Journal of Nematology 33(2–3):91–103. 2001.

Schistonchus aureus n. sp. and *S. laevigatus* n. sp. (Aphelenchoididae): Associates of Native Floridian *Ficus* spp. and Their *Pegoscapus* Pollinators (Agaonidae)[1]

Nicole DeCrappeo[2] and Robin M. Giblin-Davis[2]

Abstract: Two new species of *Schistonchus* were recovered from the hemocoel of adult female fig wasps, *Pegoscapus* spp. (Agaonidae), and the syconia of *Ficus* spp. native to Florida. They are described here as *Schistonchus aureus* n. sp., associated with *Pegoscapus mexicanus* and *Ficus aurea,* and *Schistonchus laevigatus* n. sp., associated with *Pegoscapus* sp. and *Ficus laevigata.* The Florida species of *Schistonchus* are differentiated from each other by host plant and fig wasp associates, number of incisures in the lateral field, male spicule shape, and mucronate male tail of *S. aureus* n. sp. *Schistonchus aureus* n. sp. and *S. laevigatus* n. sp. are differentiated from other members of the genus by the absence of a lip sector disc as observed with scanning electron microscopy for comparison with *S. caprifici* and *S. macrophylla.* Excretory pore position for both Florida species, *S. altermacrophylla,* and *S. africanus* is less than 50% (one half) the distance between the top of the head and the top of the metacorpus, as opposed to other species (*S. caprifici, S. hispida, S. macrophylla,* and *S. racemosa*), where it is located near or below the level of the metacorpus. Males of both species from Florida can be distinguished from *S. altermacrophylla* and *S. africanus* by the more posteriad positioning of the three pairs of caudal papillae; the anteriormost pair of papillae in the Florida species are adcloacal vs. precloacal in the other two species.

Key words: Agaonidae, Aphelenchoididae, *Ficus,* fig, nematode, new species, parasitism, *Pegoscapus,* phoresy, scanning electron microscopy, *Schistonchus aureus* n. sp., *Schistonchus laevigatus* n. sp., taxonomy.

Ficus spp. (Moraceae) and their fig wasp pollinators (Agaonidae) have coevolved to form highly mutualistic relationships that are distributed worldwide with associated nematode symbionts (Giblin-Davis et al., 2001). Fig trees produce syconia, or figs, that release semiochemicals attracting a species-specific pollinator wasp. A female fig wasp enters the lumen of the syconium through the ostiole, or entrance pore, and pollinates and oviposits in female florets. The two native Florida fig species are monoecious (both male and female florets are present in each individual syconium), and each syconium contains female florets with different style lengths, allowing the wasp to parasitize only a fraction of the total receptive florets. Two underscribed species of *Schistonchus* were found to be associated with fig wasp pollinators from the two species of *Ficus* in Florida (Giblin-Davis, 1993; Giblin-Davis et al., 1992, 1995, 2001). Molecular analysis of the D3 expansion segment of the 28S rRNA gene supports the designation of separate species for the two Florida isolates of *Schistonchus* (Giblin-Davis et al., 2001).

The nematode associated with *Pegoscapus mexicanus* Ashmead (*P. jimenezi* (Grandi) sensu Wiebes) and *Ficus aurea* Nuttall is described here as *Schistonchus aureus* n. sp., and the nematode associated with *Pegoscapus* sp. and *Ficus laevigata* Vahl (*F. citrifolia* Miller sensu DeWolf, 1960) is described as *Schistonchus laevigatus* n. sp.

Materials and Methods

Syconia from *Ficus aurea* were collected in Broward County, Florida, between May 1996 and May 1997; syconia from *F. laevigatus* were collected in Dade County, Florida, during the same time period. Syconia were staged in individual petri dishes, chopped with a scalpel, and allowed to sit in distilled water for 5 to 10 minutes. Emerging phytoparasitic life stages and preinfective, pre-reproductive entomophilic female nematodes were collected individually or on a Baermann funnel. All nematodes used for measurements by light microscopy were heat-killed and mounted in temporary water mounts. Adult female fig wasps were dissected in distilled water, and pre-reproductive entomophilic female nematodes found in the hemocoel were prepared for measurements as above. Drawings were done with the aid of a camera lucida, stage micrometer, and Adobe Photoshop 4.0. Specimens for permanent mounts were processed slowly into 100% glycerol and mounted (Southey, 1970).

Scanning electron microscopy (SEM) was used to examine and compare the ultrastructure of the two new species with *S. caprifici* Gasparrini. Adult males and females were collected from phase C and D syconia, heat-killed, and fixed in 5% formalin glycerol (Southey, 1970). Specimens were then placed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for several days and postfixed in 2% OsO_4 overnight. Fixed specimens of *S. caprifici* were provided by N. Vovlas from Italy. Specimens were dehydrated to 100% ethanol through a graded series, critical-point dried using carbon dioxide, mounted on stubs, sputter-coated with 20 nm of gold-palladium, and observed with a Hitachi S-4000 Field Emission SEM at 15 kv.

Morphometric data were statistically analyzed using

Received for publication 2 November 2000.

[1] Contribution of the Florida Agricultural Experiment Station, Journal Series R-06177.

[2] Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Ave., Davie, FL 33314.

The authors thank Nicola Vovlas for providing specimens of *Schistonchus caprifici* from Italy; Scott Whittaker and Greg Erdos, Interdisciplinary Core for Biotechnology Research (ICBR), University of Florida, for assistance with the scanning electron microscope; Kerrie Davies, Renato Inserra, and Nicola Vovlas for critical review of the manuscript; the U.S. Department of Interior's SCA/AmeriCorps program for providing support for N. DeCrappeo; and Everglades National Park for providing collecting sites and permits. This research was supported in part by USDA Special Grant in Tropical and Subtropical Agriculture CRSR-99-34135-8478.

E-mail: giblin@ufl.edu

This paper was edited by R. T. Robbins.

t-tests ($P < 0.05$) with a general linear models procedure (Proc GLM) for unbalanced data sets (SAS Institute, Cary, NC). For both species, measurements of pre-infective, pre-reproductive entomophilic females isolated from figs were compared with those of pre-reproductive entomophilic females isolated from wasps to determine if nematodes grew while inside the wasp. Morphometrics of pre-reproductive entomophilic females were compared to those of reproductive females from early phase C figs to demonstrate that females go through a major growth period once inside the next generation of figs. Measurements of both sexes and all stages of both new species of *Schistonchus* were compared to determine if any significant morphometric variation occurs between the species.

Head pattern terminology used in this description is as proposed by Giblin-Davis et al. (1989). The labial disc described in previous *Schistonchus* descriptions is referred to as the lip sector disc; we use the term labial disc to describe the area immediately surrounding the oral aperture, enclosed by the inner labial sensillae. Stylet length was measured from the base of the cephalic framework to the bottom of the stylet knobs because the tip of the stylet cone was usually not visible. Esophagus length is the distance from the top of the head to the bottom of the metacorpus because the esophageal-intestinal junction is difficult to discern in most specimens. Spicule length is the distance between the condylus and the posterior-most point of the spicule measured in a straight line, and spicule width is the length of the capitulum (Giblin-Davis et al., 1993).

Systematics

Schistonchus aureus n. sp.
(Figs. 1–7)

Measurements of males, females, and pre-reproductive entomophilic females from figs and fig wasps in temporary water mounts are presented in Tables 1–4.

Males from fig (Table 1): Body cylindrical, tapered at both ends, J-shaped when heat-killed. Cuticle with fine annulation, annules about 0.3 µm wide at mid-body. Lateral field with three very faint incisures (Fig. 7G), not visible with light microscope, extending posteriorly to tail tip. Cuticle of lateral field slightly thickened between last pair of papillae and tail tip, but not developed into a terminal bursal flap = caudal ala (Fig. 6A,C). Head not offset from body in SEM but may appear so under light microscope. En face pattern with SEM consisting of a labial disc about 0.6 to 0.9 µm diam. with clearly defined oral aperture (ca. 0.2 µm) (Figs. 2A;3A,B). Six inner labial sensilla present at the margin of the labial disc. Six obscured outer labial sensilla located on the fused lip sectors surrounding the labial disc (two subdorsal, two lateral, two subventral) (Fig. 2A). Six cephalic sectors, laterally compressed, surround fused lip sectors and labial disc (Figs. 2A;3A,B). Amphidial apertures dorso-medially located on each lateral cephalic sector. One cephalic papilla on each of the two subventral and two subdorsal cephalic sectors (Figs. 2A;3A,B). Six head annules visible, not always contiguous (annules counted from anteriormost body annule). Stylet consisting of a conus greater than 1/2 of total stylet length and a shaft with well-developed basal knobs. Under light microscope, tip of stylet is difficult to see, obscured by well-developed stomal wall and appearing flange-like (Figs. 1A;4A,C). Excretory pore 1 to 2 head diam. behind top of head (Figs. 1A;3A;4A). Procorpus about 1.5 stylet lengths long, ending in well-developed metacorpus with valves posterior to center. Dorsal esophageal gland orifice opens into lumen of metacorpus about one half metacorpal valve length above metacorpal valve; postcorpus glandular, well developed. Esophago-intestinal junction just posterior to metacorpus. Isthmus rudimentary. Deirids, hemizonid, and phasmids not seen. Testis outstretched or reflexed. Sperm amoeboid. Tail arcuate, about two anal body widths long. Spicules paired, arcuate, rose-thorn-shaped with low distinct rostrum (Fig. 5A,B). Gubernaculum faintly visible. Three pairs of genital papillae: one pair adcloacal (Fig. 6B,C,D), inconspicuous under light microscope; one pair halfway between cloaca and tail tip (Fig. 6A,B,C); and one pair about 1.5 µm from tail terminus (Figs. 5A;6A,C). Tail tip mucronate (Figs. 5A;6A,C).

