Nematodes Associated with Fig Wasps, *Pegoscapus* spp. (Agaonidae), and Syconia of Native Floridian Figs (*Ficus* spp.)[1]

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Abstract: Syconia in successive developmental phases from *Ficus laevigata* Vahl (*F. citrifolia* Miller sensu DeWolf 1960) (Moraceae) and successive life stages of its fig wasp pollinator, *Pegoscapus* sp. (*P. assuetus* (Grandi) sensu Wiebes 1983) (Agaonidae) were dissected to elucidate their association with two undescribed species of nematodes. *Parasitodiplogaster* sp. (Diplogasteridae) are transported by female *Pegoscapus* sp. into the cavity of a phase B syconium as third-stage juveniles (J3), where they molt to the J4 stage and greatly increase in size in the hemocoel of the fig wasp after it begins to pollinate and oviposit in female florets. The J4 exit the wasp cadaver in a phase B or early phase C syconium, and molt to adults that mate and lay eggs. New J3 infect the next generation of female or male wasps as they emerge from their galls in phase D figs. Mated entomogenous females of *Schistonchus* sp. (Aphelenchoididae) are transported in the hemocoel of female wasps to the fig cavity of a phase B syconium. Female *Schistonchus* sp. exit the wasp and parasitize immature male florets causing an exudate, the development of hypertrophied epidermal cells of the anther filaments and anthers, and aberrations of the anther filament, anthers, and pollen. At least one generation of *Schistonchus* sp. occurs in the male florets. Entomogenous females appear at about the time that fig wasps molt to adults in their galls in late phase C syconia. Another *Schistonchus* sp. was recovered from females of *P. mexicanus* (Ashmead) (*P. jimenezi* (Grandi) sensu Wiebes 1983) and from the syconia of *F. aurea* Nuttall and appears to have a life cycle similar to that described for the *Schistonchus* sp. from *F. laevigata*.

Key words: Agaonidae, Aphelenchoididae, Diplogasteridae, *Ficus aurea*, *Ficus laevigata*, fig, life history, nematode, parasitism, *Parasitodiplogaster* sp., *Pegoscapus* spp., *Schistonchus* spp.

Over 700 species of figs (*Ficus* spp.) worldwide are mutualistically associated with highly host-specific fig wasps (Agaonidae) for pollination (1,2,6,22,29). A high degree of coevolution is apparent in the adaptations of both mutualists in each fig–fig wasp association. The fig tree produces enclosed inflorescences (syconia), commonly called figs (Figs. 1,2). Syconia produce semiochemicals that attract only the specific fig wasp pollinator to the flowers (florets) via an ostiole (28), an entrance pore that is filled with a whorl of bracts that screen out most other insects (Figs. 1,2A) (2,26). In all figs, flowering is protogynous with pistillate (female) florets being receptive to pollination and parasitism by female wasp foundresses several weeks before staminate (male) florets produce pollen. About one-half of all fig species are gynodioecious, whereas the rest (including the native Neotropical species) are monoecious, with both male and female florets occurring in an individual syconium. Each syconium contains female florets with dif-

