbus Zeng, Ye, Li, Wang, Du & Giblin-Davis 2013a) and S. caprifici (Gasparrini 1864) Fuchs 1937; v) the genus Schistonchus shares a more recent common ancestor with Aphenelchoides and Laimaphelenchus (Aphenelchoididae according to Hunt 1993) than with Bursaphelenchus (Parasitaphelenchidae according to Hunt 1993).

The tree inferred from D3 of LSU (Fig. 2) using Aphenelchoides besseyi Christie 1942 to root the tree to investigate the relationships of the species in the genus Schistonchus suggested that: i) all the sequenced Schistonchus species are divided into two monophyletic clades with 100% support, i.e., S. aculeata Davies, Bartholomaeus, Ye, Kanzaki & Giblin-Davis 2010, S. altermacrophylla Lloyd & Davies 1997, S. altissimus n. sp., S. aureus, S. baculum Davies, Bartholomaeus, Kanzaki, Ye & Giblin-Davis 2013, S. benjamina Bartholomaeus, Davies, Ye & Giblin-Davis 2012, S. centerae, S. fistulosus, S. guanghouensis, S. laevigatus DeCrappeo & Giblin-Davis 2001, S. microcarpus and S. virens Bartholomaeus, Davies, Ye, Kanzaki & Giblin-Davis 2009 are in a monophyletic clade; and S. caprifici, S. hirtus, S. macrophylla Lloyd & Davies 1997 and S. superbus are in a second monophyletic clade; ii) S. altissimus n. sp. appears to be closer to S. centerae, S. fistulosus, S. guanghouensis and S. microcarpus than to the other two Chinese species (S. hirtus and S. superbus); and iii) S. baculum, S. fistulosus and S. guanghouensis are in a monophyletic clade with 99% support.

A comparison of the sequence of a 591-bp fragment of mtCOI of S. altissimus n. sp. (GenBank Accession No. KC526930) with S. caprifici, S. fistulosus, S. guanghouensis, S. hirtus, S. microcarpus and S. superbus yielded identity of 85%, 81%, 81%, 84% and 87%, respectively. Currently, there are too few mtCOI sequences available for Schistonchus for meaningful phylogenetic analysis.

Description of Schistonchus altissimus1 n. sp.
(Figs 3, 4)

Measurements. See Table 1.

Material examined. Holotype male, paratype male, paratype entomophilic female and paratype reproductive female deposited in the Department of Nematology, University of California, Riverside, CA, USA. Type materials were collected from phase C–D syconia from a single tree of Ficus altissima in the residential area of Huajingxincheng, Guangzhou, (23°8’45.50”N, 113°21’48.50”E), Guangdong Province, P. R. China, on November 22, 2012. One paratype each of a male, entomophilic female and reproductive female deposited at the Department of Nematology, University of California, Davis, CA, USA; and the Nematology Laboratory, USDA, ARS, Beltsville, MD, USA, and the remaining type materials were deposited in the Plant Pathology Laboratory, Department of Plant Protection, Zhongkai University of Agriculture and Engineering, Guangzhou, P. R. China.

1. The species epithet of this new species is derived from the species name of the type host.
**TABLE 1.** Measurements and morphometrics of male holotype and male and female paratypes of *Schistonchus altissimus* n. sp. mounted in formalin-glycerin. All measurements in mm and in the format: mean ± s.d. (range).