Reproductive females from fig (Table 2): Body ventrally arcuate to C-shaped when heat-killed. Female cuticular features and cephalic regions indistinguishable from those of adult male. Lateral field with three incisures as in males; extends to tail terminus. Ovary monodelphic, prodelphic, about 15–20 vulval body widths long, usually reflexed, oocytes in single file. Eggs 2 to 3 times longer than wide, 64 ± 5 µm long, 24 ± 2 µm wide (n = 8). Vulva at 72% of body length, vagina posteriorly directed. Vulval lips slightly protruded. Post-uterine sac short, less than 1 body width long. Anus dome-shaped slit in ventral view (Fig. 7D). Tail conoid, about 3 times longer than anal body width, tail tip rounded (Fig. 7D).

Pre-reproductive entomophilic females from fig and wasp (Tables 3–4): Nematodes straight or with slight ventral curve in posterior region when heat-killed. Cuticle, lateral fields, head, stylet, and excretory pore as in reproductive females. Total length, body width at level of vulva (VBW), length from head to vulva, body width at level of anus (ABW), and tail length all smaller (P = 0.0001) in pre-reproductive entomophilic stage from fig than in egg-producing female from fig. Esophagus length (P = 0.0315) and dorsal-esophageal gland (not compared statistically) longer than in egg-producing females (dorsal-esophageal gland about 4 to 5 stylet lengths long). Ovary about 2 to 4 vulval body widths

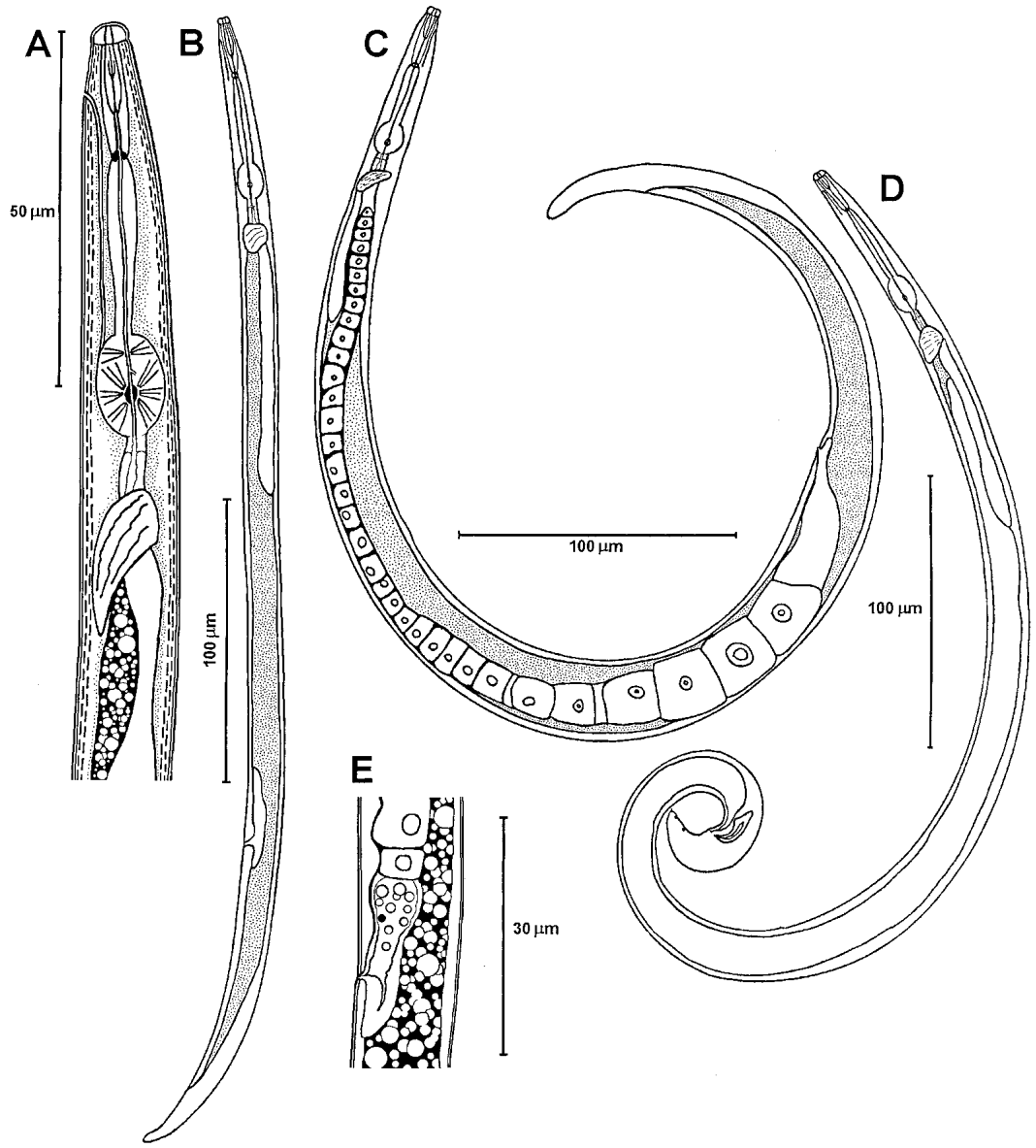

FIG. 1. *Schistonchus aureus* n. sp. A) Anterior body portion of female. B) Entomophilic female. C) Reproductive female. D) Male. E) Vulva and post-uterine sac of entomophilic female.

long. Vulva 65 to 74% of body length, vagina posteriorly directed. Vulva lips barely discernible (Fig. 7A). Tail conoid and straight, tail tip rounded.

Type host and locality

Nematodes were collected on 8 January 1997 from phase C syconia of *Ficus aurea* Nuttall or the wasp vector, *Pegoscapus mexicanus* (Ashmead) from Davie, Broward County, Florida.

Type specimens

Holotype male and allotype female and paratypes deposited at the University of California-Riverside Nematode Collection.

Schistonchus laevigatus n. sp.
(Figs. 2–8)

Measurements of males, females, and pre-reproductive entomophilic females from temporary water mounts are presented in Tables 1–4.

Males from fig (Table 1): Body cylindrical, tapered at both ends, J-shaped when heat-killed. Cuticle with fine annulation. Lateral field consists of five or more discontinuous incisures (Fig. 7H), inconspicuous under light microscope, extending posteriorly to tail tip. Cuticle of lateral field slightly thickened between last pair of papillae and tail tip but is not developed into a terminal bursal flap (Fig. 6E,H). Head not offset from body in SEM but may appear to be with light microscope. En face pattern indistinguishable from *S. aureus* n. sp.