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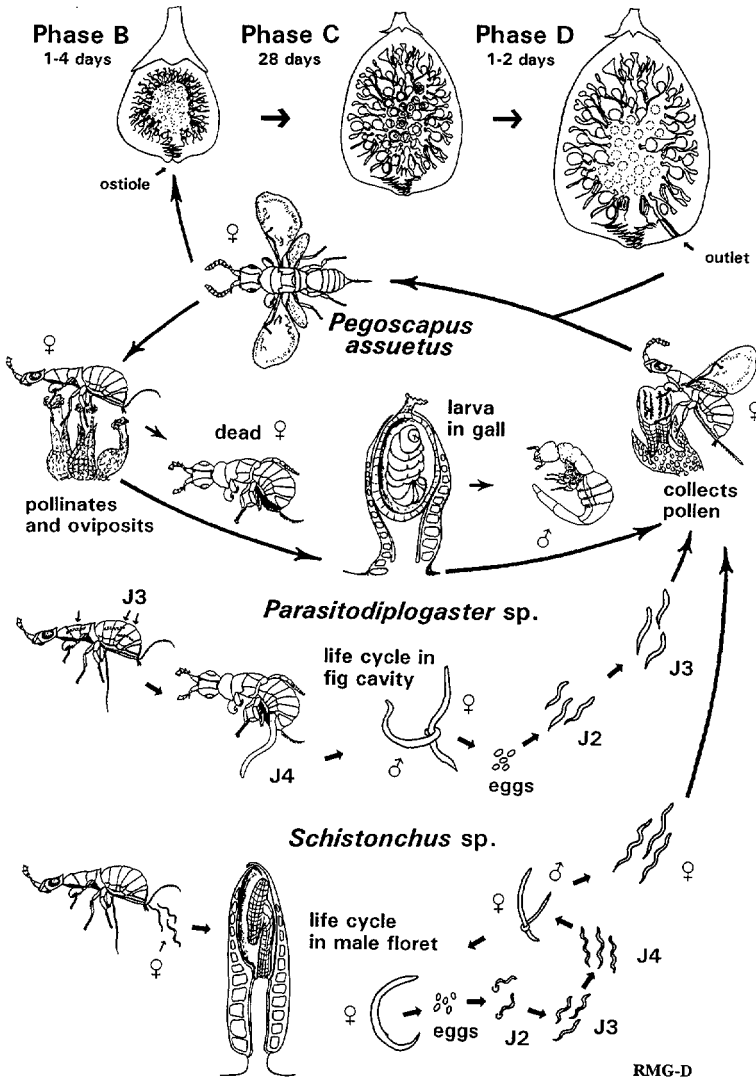
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Ficus laevigata