<table>
<thead>
<tr>
<th>Character</th>
<th>Male Holotype</th>
<th>Male Paratypes</th>
<th>Entomophilic female Paratypes</th>
<th>Reproductive female Paratypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1</td>
<td>18</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>L</td>
<td>487</td>
<td>467.4 ± 26.5 (424–515)</td>
<td>720.1 ± 47.4 (610–788)</td>
<td>593.9 ± 94.8 (485–787)</td>
</tr>
<tr>
<td>a</td>
<td>30.4</td>
<td>30.0 ± 4.2 (23.0–39.5)</td>
<td>35.6 ± 4.7 (26.3–43.3)</td>
<td>24.8 ± 7.1 (13.4–39.4)</td>
</tr>
<tr>
<td>b₁</td>
<td>8.9</td>
<td>8.6 ± 1.5 (6.3–11.6)</td>
<td>12.4 ± 2.1 (8.3–16.9)</td>
<td>11.1 ± 3.0 (5.1–16.0)</td>
</tr>
<tr>
<td>c</td>
<td>18.7</td>
<td>20.4 ± 6.2 (15.6–41.8)</td>
<td>20.6 ± 3.6 (13.5–26.0)</td>
<td>17.5 ± 3.7 (7.8–21.7)</td>
</tr>
<tr>
<td>c’</td>
<td>1.7</td>
<td>1.6 ± 0.4 (0.5–2.0)</td>
<td>3.9 ± 0.5 (3.1–4.7)</td>
<td>3.2 ± 0.7 (2.2–4.8)</td>
</tr>
<tr>
<td>V</td>
<td>–</td>
<td>–</td>
<td>73.2 ± 2.2 (69.2–77.2)</td>
<td>73.8 ± 2.1 (70.1–77.1)</td>
</tr>
<tr>
<td>Body diam. (males =greatest body diam.; females=vulval body diam.)</td>
<td>16.0</td>
<td>15.9 ± 2.2 (12–20)</td>
<td>20.6 ± 3.4 (17–29)</td>
<td>25.1 ± 5.3 (18–40)</td>
</tr>
<tr>
<td>Stylet length</td>
<td>16.0</td>
<td>16.0 ± 1.0 (15–18)</td>
<td>18.6 ± 1.9 (16–22)</td>
<td>19.2 ± 1.7 (17–22)</td>
</tr>
<tr>
<td>Stylet shaft length</td>
<td>6.0</td>
<td>5.4 ± 1.2 (4–7)</td>
<td>6.5 ± 0.7 (5.5–8)</td>
<td>6.7 ± 0.9 (5.5–8)</td>
</tr>
<tr>
<td>Pharynx length (head to metacorpus base)</td>
<td>55</td>
<td>55.2 ± 8.9 (43–73)</td>
<td>59.6 ± 11.5 (42–88)</td>
<td>56.6 ± 16.4 (40–104)</td>
</tr>
<tr>
<td>Spicule length*</td>
<td>15</td>
<td>14.1 ± 1.3 (12–16)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vulva to anus distance (VA)</td>
<td>–</td>
<td>–</td>
<td>156.6 ± 19.0 (125–191)</td>
<td>120.6 ± 31.1 (66–182)</td>
</tr>
<tr>
<td>Post uterine sac length (PUS)</td>
<td>–</td>
<td>–</td>
<td>9.2 ± 2.0 (7–14)</td>
<td>11.3 ± 3.1 (8–17)</td>
</tr>
<tr>
<td>Anal body diam. (males=cloacal body diam.)</td>
<td>15.0</td>
<td>15.4 ± 2.4 (12–20.5)</td>
<td>9.2 ± 2.0 (7–14)</td>
<td>11.3 ± 3.1 (8–17)</td>
</tr>
<tr>
<td>Tail length</td>
<td>26.0</td>
<td>25.0 ± 3.9 (16–32)</td>
<td>35.9 ± 6.6 (27–51)</td>
<td>35.9 ± 11.2 (24–66)</td>
</tr>
<tr>
<td>Excretory pore from anterior end</td>
<td>7.0</td>
<td>6.7 ± 1.2 (5–9)</td>
<td>9.2 ± 1.1 (7.5–11)</td>
<td>9.0 ± 0.9 (8–11)</td>
</tr>
<tr>
<td>Lip diam.</td>
<td>5.0</td>
<td>4.9 ± 0.4 (4–6)</td>
<td>5.8 ± 0.4 (4.5–6)</td>
<td>5.8 ± 0.4 (5–6)</td>
</tr>
<tr>
<td>Lip height</td>
<td>3.0</td>
<td>2.7 ± 0.3 (2–3)</td>
<td>2.9 ± 0.4 (2.5–4)</td>
<td>2.9 ± 0.2 (2.5–3)</td>
</tr>
<tr>
<td>Metacorpus length</td>
<td>14.0</td>
<td>12.9 ± 2.2 (8–18.5)</td>
<td>14.6 ± 1.9 (12–18)</td>
<td>15.8 ± 2.7 (12–22)</td>
</tr>
<tr>
<td>Metacorpus diam.</td>
<td>11.0</td>
<td>8.4 ± 1.4 (6–11)</td>
<td>9.8 ± 1.7 (8–14)</td>
<td>10.8 ± 2.4 (8–18)</td>
</tr>
<tr>
<td>PUS/VBD</td>
<td>–</td>
<td>–</td>
<td>0.3 ± 0.0 (0.2–0.4)</td>
<td>0.2 ± 0.1 (0.1–0.4)</td>
</tr>
<tr>
<td>PUS/VA (%)</td>
<td>–</td>
<td>–</td>
<td>4.2 ± 0.7 (3.1–6.0)</td>
<td>5.0 ± 1.0 (3.3–6.8)</td>
</tr>
<tr>
<td>Metacorpus L/D</td>
<td>1.3</td>
<td>1.5 ± 0.2 (1.2–2.0)</td>
<td>1.5 ± 0.2 (1.3–1.8)</td>
<td>1.5 ± 0.2 (1.2–1.8)</td>
</tr>
</tbody>
</table>

* The distance between the condylus and the posterior-most point of the lamina measured in a straight line.