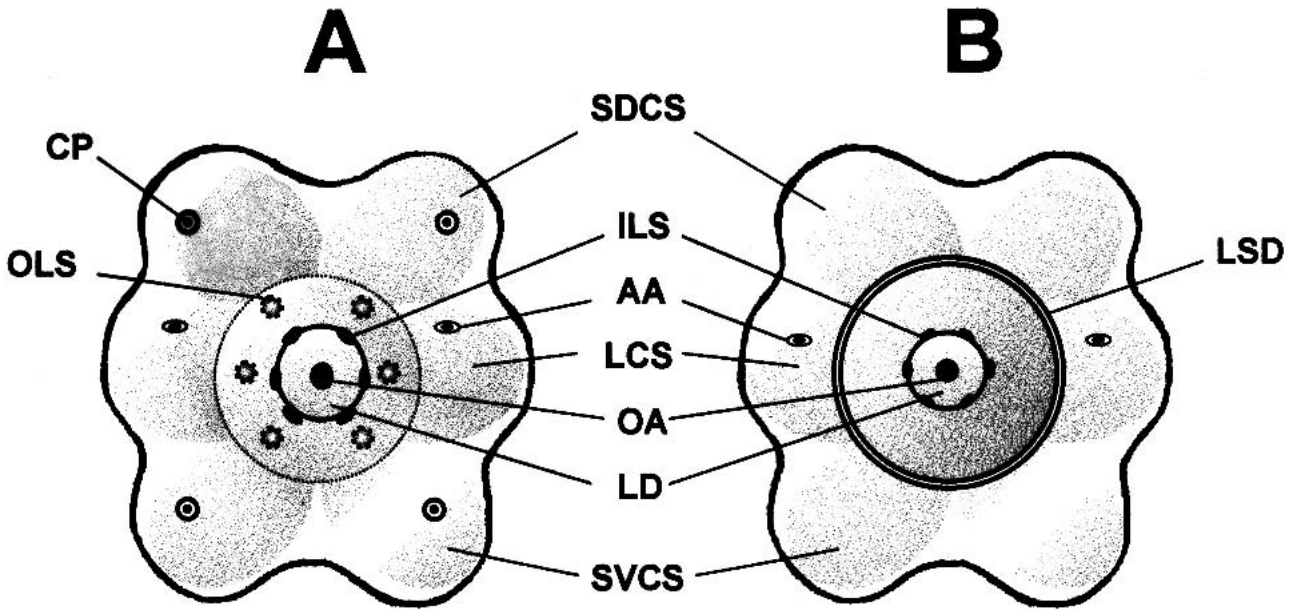

FIG. 2. Comparative diagrams of en face patterns from scanning electron micrographs. A) *Schistonchus aureus* n. sp. and *S. laevigatus* n. sp. B) *S. caprifici* and *S. macrophylla.* AA = amphidial aperture, CP = cephalic papilla, ILS = inner labial sensilla, LCS = lateral cephalic sector, LD = labial disc, LSD = lip sector disc, OA = oral aperture, OLS = outer labial sensilla (obscured), SDCS = subdorsal cephalic sector, SVCS = subventral cephalic sector.

(Figs. 2A;3C,D). Six head annules visible, not always contiguous. Stylet comprised of a conus about 2/3 of total stylet length and a shaft with well-developed basal knobs. Under light microscope, tip of stylet is difficult to see, as in *S. aureus* n. sp., obscured by well-developed stomal wall and appearing flange-like (Figs. 4E;8A). Excretory pore about 1 to 3 head diam. behind top of head (Figs. 3C;8A). Procorpus about 2 stylet lengths long, ending in well-developed metacorpus with valves posterior to center. Dorsal esophageal gland orifice opens into lumen of metacorpus about 1 metacorpal valve length anterior to the metacorpal valve. Esophageal-intestinal junction just posterior to metacorpus. Isthmus rudimentary. Deirids, hemizonid, and phasmids not seen. Testis outstretched or reflexed. Spermatozoa amoeboid. Tail arcuate, conoid, less than 2 anal body widths long. Spicules paired, arcuate, rose-thorn-shaped; rostrum pointed (Fig. 5C,D). Gubernaculum faintly visible. Three pairs of papillae: one pair adcloacal (Fig. 6E,F,G,H), inconspicuous under light microscope; one pair halfway between cloaca and tail tip (Fig. 6E,H); and one pair about 1.5 µm from tail terminus (Fig. 6H). Papilla-like structure protruding laterally from cloaca when spicules are extended (Fig. 6F). Tail tip rounded, no mucron (Figs. 5C;6E,H).

Reproductive females from fig (Table 2): Body ventrally arcuate to C-shaped when heat-killed. Female cuticular features and cephalic regions indistinguishable from those of the male. Lateral field with five discontinuous incisures as in males; extends to tail terminus. Ovary monodelphic, prodelphic, about 15–20 vulval body widths long, usually reflexed, oocytes in single file. Eggs 2 to 3 times longer than wide, 64 ± 4 µm long, 27 ± 4 µm wide (n = 11). Vulva at 71% of body length, vagina posteriorly directed. Vulval lips slightly protruded (Fig. 7B). Post-uterine sac short, less than 1 body width long. Anus dome-shaped slit in ventral view. Tail conoid, about 3 to 4 times longer than anal body width, tail tip rounded (Fig. 7E).

Pre-reproductive entomophilic females from fig and wasp (Tables 3–4): Nematodes straight or slightly ventrally curved in posterior region when heat-killed. Cuticle, lateral fields, head, stylet, and excretory pore as in females. Total length, VBW, length from head to vulva, ABW, and tail length all smaller in pre-reproductive entomophilic stage from fig than in egg-producing female ($P \leq 0.001$). Esophagus length ($P \leq 0.001$) longer in entomophilic females than in egg-producing females from figs. Ovary about 2 vulval body widths long. Vulva 66 to 73% of body length, vagina posteriorly directed. Vulva lips barely discernible. Tail conoid and straight, tail tip rounded.

Type host and locality

Nematodes were collected from phase C syconia of *Ficus laevigata* Vahl (*F. citrifolia* Miller sensu DeWolf, 1960) or the wasp vector, *Pegoscapus* sp. from Florida City, Dade County, Florida. Mature females were collected 18 July 1997, and pre-reproductive entomophilic females and males were collected 29 January 1997.

Type specimens

Holotype male and allotype female and paratypes deposited at the University of California-Riverside Nematode Collection.

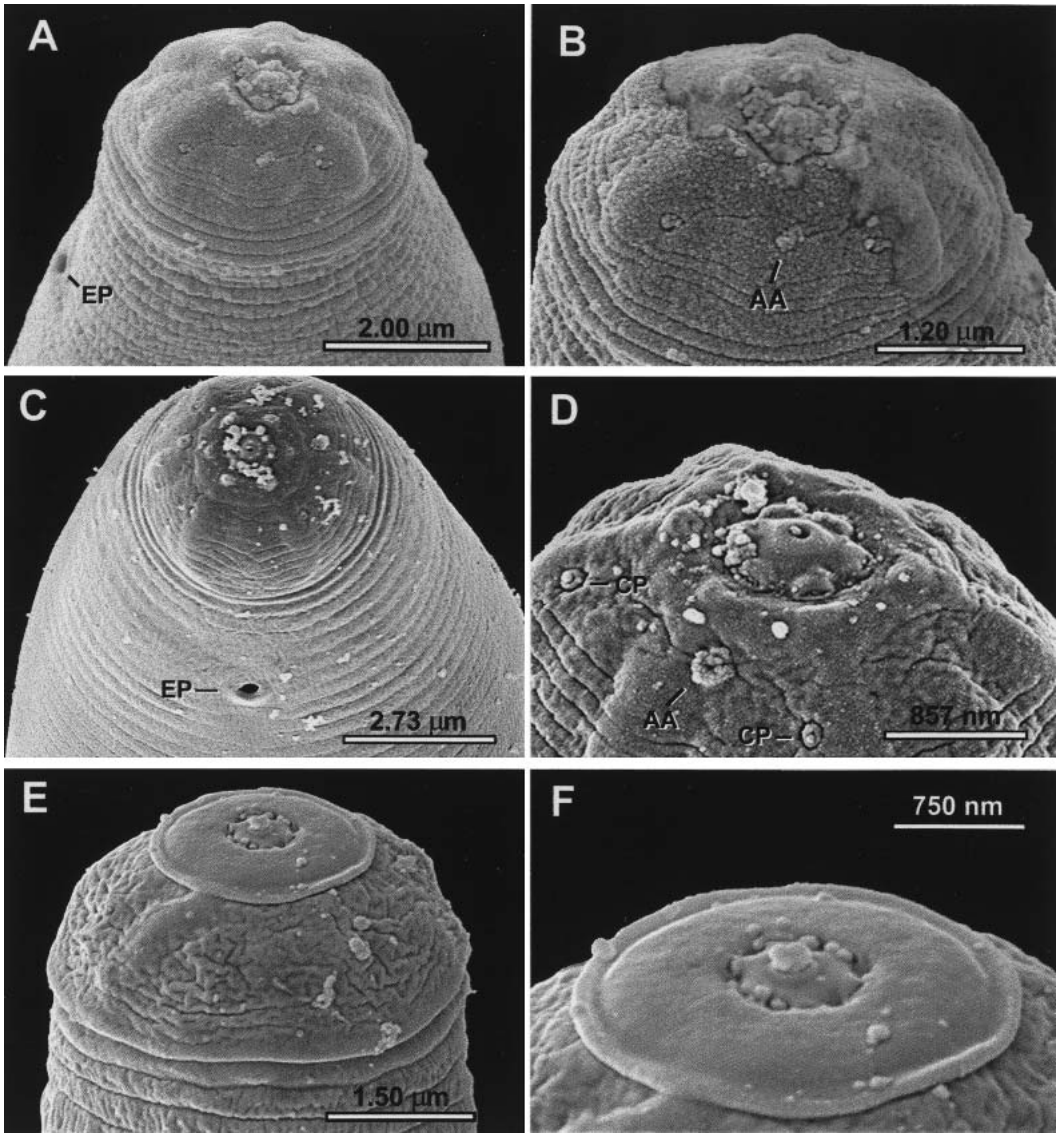

FIG. 3. Scanning electron micrographs of head regions. A-B) *Schistonchus aureus* n. sp. female. C-D) *S. laevigatus* n. sp. female. E-F) *S. caprifici* female. AA = amphidial aperture, CP = cephalic papillae, EP = excretory pore.