FIG. 1. Diagram of successive developmental phases of *Ficus laevigata* syconia, with associated life cycles of its pollinator, *Pegoscapus* sp., and the nematodes *Parasitodiplogaster* sp. and *Schistonchus* sp. The phase B syconium contains receptive female florets and produces semiochemicals that attract the female foundress wasp, which penetrates the ostiole, pollinates, oviposits, and dies. J3 of *Parasitodiplogaster* sp. and entomogenous adult females of *Schistonchus* sp. are carried in the hemocoel of the wasp into the syconial cavity. J3 of *Parasitodiplogaster* sp. molt to the J4 stage and greatly increase in size in the wasp, whereas females of *Schistonchus* sp. leave and parasitize immature male florets. In the phase C syconium, the fig embryos and wasp larvae develop within their respective fig ovaries. The J4 of *Parasitodiplogaster* sp. exit the cadaver of the foundress wasp and molt to adults, which mate and lay eggs in the syconial cavity. The eggs eclose to J2, which molt to the insect-parasitic J3 by the end of phase C. *Schistonchus* sp. entomogenous females enter an ectoparasitic phytophagous cycle and produce eggs, which cycle through J2, J3, and J4 to adult females and males. The cycle may be repeated once more and ends with the production of mated entomogenous females by the end of phase C. In the phase D syconium, male florets mature and present pollen, male wasps mate with unemerged females, and female wasps emerge, collect pollen, and exit the syconium via holes made by the male(s). J3 of *Parasitodiplogaster* sp. and entomogenous females of *Schistonchus* sp. enter the hemocoel of the female wasp about the time she leaves the phase D syconium to search for a new phase B syconium.
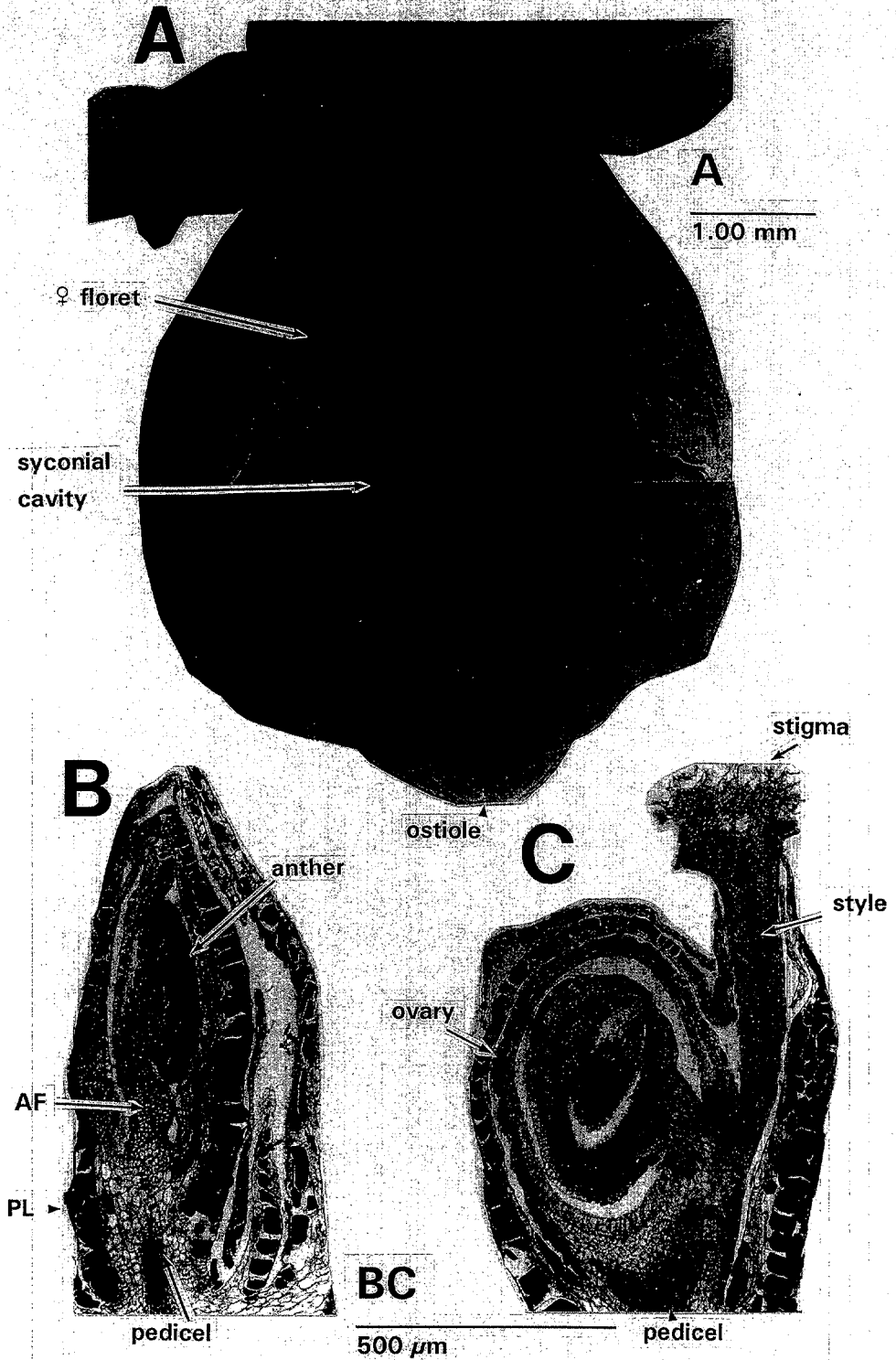
FIG. 2. Female phase B syconium of *Ficus laevigata*. A) Scanning electron micrograph of opened syconium (lateral view). B) Longitudinal section through immature male floret from phase B syconium. C) Longitudinal section through receptive female floret from phase B syconium. AF = anther filament, PL = perianth lobe.

ferent style lengths and (or) firmness, which together with other factors may help to control the proportion parasitized by ovipositing wasps (26). Female *Pegoscapus* wasps are morphologically and behaviorally adapted to collect pollen, disperse, and locate a new receptive syconium, after which they open and squeeze through the ostiolar scales into the syconial cavity to pollinate and oviposit in female florets (2,22). A female floret that receives a wasp egg develops into a gall that contains multinucleate cells and irregular masses of tissue that are consumed by the developing wasp larva (16,26). Male *Pegoscapus* emerge before the female, are wingless, and have long telescoping abdomens to mate with females that are still inside their galls. Male wasps also cut an emergence hole in the syconial wall to allow the females to escape after they have emerged from their galls and collected pollen from the now mature staminate flowers (Fig. 1) (22,23).