**Description.** Males (from figs). Body cylindrical, posterior end strongly curved ventrally when heat-killed. Cuticle with fine annulation, annuli about 1 µm wide at mid-body. Three incisures visible in lateral field under light microscopy. Head slightly offset. Amphids prominent, situated laterally on the head, 3–4 µm from anterior. Amphid structure indistinct under light microscope. Stylet 15–18 µm long, robust, conus 57–75% of total stylet length, with strong, sub-diamond-shaped basal swellings (knobs) smoothly connected to shaft. Procorpus sub-cylindroid, 1.5–2.0 times as long as stylet. Metacorpus well-developed with valve posterior to centre. Excretory pore (EP) near the lip or head. Dorsal pharyngeal gland well-developed with one lobe, overlapping intestine, one gland nucleus observed. Pharyngo-intestinal junction with small and vague valve apparatus just posterior to metacorpus. Dorsal pharyngeal gland orifice indistinct under light microscope. Deirids and hemizonid not observed. Testis usually reflexed to left of intestine, anterior part usually reflexed once to right, *vas deferens* not clearly differentiated from seminal vesicle in specimens with less developed reproductive systems. Sperm amoeboid (4–5 µm by 2–3 µm). Spicules slender, paired, separate, hook-shaped with a thorn-shaped rostrum, arcuate. Capitulum strongly bent towards the lamina or depressed. Condylus small, rounded, rostrum prominent,
conical to pointed, lamina dorsal line smoothly and symmetrically curved, calomus smoothly curved, junction of rostrum and calomus slightly angular to smoothly rounded. Spicule tip finely rounded, with cucullus. Gubernaculum absent. Three pairs of papilla-shaped subventral caudal papillae, one pair adcloacal, one pair postcloacal mid-distance between cloaca and tail tip, one pair near tail tip. Tail conoid, length 1.6 times cloacal body diam., tail tip rounded, without a mucron. Bursa or bursal flap absent.

Entomophilic female from figs (presumed to be associated with fig wasp pollinators for dispersal): Body cylindrical, straight or slightly ventrally arcuate when heat-killed. Cuticle, lateral field, head, stylet, pharynx and intestine similar to male. EP 7–11 µm posterior to anterior end, near the lip or head. Deirids, hemizonid and phasmids not observed. Vulva posteriorly situated at 69–77% of total body length. Vagina slightly anteriorly directed. Ovary situated on left of intestine, monodelphic, prodelphic, outstretched, without reflex. Uterus long, smooth, crustaformeria absent. Oviduct with ovoid spermatheca filled with sperms, Oocytes in single file throughout most of ovary. Post-uterine sac (PUS) short, one-third vulval body diam. (VBD) long. Tail bluntly conoid, length 3.1–4.7 times body diam. at anus (ABD), tail tip broadly rounded with a mucron.

Reproductive female (from figs): Body ventrally arcuate or C-shaped when heat-killed. Features of cuticle, lateral field, head, stylet, pharynx, EP and tail similar to entomophilic female. Vulva posteriorly located at 70–77% of total body length, vulval lips slightly protruding. Ovary situated on left of intestine, anterior part usually reflexed once to right, monodelphic, prodelphic, well-developed. Uterus long, smooth, crustaformeria absent. Oviduct with ovoid spermatheca. Oocytes in single file throughout most of ovary. PUS length one-third diameter of body at vulva (VBD), Tail conoid, length 2.2–4.8 times ABD, tail tip rounded with a mucron.

**Diagnosis and relationships.** *Schistonchus altissimus* n. sp. is typologically characterised by the combined characters of a short PUS (4.5–8 µm or 0.1–0.4 VBD long), and conoid tail with a mucron in females; excretory pore located near the lip or head; amoeboid sperm, conoid tail without mucron, three pairs of subventral papillae, no gubernaculum, strongly recurved spicules with a thorn-shaped rostrum and capitulum strongly bent towards the lamina and a cecula on the tip in males. It is also distinguished by its biogeography and Ficus host species, being collected from *F. altissima* in southern China in association with the pollinator fig wasp *E. altissima*. Its status as a distinct species is corroborated by unique molecular sequences of partial SSU and the LSU D3 expansion segment (Figs 1, 2).