Diagnosis

The following combination of characteristics distinguish *Schistonchus aureus* n. sp. and *S. laevigatus* n. sp. from the six other described *Schistonchus* species. Both new species and sexes en face pattern are characterized by having an oral aperture surrounded by a labial disc ringed with six inner labial sensilla, six outer labial sensilla (obscured), two dorso-lateral amphidial apertures, and four cephalic papillae (Fig. 2A). The lip sector disc is not present. Contrastingly, for other *Schistonchus* species that have been observed with SEM (*S. caprifici* (Gasparrini) [De Ley and Coomans, 1996; this study] and *S. macrophylla* Lloyd & Davies [Lloyd and Davies, 1997; Davies, pers. comm.]), the en face pattern consists of an oral aperture surrounded by a labial disc ringed with six inner sensilla and a distinct lip sector disc without visible outer labial sensilla or distinct lips (Figs. 2B;3E,F). Two dorso-lateral amphidial apertures were covered by possible secretions, and cephalic papillae were not resolved (Figs. 2B;3E,F). Excretory pore position for both new Florida species, *S. altermacrophylla* Lloyd & Davies, and *S. africanus* Vovlas, Troccoli, van Noort & van den Berg, is less than one half the distance between the top of the head and the top of the metacorpus, as opposed to the other species (*S. caprifici, S. hispida* Kumari & Reddy, *S. macrophylla,* and *S. racemosa* Reddy & Rao) where it is located near or below the level of the metacorpus. Males of *S. aureus* n. sp. and *S. laevigatus* n. sp. have three pairs of caudal papillae: one adcloacal and two postcloacal; whereas *S. hispida* and *S. racemosa* have two pairs occurring pre- and postcloacal or both postcloacal, respectively. Males of *S. africanus, S. altermacrophylla, S. caprifici,* and *S. macrophylla* have three pairs of caudal papillae with one pair precloacal, one pair adcloacal, and one pair postcloacal.

Schistonchus aureus n. sp. differs from *S. laevigatus* n.

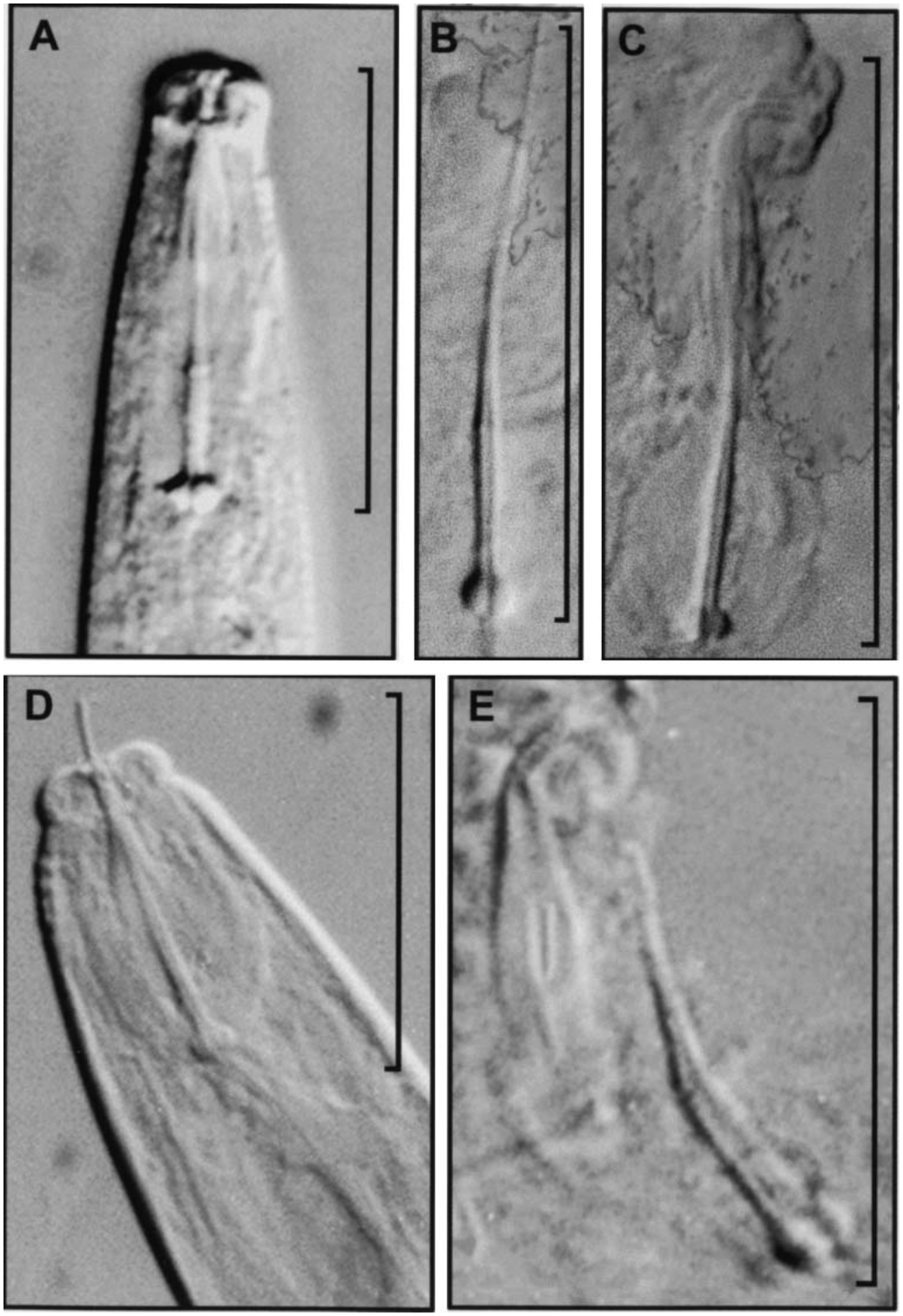

FIG. 4. Photomicrographs of head region and extracted stylets showing flange-like structure of the stomal wall. A-C) *Schistonchus aureus* n. sp. males. D-E) *S. laevigatus* n. sp. females. All scale bars = 20 µm.

sp. by the number of incisures in the lateral field of males and females (three faint incisures in *S. aureus* n. sp., visible only with SEM, compared with five discontinuous lines in *S. laevigatus* n. sp.), spicule shape (rostrum small and rounded in *S. aureus* n. sp. vs. pointed in *S. laevigatus* n. sp.), male tail with mucron in *S. aureus* n. sp. (absent in *S. laevigatus* n. sp.), and *Ficus* and *Pegoscapus* host range. In addition, recent phylogenetic analysis using sequence data from the D3 expansion segment of the 28S rRNA gene support that *S. aureus* n. sp. and *S. laevigatus* n. sp. are different species (Giblin-Davis et al., 2001).