Monoecious synconia are categorized into five successive developmental phases, which are synchronized with the parasitic habits of the pollinating fig wasp (7). The prefemale phase A occurs when the young syconium has immature florets, before loosening of the ostiolar scales. The female phase B occurs when ostiolar scales become relaxed and female florets become receptive inside the syconium. Female wasp(s) (foundresses) penetrate the ostiole into the syconial cavity and pollinate and oviposit in female florets (Figs. 1,2C), then die. The interfloral phase C occurs when fig embryos and wasp larvae develop within their respective fig ovaries (Fig. 1). Male phase D occurs when the male florets mature; wasp pupae molt inside their galls to adults; male wasps emerge and mate with females that are still in their galls; and female wasps emerge, "collect" pollen, and exit the synconium via holes made by male wasp(s) (Fig. 1). Last, the postfloral phase E occurs when the syconium and seeds ripen. The mature fig may then be consumed by birds or mammals, which disperse the seeds. The duration of the five

syconial phases is similar in *F. aurea* Nuttall and *F. laevigata* Vahl (*F. citrifolia* Miller sensu DeWolf 1960) (2). Prefemale phase A in *F. aurea* can last from 1 week to more than 8 months. "Dormant" phase A syconia remain on an *F. aurea* tree for months, and then resume growth and enter phase B within 2 days (3,4). The female phase B lasts 1 to 2 days in *F. aurea;* the interfloral phase C lasts 36 ± 7 days in *F. aurea* and 28 ± 5 days in *F. laevigata;* the male phase D in *F. aurea* lasts 1 to 2 days; and the postfloral phase E lasts 5 to 34 days (2,3,4). These phases are temperature-dependent (3,4).

Associations of several nematode species with fig wasps have been reported (9,11,12, 17–21,24,27). *Parasitodiplogaster sycophilon* Poinar (Diplogasteridae) parasitizes females of the fig wasp *Elisabethiella stuckenbergi* Grandi that pollinates *F. burkei* (Miq.) in Zimbabwe (20). Recently, 10 species of *Parasitodiplogaster* were described from Panama parasitizing females of 10 species of *Pegoscapus* from 10 species of *Ficus* (21).

Schistonchus caprifici (Gasperrini) (Aphelenchoididae) causes necrosis in the cortical parenchyma of the peduncles of female florets and anther filaments in the syconia of the gynodioecious fig, *Ficus carica* L. (27). This nematode is carried as eggs, juveniles, and (or) adults in the hemocoel of the female fig wasp, *Blastophaga psenes* L. (27). Little is known about *S. hispida* Kumari and Reddy, which is associated with a fig wasp in the syconia of *F. hispida* L. (17). *Schistonchus racemosa* Reddy & Rao is associated with the female fig wasp, *Ceratosolen* sp., which pollinates *F. racemosa* L. in India (24). The nematodes are carried as second-stage juveniles (J2) in the "abdominal folds" of the wasp, but the association between the nematode and the fig was not investigated.

Ficus laevigata and *F. aurea* are members of the monoecious subgenus *Urostigma,* section *Americana,* and are pollinated by *Pegoscapus* species. They are the only figs native to North America, but they range south to the West Indies (1,2,6,30). De-Wolf (5) lumped *F. laevigata, F. hemsleyana*

Standley, and *F. turbinata* Pitt. into one species, *F. citrifolia*. This has caused taxonomic confusion (23). The actual identities of *P. assuetus* (Grandi) and *F. citrifolia* from Florida (2–4,6,11,12,19,30) are *Pegoscapus* sp. (undescribed) and *F. laevigata* Vahl, whereas the actual identities of *P. assuetus* and *F. citrifolia* from Panama (13,14,21) are most likely *P. tonduzi* Grandi and *F. hemsleyana* (22,23).