*Schistonchus altissimus* n. sp. is typologically distinguished from all other described species except *S. microcarpus* by having a spicule with a cecula on the tip. It has a PUS 0.1–0.4 VBD long in females. Species with a similar PUS are *S. aculeata* and *S. microcarpus*, which also have a PUS <0.4 VBD in reproductive females. *Schistonchus altissimus* n. sp. differs from *S. microcarpus* by having a longer (7–17 vs 13–17 µm) in reproductive females, and a spicule tip with vs without a cecula in males; from *S. stenomympha* by having a shorter PUS (0.4 vs 0.6 VBD long) in females, a smaller spicule (12–16 vs 18–24 µm), spicule tip with vs without cecula, and tail tip without vs with mucron in males; from *S. aureus* by a shorter PUS (0.4 vs 0.4–0.6 VBD long) in females, spicule tip with vs without cecula, and tail tip without vs with mucron in males; from *S. benjamina* by having a shorter PUS (0.4 vs 0.4–0.6 VBD long), fewer incisures in the lateral field (3 vs 4), spicule tip with vs without cecula in males, and reproductive system with crustaformeria absent vs present, with spermatheca vs no true spermatheca in reproductive females; from *S. centerae* by having fewer spicules in the lateral field (3 vs 4), spicule tip with vs without cecula, tail tip without vs with mucron in males, and tail tip with vs without a mucron in females; from *S. laevigatus* by having a shorter PUS (0.4 vs 0.4–0.6 VBD long) in females, and spicule tip with vs without cecula in males; from *S. microcarpus* by having a smaller spicule (12.0–16.0 vs 16.0–18.5 µm), 3 distinct vs indistinct spicule rostrum.
FIGURE 3. Adult male and reproductive female of *Schistonchus altissimus* n. sp. in lateral view. A: Anterior body of reproductive female; B: Vulva, post-uterine sac of reproductive female reproductive system; C–E: Male spicules; F: Anterior body of male; G: Male tail; H: Reproductive female tail; I: Entire reproductive female; J: Entire male (Scale bars: A–H = 20 µm; I, J = 50 µm).
FIGURE 4. Photomicrographs of adult entomophilic, reproductive female and male of *Schistonchus altissimus* n. sp. in lateral view. A, B: Anterior body of reproductive female (arrow=excretory pore); C: Vulva, post-uterine sac of reproductive female reproductive system; D: Reproductive female tail; E: Lateral incisures of reproductive female; F: Entomophilic reproductive female tail; G: Anterior body of male (arrow=excretory pore); H: Male tail (first arrow=cucullus; other three arrows=papillae) (All scale bars = 10 µm).
incisures in the lateral field, and tail tip in males without two morphotypes vs with two morphotypes; and from S. virens by having a longer stylet (16–22 vs 14–16 m) in females, and spicule tip with vs without cucullus in males.  


Males of S. altissimus 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), similar to the arrangement in S. aculeata, S. aureus, S. benjamina, S. fistulosus, S. fleckeri, S. hirtus, S. laevigatus, S. microcarpus and S. virens. However, it differs from the situation in S. africanus, S. altimacrophylla, S. caprifici, S. centerae and S. macrophylla, in which one pair is precloacal, one pair adcloacal, and one pair postcloacal, and from S. cassowaryi in which one pair is pre-cloacal, one almost lateral at mid-tail, and one subventral near the tail tip.

Discussion

In overall morphological appearance, S. altissimus n. sp. is closest to S. microcarpus. Phylogenetically, S. altissimus n. sp. is monophyletic with S. microcarpus (Figs 1, 2), but its sequences are significantly different for the multiple copy loci that we examined. Although S. altissimus n. sp. and S. microcarpus were collected at the same location (Guangzhou, China), they are from different fig host species [F. altissima vs F. microcarpa; both from the same taxonomic section (Urostigma) and subsection (Conosycea) of the subgenus (Urostigma)], and are putatively associated with different species of pollinator wasps (respectively, Eupristina altissima and Paraprinstina verticillata). The unique molecular sequences of the partial SSU, the LSU D3 expansion segment and the fragment of mtCOI support our assertion that S. altissimus n. sp. is indeed a separate species from S. microcarpus. For example, the 591-bp fragment of mtCOI for S. altissimus n. sp. was equally dissimilar to S. microcarpus (16%) when compared with the highly divergent S. caprifici (15%) which is consistent with the hypothesis that these two species are on non-coalescing trajectories.

Numbers and relative positions of genital papillae on male tails appear to be important characters in species taxonomy in Schistonchus. There are three pairs of genital papillae in male tails of all described species belonging to the group of Schistonchus with the EP located near the lip or head, including S. aculeata, S. altimacrophylla, S. altissimus n. sp., S. aureus, S. benjamina, S. centerae, S. fleckeri, S. laevigatus, S. microcarpus and S. virens, with similar arrangements of the papillae being observed in S. aculeata, S. aureus, S. benjamina, S. fleckeri, S. laevigatus, S. microcarpus and S. virens (i.e., one pair adcloacal, one pair halfway between cloacal aperture and tail terminus, and one pair near the tail tip). The papillae arrangement in S. altimacrophylla and S. centerae are divergent from the above-mentioned pattern (i.e., one precloacal pair, one pair adcloacal, and one pair of postcloacal papillae). However, some papillae are too small or hidden to observe clearly with the light microscope, emphasizing the need for scanning electron microscope observations on genital papillae in male tails in Schistonchus to help accurately delineate species boundaries given their highly conserved general morphology.

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