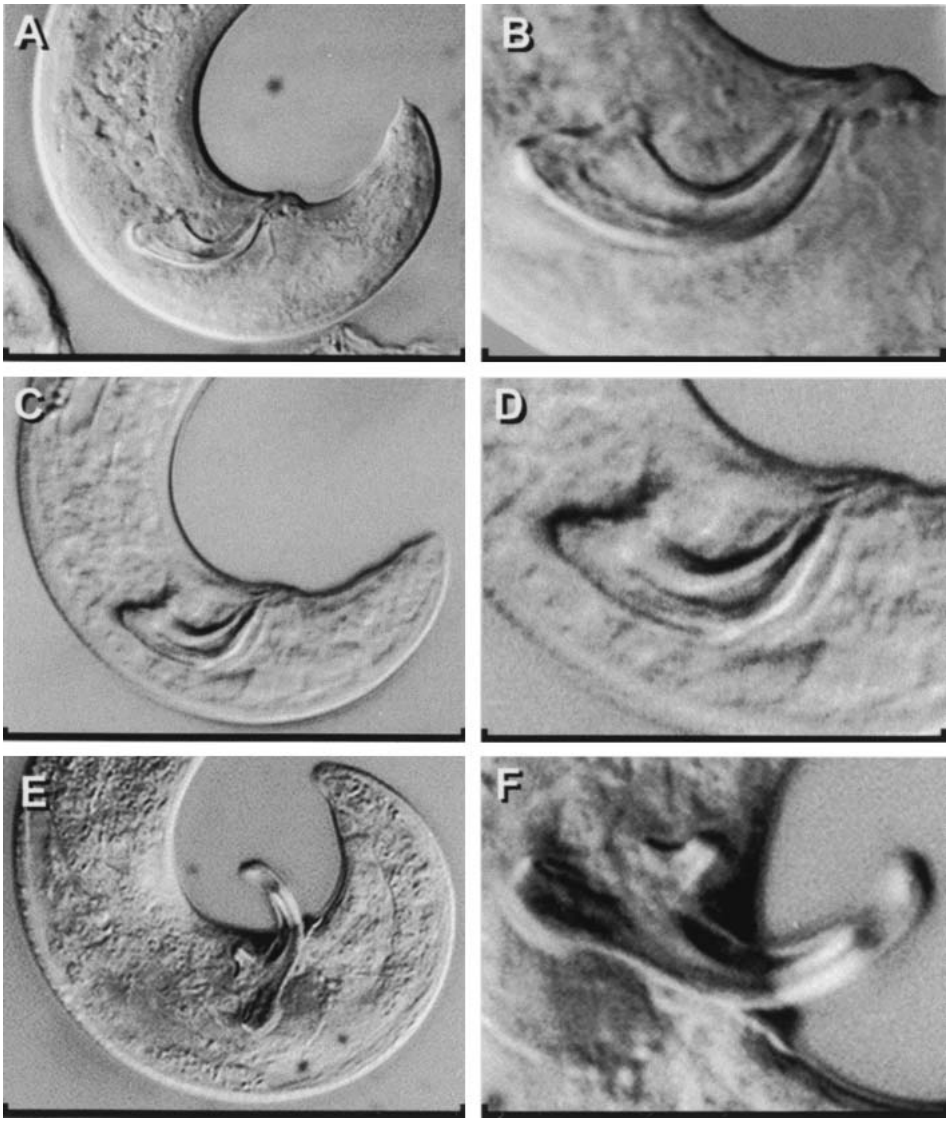

FIG. 5. Comparison of male tails and spicules. A-B) *Schistonchus aureus* n. sp. C-D) *S. laevigatus* n. sp. E-F) *S. caprifici*. Scale bars for A, C, E = 50 µm, B, D, F = 20 µm.

Morphometric comparisons show that, on average, males of *S. aureus* n. sp. were thinner, with a longer stylet, wider spicules, narrower ABW, and smaller b ratios than males of *S. laevigatus* n. sp. (Table 1). Reproductive females of *S. aureus* n. sp. had a narrower VBW and shorter tail than *S. laevigatus* n. sp. (Table 2). Pre-reproductive entomophilic females of *S. aureus* n. sp. from figs were shorter and narrower with a longer stylet; shorter esophagus, vulva-anus distance, and anal body width; smaller b ratio; and larger V% than *S. laevigatus* n. sp. (Table 3). Pre-reproductive entomophilic females of *S. aureus* n. sp. from fig wasps were wider, with a longer stylet and esophagus, shorter vulva-anus distance and tail length, smaller a and b ratios, and larger c ratio and V% than *S. laevigatus* n. sp. (Table 4). Although these mean differences were significant ($P \leq 0.05$), there were no discontinuities in the ranges for these measurements (except for a shorter vulva-anus difference distance in pre-reproductive entomophilic females of *S. aureus* n. sp. from figs than *S. laevigatus* n. sp.).

Relationships

Schistonchus aureus n. sp. and *S. laevigatus* n. sp. are similar to the Australian *S. altermacrophylla* and African *S. africanus* in excretory pore position, amoeboid spermatozoa, and short postuterine sac. Males of both new *Schistonchus* species from Florida can be distinguished from *S. altermacrophylla* and *S. africanus* by the more posteriad positioning of the three pairs of caudal papillae; the anteriormost pair of papillae in the Florida species are adcloacal vs. precloacal in the other two species.

The morphological similarities between *S. altermacrophylla* and *S. africanus* and the Neotropical species (*S. aureus* n. sp. and *S. laevigatus* n. sp. from Florida; *Schis-*

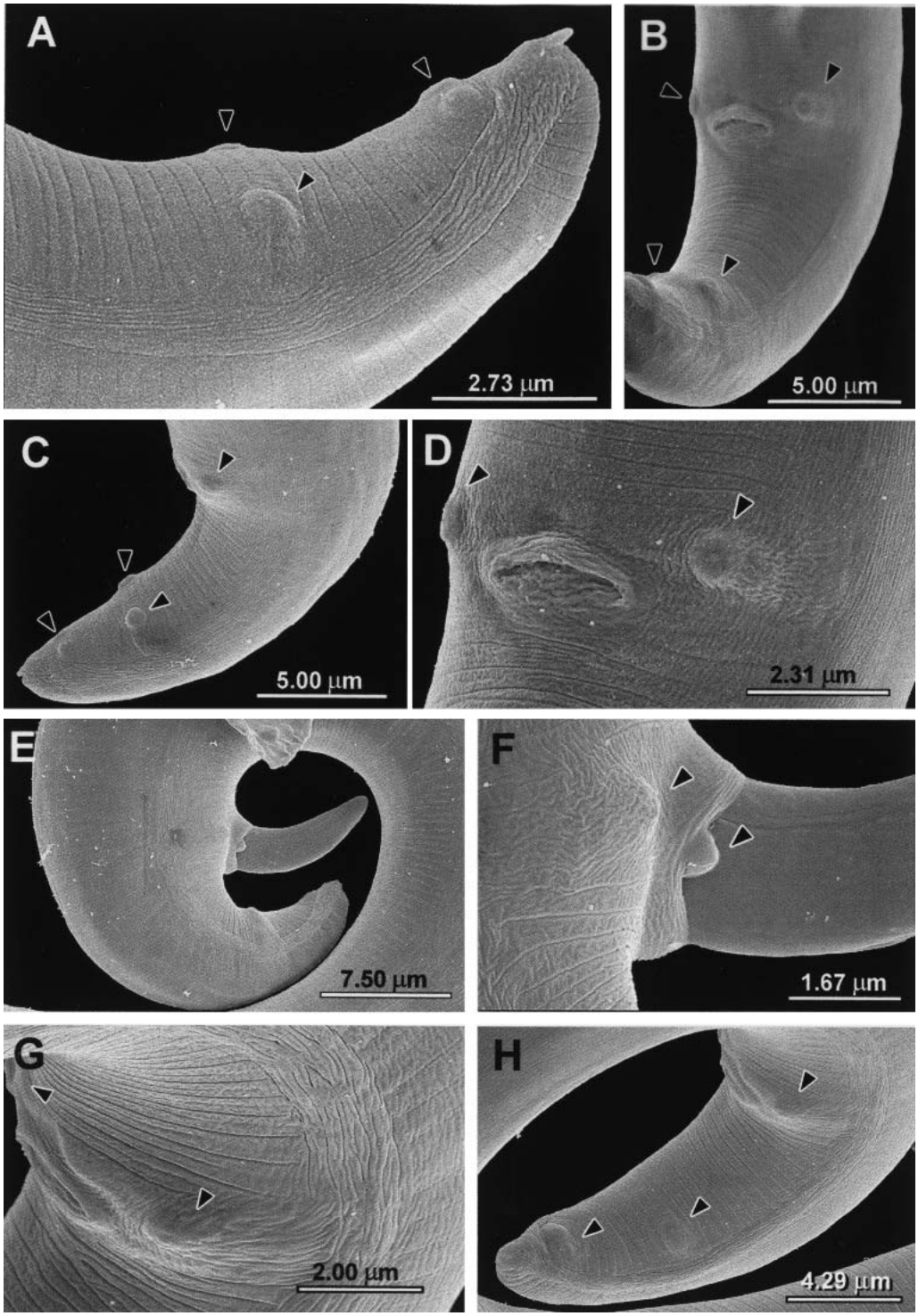

FIG. 6. Scanning electron micrographs of male tails. A-D) *Schistonchus aureus* n. sp. E-H) *S. laevigatus* n. sp. Arrows = caudal papillae.

tonchus spp. from *Ficus hemsleyana* and *F. pertusa* L. from Costa Rica; and *Schistonchus* spp. from *F. dugandii* Standl., *F. popenoei* Standl., *F. trigonata* L., *F. glabrata* H.B.K., *F. maxima* P. Mill, and *F. yoponensis* Desv. from Panama) may indicate common Gondwanaland ancestors or convergence. If the former is true, then we might expect the lip sector disc to be absent in SEM for *S. altermacrophylla* or *S. africanus* (SEM work on these species was not reported).