Our study expands the observations previously reported in an abstract (12) concerning a new species of *Parasitodiplogaster*, which parasitizes *Pegoscapus* sp. (*P. assuetus* (Grandi) sensu Wiebes 1983), and a new species of *Schistonchus*, which parasitizes male florets of the shortleaf fig, *F. laevigata*, and is carried in the hemocoel of females of the fig wasp, *Pegoscapus* sp., in Florida. In addition, we expand observations (11) on a new *Schistonchus* sp. associated with the strangler fig, *F. aurea*, and the fig wasp, *P. mexicanus* (Ashmead) (*P. jimenezi* (Grandi) sensu Wiebes 1983).

MATERIALS AND METHODS

Biology: Syconia were collected from *F. laevigata* and *F. aurea* for dissection from several sites in southern Florida during this study, starting in June 1989. Although all syconial phases were examined, proportionally more interfloral phase C syconia were dissected (Table 1) because most of the development of the fig wasp and nematodes occurs during this phase.

Syconia were opened with a scalpel, staged, chopped, and placed individually into distilled water in petri dishes for at least 20 minutes. Emerging nematodes were collected, numbers counted or estimated, heat killed, and fixed in 5% formalin glycerol (25). Adult wasps inside a syconium were collected with an aspirator or with forceps and placed in a capped vial until dissection.

In total, 613 *F. laevigata* syconia and 342 of its *Pegoscapus* sp. pollinator in successive developmental phases or stages were dis-

TABLE 1. Association of *Parasitodiplogaster* sp. and *Schistonchus* sp. with successive developmental phases of *Ficus laevigata* from Florida.

	Developmental phase of dissected syconia†			
	B	C	D	E
Total no. of syconia	80	364	50	31
% with *Parasitodiplogaster* sp. only	30 (24/24)‡	29 (81/106)	12 (5/6)	19 (3/3)
% with *Schistonchus* sp. only	11 (9/9)	10 (24/35)	22 (4/11)	10 (1/6)
% with both nematode species	1 (1/1)	7 (22/24)	18 (9/9)	0
No. pollinators per syconium	1.5 ± 1.1§ (77)	1.5 ± 0.9 (92)	1.3 ± 0.6 (3)	—
No. of *Parasitodiplogaster* sp. per infested syconium				
Eggs	0	9 ± 5 (18)	0	0
J2–J3	0	74 ± 67 (77)	59 ± 40‖ (14)	4 ± 5‖ (3)
J4	6 ± 4 (24)	7 ± 8 (2)	0	0
♂	2 ± 1 (2)	4 ± 3 (78)	0	0
♀	0	4 ± 3 (88)	0	0
No. of *Schistonchus* per infested syconium				
Eggs	0	50 ± 48 (22)	0	0
J2–J4	0	115 ± 155 (38)	1,000 (1)	0
♂	0	7 ± 7 (19)	35 ± 57 (3)	10 (1)
♀	4 ± 5 (5)	15 ± 19 (34)	71 ± 112 (3)	0
Entomogenous ♀	3 ± 3 (8)	0	339 ± 538 (9)	20 (1)

† See introduction for a description of the developmental phases of monoecious figs. Eighty-eight phase A syconia were dissected and yielded no nematodes.

‡ Number of syconia observed; numerator is number of syconia dissected with nematode(s) where nematodes were staged and counted; denominator is total number of syconia with nematode(s).

§ Mean ± standard deviation.

‖ J3 stage only.

sected and examined. Most of the syconia were collected from 10 young *F. laevigata* along a canal adjacent to Palm Avenue, east of the turnpike, in Florida City, Dade County, Florida (n = 457, phase A-E syconia; May 1990 through April 1993). Additional collections of *F. laevigata* syconia were made in Florida from Monroe Station, Collier County (n = 118 phase C and D syconia; April 1990), Key Largo, Monroe County (n = 30 phase A syconia; June 1989), and Everglades National Park, Shark Valley, Dade County (n = 6 phase D syconia; February 1993). Phase C and D syconia from *F. aurea* were collected from Davie, Broward County (n = 8; January 1991), and Captiva Island, Lee County (n = 31; August 1993).

Newly emerged females of *Pegoscapus* sp. were collected from phase D syconia from the Florida City site and dissected (n = 213; June 1989 through April 1993). Newly emerged males of *Pegoscapus* sp. were also collected from phase D syconia from the Florida City site and dissected (n = 10; November 1990 through February 1993). Live females of *Pegoscapus* sp. were dissected out of their galls from phase D syconia from the Florida City site (n = 22; June 1989 through February 1993). Live pupae of *Pegoscapus* sp. were dissected out of their galls from a phase C synconium from the Florida City site (n = 24; May 1991). Dead foundress females of *Pegoscapus* sp. were dissected from phase B or early phase C syconia from the Florida City site and examined (n = 42; November 1990 through April 1993). Live foundress females of *Pegoscapus* sp. were dissected from phase B syconia from the Florida City site and examined (n = 31; March through April 1993).

Attempts were made to culture J3 of *Parasitodiplogaster* sp. from phase D syconia of *F. laevigata* and from infested females of *Pegoscapus* sp. on 10% glycerol supplemented potato dextrose agar (GPDA) (10) and 10% strength tryptic soy broth agar (TSB). Attempts were also made to culture entomogenous females and propagating *Schistonchus* sp. from phase D syconia of *F. laevigata* and entomogenous females from *Pegoscapus* sp. on 10% GPDA and GPDA seeded with the fungus *Monilinia fructicola* (Wint.) Honey (10).

Twenty *Parasitodiplogaster* sp.-infested females of *Pegoscapus* sp. from a phase D syconium of *F. laevigata* were placed in two groups of 10 each onto 1% water agar in petri dishes and observed for 72 hours at 25 C to determine whether any nematode development occurred. The nematode infestation was confirmed by examining the live wasps in a droplet of water under a compound microscope before they were confined on agar. The nematodes were associated around the intersegmental membranes and in the posterior region of the abdomen and were visible through the integument.

Histopathology: Phase C syconia of *F. laevigata* were cut in half and one half placed into FAA (formalin, acetic acid, ethanol; 5:5:90) and the other half chopped and placed in water for >20 minutes to determine whether the fig was infested with *Schistonchus* sp. Infested and uninfested (control) syconia were retained in FAA for several days, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin (15). Embedded fig halves were sectioned 10–15 μm thick, mounted on slides treated with Mayer's albumin (50 ml fresh egg albumin, 50 ml glycerin, 1 g sodium salicylate), stained with 1% safranin in water and 0.5% fast green in clove oil and 100% ethanol (1:1), and examined and photographed with a compound photomicroscope. Several uninfested phase A and B syconia were processed for sectioning and observations as described herein. *Pegoscapus* sp. with *Schistonchus* sp. or *Parasitodiplogaster* sp. or no nematodes were collected from *F. laevigata* syconia in phase D, checked for nematodes, placed into FAA, and processed and sectioned as described herein except that 1% fast green in 50% ethanol was used.

RESULTS AND DISCUSSION

Biology of Parasitodiplogaster sp.: The life history of *Parasitodiplogaster* sp. from *Pego-*

scapus sp. and *F. laevigata* in Florida is summarized in Fig. 1. Individuals of *Parasitodiplogaster* sp. molt from the J3 to the J4 stage and greatly increase in size in the hemocoel of the foundress female wasp after her entry into a phase B syconium. The J4 exit the wasp cadaver inside a phase B or early phase C syconium, and molt to adults that mate and lay eggs (Fig. 1, Table 1). Eggs, J2, and J3 of *Parasitodiplogaster* sp. appear in the syconial cavity during the mid-interfloral phase C (Fig. 1, Table 1). Egg hatching of *Parasitodiplogaster* sp. was not synchronized with *Pegoscapus* sp. emergence, as reported for the Panamanian species of *Parsitodiplogaster* (14). It is not known whether *Parasitodiplogaster* sp. feeds while in the syconial cavity or survives solely on reserves obtained during parasitism. All stages, except the J3, have an open stoma with two large teeth, suggesting that they may feed. In late phase C and phase D syconia, *Parasitodiplogaster* sp. were all in the J3 stage and were nictating on fruitlet surfaces in the syconial cavity, with respective mean numbers of 74 and 59/fig (Table 1). No stage of *Parasitodiplogaster* sp. was ever observed penetrating or inside the floret tissues of *F. laevigata* syconia. Almost