Biological characteristics

The biology of the two new *Schistonchus* species from Florida varies from the other described *Schistonchus* species. Pre-reproductive, entomophilic females of these new *Schistonchus* species are carried in the hemocoel of their respective fig wasp hosts; the association with the wasps appears to be one of internal phoresy (Giblin-Davis et al., 1995). The female nematodes exit the he-

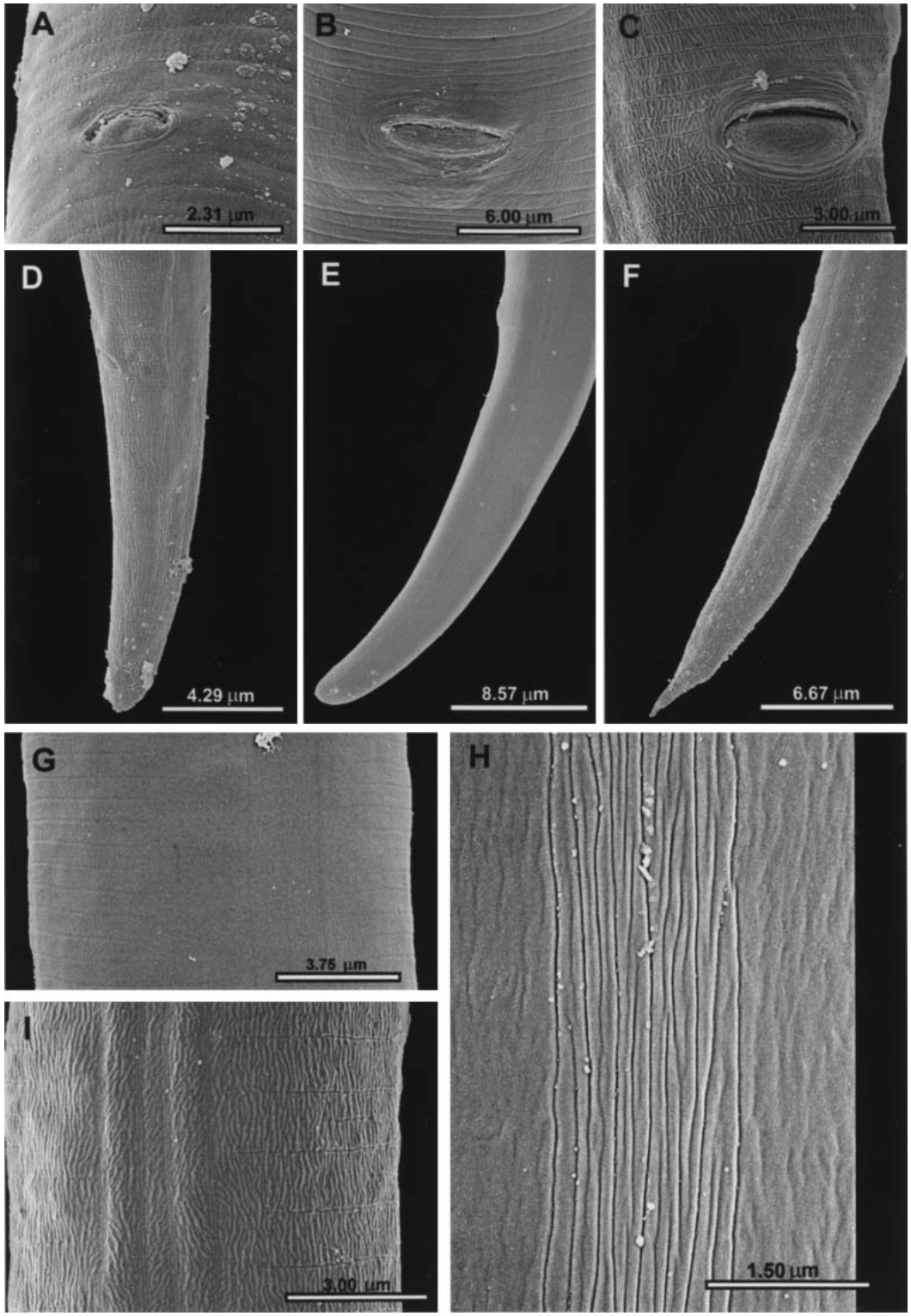

FIG. 7. Scanning electron micrographs of vulvas, tails, and lateral fields. A) *Schistonchus aureus* n. sp., vulva of entomophilic female. B) *S. laevigatus* n. sp., vulva of reproductive female. C) *S. caprifici,* vulva of reproductive female. D) *S. aureus* n. sp., female tail and anus. E) *S. laevigatus* n. sp., lateral view of female tail and anus. F) *S. caprifici,* lateral view of female tail and anus. G) Lateral field with three faint incisures from male *S. aureus* n. sp. H) Lateral field with five discontinuous lines from female *S. laevigatus* n. sp. I) Lateral field with four clear incisures from female *S. caprifici.*

TABLE 1. Morphometrics of males of *Schistonchus aureus* n. sp. and *S. laevigatus* n. sp. from figs measured in temporary water mounts.

	Schistonchus aureus n. sp.				*Schistonchus laevigatus* n. sp.			
Measure	*n*	Mean	SD	Range	*n*	Mean	SD	Range
Length	23	493	54.2	378–621	14	517	32.9	462–573
Width	22	17*	3.0	10–23	12	20	2.8	16–26
Stylet length	23	17*	1.2	14–18	14	15	1.9	12–19
Esophagus length	22	60	4.8	51–70	15	56	6.4	40–65
Spicule length	22	14	1.4	12–18	15	14	0.8	12–15
Spicule width	23	6*	0.7	5–8	15	5	0.6	4–6
Anal body width	23	14*	1.5	12–18	15	16	1.4	14–19
Tail length	23	26	2.3	20–30	15	27	2.9	22–33
a	22	29	5.7	21–51	12	26	4.0	18–32
b	22	8*	0.7	7–10	14	9	1.0	8–12
c	23	19	1.6	16–22	14	19	2.2	16–24

All measurements in µm.
Means in a row followed by an asterisk are significantly different at $P \leq 0.05$ with a *t*-test.

mocoel of the female wasp after she enters the lumen of a phase B syconium (female phase; female florets receptive to pollination) and parasitize either the syconial wall (*S. aureus* n. sp.) or the anthers (*S. laevigatus* n. sp.) (Giblin-Davis et al., 1995; Center et al., 1999). After 1 to 4 days, the phase B syconium reaches phase C. At least one generation of *Schistonchus* develops at the expense of the fig during phase C (interfloral phase; developing fig embryos and wasp larvae and pupae). After about 30 days, the phase C syconium matures to phase D (male phase; male florets with mature pollen), which contains adult male and female *Schistonchus,* adult male and female wasps, and mature fig seeds. It is presumed that after mating, pre-reproductive entomophilic female nematodes enter the hemocoels of emerging female wasps of the next generation. These wasps collect pollen, exit the syconium through an emergence hole created by the wingless male wasps, and seek out a new phase B syconium for oviposition. In the process, an infested female wasp vertically transmits the nematodes to the next generation of figs and fig wasps (Giblin-Davis et al., 1995, 2001). The biology for *S. aureus* n. sp. appears to be identical to that of *S. laevigatus,* although female:male sex ratios for the two species in phase D figs are significantly different (about 10:1 for *S. laevigatus* n. sp. vs. 3:1 for *S. aureus* n. sp.).

Schistonchus macrophylla and *S. altermacrophylla,* from Australia, appear to have life-history traits similar to those of the Florida *Schistonchus* species. Young mated females of these species have been found in female wasps; the nematode probably has a phoretic relationship with the pollinator wasp, *Pleistodontes froggatti* Mayr (Lloyd and Davies, 1997). *Schistonchus caprifici,* from the gynodioecious fig, *Ficus carica* L. var. *sylvestris* Shinn in Italy, is able to parasitize and develop inside both the syconium and the pollinator wasp, *Blastophaga psenes* L. (Vovlas et al., 1992). Because the nematodes grow and reproduce in the wasp, they are probably parasites—although no damage to the insect was reported by Vovlas et al. (1992). *Schistonchus caprifici* also has been found in the hemocoel of *Philotrypesis caricae* (L.), a cleptoparasitic wasp in the *F. carica* system (Vovlas and Larizza, 1996). The African species, *S. africanus,* inhabited about 45% of the dissected wasp pollinators (*Elisabethiella stuckenbergi* Grandi). Each female wasp emerging from its gall had 5–88 juvenile, male, and female

TABLE 2. Morphometrics of reproductive females of *Schistonchus aureus* n. sp. and *S. laevigatus* n. sp. from figs measured in temporary water mounts.

	Schistonchus aureus n. sp.				*Schistonchus laevigatus* n. sp.			
Measure	*n*	Mean	SD	Range	*n*	Mean	SD	Range
Length	15	573	60.4	493–686	18	614	121.7	405–851
Width (at vulva)	15	22*	3.4	18–29	18	26	5.8	18–36
Stylet length	15	15	0.8	14–17	17	15	2.4	10–21
Esophagus length	15	58	4.1	52–66	18	57	7.0	44–70
Vulva-anus distance	13	130	18.0	100–164	17	142	31.0	79–201
Anal body width	15	10	1.0	9–12	18	11	1.7	8–14
Tail length	15	30*	4.9	23–40	18	37	8.3	22–51
a	15	26	2.3	23–30	18	24	4.3	15–32
b	15	10	1.1	8–12	18	11	1.6	8–13
c	15	19*	2.0	15–22	18	17	1.8	13–19
V	13	72	1.7	69–75	17	71	3.9	59–78

All measurements in µm.
Means in a row followed by an asterisk are significantly different at $P \leq 0.05$ with a *t*-test.

TABLE 3. Morphometrics of pre-reproductive entomophilic females of *Schistonchus aureus* n. sp. and *S. laevigatus* n. sp. from figs measured in temporary water mounts.

	Schistonchus aureus n. sp.				*Schistonchus laevigatus* n. sp.			
Measure	*n*	Mean	SD	Range	*n*	Mean	SD	Range
Length	15	384*	22.0	342–416	15	453	44.9	399–584
Width (at vulva)	15	13*	1.6	11–18	13	15	2.1	11–18
Stylet length	15	19*	0.5	18–20	15	16	2.2	13–21
Esophagus length	15	61*	4.2	52–69	14	66	4.7	60–77
Vulva-anus distance	15	85*	6.3	73–93	13	111	7.3	100–126
Anal body width	15	7*	0.6	6–9	15	8	1.2	6–11
Tail length	15	22*	2.7	19–28	15	27	3.0	22–34
a	15	30	2.7	22–35	13	31	4.0	24–36
b	15	6*	0.4	5–7	14	7	0.5	6–8
c	15	18	1.9	14–20	15	17	1.4	15–19
V	15	72*	1.4	68–74	13	70	1.9	66–73

All measurements in μm.
Means in a row followed by an asterisk are significantly different at $P \leq 0.05$ with a *t*-test.

nematodes (5 females: 1 male) (Vovlas et al., 1998). The two Indian *Schistonchus* species, *S. hispida* Kumari and Reddy and *S. racemosa* Reddy and Rao, are carried in the abdominal folds of the wasp as second-stage juveniles (Kumari and Reddy, 1984; Reddy and Rao, 1986).

Etymology

The species names were derived from the names of the fig species with which these nematodes are associated.

As new *Schistonchus* species are being discovered continuously, it may be necessary to update the genus diagnosis on a regular basis. The Florida species have morphological characteristics not included in the last genus emendation; therefore, a reworking of *Schistonchus* is in order.

Genus Schistonchus Cobb, 1927 (Fuchs, 1937)

Diagnosis, emended from Hunt (1993) and Lloyd and Davies (1997).

Aphelenchoidinae. Primarily associated with the syconia of *Ficus* and fig wasps (Agaonidae). Nematodes about 0.4–0.8 mm long. Straight or ventrally arcuate to C-shaped (females) or J-shaped or coiled (males) on heat relaxation. Cuticle finely annulated. Lateral fields consist of three, four, or five incisures, discontinuous in some species, thickened slightly ventrally in males between the last pair of papillae and the tail tip, but not enough to be called a terminal bursa. Cephalic framework symmetrically hexaradiate. Lip sector disc (=labial disc) present in some species, absent in others. Head region offset or tapered. Number of head annules varies from 0–6. Stylet robust, 13–26 μm long, with strong basal knobs. Relative length of conus variable, from 52–71% of total stylet length. Procorpus fairly short, cylindrical (except in some species), leading to a strong, ovoid, metacorpus with post-median valve plates. Esophago-intestinal valve near bottom of metacorpus. Isthmus rudimentary. Excretory pore position varies from about the level of the nerve ring and below the metacorpus to two to four head lengths behind the head. Dorsal esophageal gland lobe well developed, subventral lobes sometimes elongated. Vulva usually

TABLE 4. Morphometrics of pre-reproductive entomophilic females of *Schistonchus aureus* n. sp. and *S. laevigatus* n. sp. from wasps measured in temporary water mounts.

	Schistonchus aureus n. sp.				*Schistonchus laevigatus* n. sp.			
Measure	*n*	Mean	SD	Range	*n*	Mean	SD	Range
Length	15	408	26.5	336–448	20	417	35.0	339–470
Width (at vulva)	14	14*	1.8	13–18	20	12	1.0	11–15
Stylet length	15	19*	1.6	13–20	19	14	1.7	11–17
Esophagus length	14	66*	2.5	62–72	20	59	6.4	45–69
Vulva-anus distance	14	95*	5.4	89–108	20	103	10.7	82–119
Anal body width	15	8	1.2	7–11	19	8	0.7	6–9
Tail length	15	26*	2.4	23–31	20	29	3.2	22–36
a	14	29*	3.7	23–33	20	34	2.9	30–40
b	14	6*	0.4	5–7	20	7	0.4	6–8
c	15	16*	1.1	14–17	20	14	1.3	11–17
V	14	70*	1.9	65–72	20	68	1.0	67–70

All measurements in μm.
Means in a row followed by an asterisk are significantly different at $P \leq 0.05$ with a *t*-test.

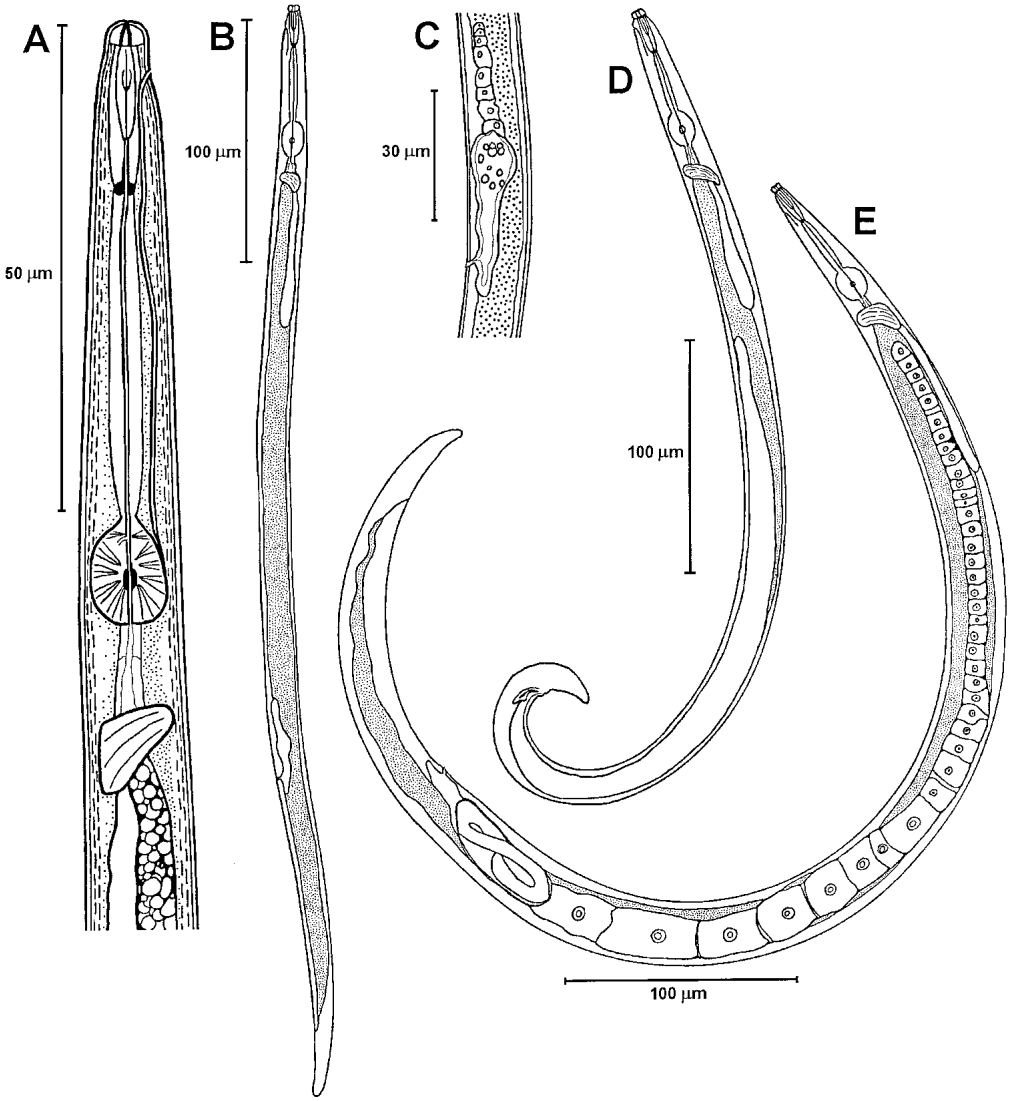

FIG. 8. *Schistonchus laevigatus* n. sp. A) Anterior body portion of female. B) Entomophilic female. C) Vulva and post-uterine sac. D) Male. E) C-shaped female with egg.

situated at 60–75% of total body length (has been reported up to 89% in *S. hispida* and 95% in *S. altermacrophylla*), usually with slightly protuberant lips. Vagina directed anteriorly or posteriorly. Genital tract monodelphic and prodelphic, outstretched or reflexed, with oocytes in one or two rows. Post-uterine sac variable in length. Female tail with a rounded or pointed tip. Male tail region sharply recurved. Spicules paired, ventrally arcuate, rose thorn-shaped with a rostrum and a broadly rounded apex continuing the line of the shaft. Gubernaculum often visible. Spermatozoa flagellate or amoeboid. Caudal papillae present. Male tail short, with a rounded or mucronate tip.

DISCUSSION

The phoretic association between the nematodes and fig wasps hypothesized by Giblin-Davis et al. (1995) was based on histological work done on *F. laevigata* and its pollinator wasp, *Pegoscapus* sp. This work suggested that *S. laevigatus* n. sp. feeds on the anthers of the fig but does not feed or cause any apparent damage while inside the fig wasp. Subsequent work has been done on *F. aurea* (the host fig of *S. aureus* n. sp.) but not on its pollinator wasp, *P. mexicanus* (Center et al., 1999).

Schistonchus aureus n. sp. was associated with necrotic lesions and hypertrophied cells in the sycone of *F. aurea,* demonstrating that this nematode also parasitizes the fig (Center et al., 1999). The morphometrics for pre-reproductive entomophilic females of *S. aureus* n. sp. suggest some growth in the wasp host because many of the measurements were significantly greater for entomophilic female nematodes collected from the wasps than for those collected from figs (analyses not shown). However, several factors should be taken into consideration. First, there is always a potential for sampling error. Second, entomophilic females from the fig were sometimes measured from late C-stage figs, meaning

they still had at least 1 or 2 days before entering the hemocoel of a wasp emerging from a D-phase fig. Given their short life spans, *S. aureus* n. sp. females could significantly increase in size in 1 or 2 days, which would explain the discrepancy. Finally, the relative health of the figs chosen could play a part in the fitness of its symbionts, so that nematodes collected from a less healthy syconium could be smaller than nematodes collected from fig wasps emerging from a healthier fig.

Conversely, morphometric comparisons of entomophilic females from *F. laevigatus* n. sp. (analyses not shown) suggest that the nematodes are shrinking after entering the fig wasps. Again, the data may be skewed by sampling error but indicate that the nematodes are not benefiting at the expense of their wasp hosts.

Adaptive radiation, described by Poinar and Herre (1991) for *Parasitodiplogaster* species from figs from Panama and Zimbabwe, might be expected for *Schistonchus* species found throughout the world. *Schistonchus,* however, shows much less morphological and morphometric variation than the corresponding host isolates of *Parasitodiplogaster* spp. This is true not only of the described *Schistonchus* species but also for members of the genus observed from Panama (Giblin-Davis and DeCrappeo, unpubl. data), Costa Rica, and Australia (Giblin-Davis and Davies, unpubl. data). Molecular data, such as sequences from the D3 and D2-D3 expansion segments of the 28S rRNA gene, may prove important for confirming the identity of different host isolates of *Schistonchus* and for hypothesizing phylogenetic relationships (Giblin-Davis et al., 2001). Scanning electron microscopy may also prove critical because many of the differentiating characteristics between the Neotropical and Old World species may be limited to structures difficult to interpret with a light microscope, such as the head region and lateral fields.

An obstacle in correctly characterizing the remaining *Schistonchus* species and determining their phylogenetic relationships lies in properly identifying their *Ficus* hosts and Agaonid associates worldwide. Coevolutionary models and cladistic analyses between the three organisms (figs, fig wasps, and nematodes) will be strengthened with independent molecular data sets (Giblin-Davis et al., 2001).

Literature Cited

Center, B. J., R. M. Giblin-Davis, E. A. Herre, and G. C. Chung-Schickler. 1999. Histological comparisons of parasitism by *Schistonchus* spp. (Nemata: Aphelenchoididae) in Neotropical *Ficus* spp. Journal of Nematology 31:393–406.

De Ley, P., and A. Coomans. 1996. Terrestrial nematodes of the Galapagos archipelago. 6. *Punchaulus gemellensis,* a new genus and species of Aphelenchina (Tylenchida). Fundamental and Applied Nematology 19:159–165.

Giblin-Davis, R. M. 1993. Interactions of nematodes with insects. Pp. 302–344 *in* W. Khan, ed. Nematode interactions. London: Chapman and Hall, Inc.

Giblin-Davis, R. M., B. J. Center, H. Nadel, J. H. Frank, and W. Ramirez B. 1995. Nematodes associated with fig wasps, *Pegoscapus* spp. (Agaonidae), and syconia of native Floridian figs (*Ficus* spp.). Journal of Nematology 27:1–14.

Giblin-Davis, R. M., K. A. Davies, G. L. Taylor, and W. K. Thomas. 2001. Entomophilic nematode models for studying biodiversity and cospeciation. In press *in* Z. X. Chen, S. Y. Chen, and D. W. Dickson, eds. Nematology, advances and perspectives. New York: Tsinghua University Press/Springer-Verlag.

Giblin-Davis, R. M., M. Mundo-Ocampo, J. G. Baldwin, K. Gerber, and R. Griffith. 1989. Observations on the morphology of the red ring nematode, *Rhadinaphelenchus cocophilus* (Nemata: Aphelenchoididae). Revue de Nématologie 12:285–292.

Giblin-Davis, R. M., M. Mundo-Ocampo, J. G. Baldwin, B. B. Norden, and S. W. T. Batra. 1993. Description of *Bursaphelenchus abruptus* n. sp. (Nemata: Aphelenchoididae), an associate of a digger bee. Journal of Nematology 25:161–172.

Giblin-Davis, R. M., H. Nadel, and J. H. Frank. 1992. New species of *Schistonchus* and *Parasitodiplogaster* parasitizing the fig wasp, *Pegoscapus assuetes,* in the syconia of *Ficus citrifolia.* Journal of Nematology 24:592 (Abstr).

Hunt, D. J. 1993. Aphelenchida, Longidoridae, and Trichodoridae: Their systematics and bionomics. Cambridge: CAB International Institute of Parasitology.

Kumari, R. V., and Y. N. Reddy. 1984. Studies on the association of a new nematode species *Schistonchus hispida* sp. n. (Aphelenchoidea, Nickle 1971) and wasp. Proceedings of the Indian Academy of Parasitology 5:21–25.

Lloyd, J., and K. A. Davies. 1997. Two new species of *Schistonchus* (Tylenchida: Aphelenchoididae) associated with *Ficus macrophylla* from Australia. Fundamental and Applied Nematology 20:79–86.

Poinar, G. O., Jr., and E. A. Herre. 1991. Speciation and adaptive radiation in the fig wasp nematode *Parasitodiplogaster* (Diplogasteridae: Rhabditida) in Panama. Revue de Nématologie 14:361–374.

Reddy, Y. N., and P. N. Rao. 1986. *Schistonchus racemosa* sp. n., a nematode parasite of wasp (*Ceratosolen* sp.) associated with the fig, *Ficus racemosa* L. Indian Journal of Nematology 16:135–137.

Southey, J. F., ed. 1970. Laboratory methods for work with plant and soil nematodes. London: Her Majesty's Stationery Office.

Vovlas, N., R. N. Inserra, and N. Greco. 1992. *Schistonchus caprifici* parasitizing caprifig (*Ficus carica sylvestris*) florets and the relationship with its fig wasp (*Blastophaga psenes*) vector. Nematologica 38:215–226.

Vovlas, N., and A. Larizza. 1996. Relationship of *Schistonchus caprifici* (Aphelenchoididae) with fig inflorescences, the fig pollinator *Blastophaga psenes,* and its cleptoparasite *Philotrypesis caricae.* Fundamental and Applied Nematology 19:443–448.

Vovlas, N., A. Troccoli, S. Van Noort, and E. Van den Berg. 1998. *Schistonchus africanus* n. sp. (Aphelenchida: Aphelenchoididae) associated with *Ficus thonningii* (Moraceae) and its pollinator wasp *Elisabethiella stuckenbergi* (Chacidoidea: Agaonidae). Journal of Nematology 30:404–410.