all of the J3 observed in the syconial cavity retained the cuticle of the previous molt. *Parasitodiplogaster* sp. J3 infest newly emerged female wasps inside the syconial cavity and are transported by the wasp to a new phase B syconium (Fig. 1). The J3 of *Parasitodiplogaster* sp. enter the hemocoel of the abdomen or thorax of the wasp some time between the emergence of the wasp from its gall in the phase D syconium and its entry into a new phase B syconium. Once the wasp successfully arrives in the cavity of a new phase B syconium, the J3 molt to the J4 stage and start a new cycle.

Histological sections and microscopic observations of *Pegoscapus* sp. showed that the J3 of *Parasitodiplogaster* sp. are associated with the thorax, abdomen, and abdominal intersegmental membranes of male and female wasps in phase D syconia (Fig. 3A,B; Table 2). Molting or development of the nematodes in the body cavity of a male wasp was never observed, indicating that males are dead-end hosts. A nonpollinating sycophilous wasp, *Idarnes* sp., was occasionally associated with J3 of *Parasitodiplogaster* sp. Because this wasp oviposits from the exterior of the syconium and because vertical transmission to
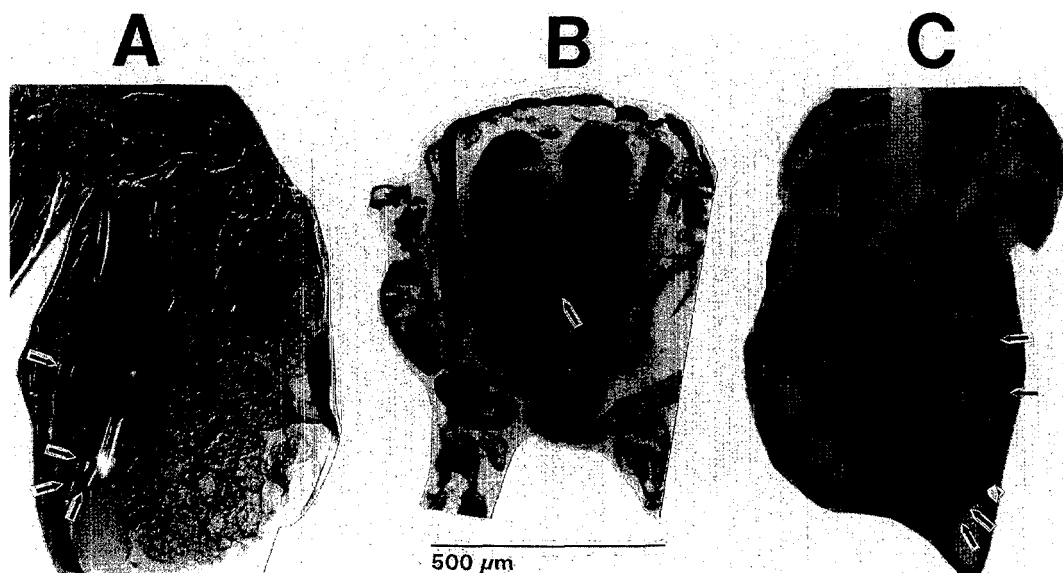

FIG. 3. Photomicrographs of sections through adult females of *Pegoscapus* sp. from male phase D syconia of *Ficus laevigata*. A) Wasp abdomen with J3 of *Parasitodiplogaster* sp. (arrows). B) Transverse section through wasp thorax with J3 of *Parasitodiplogaster* sp. (arrow). C) Dorso-longitudinal section through the thorax and abdomen of a wasp with entomogenous females of *Schistonchus* sp. (arrows).

TABLE 2. Association of *Parasitodiplogaster* sp. and *Schistonchus* sp. with adults and pupae of *Pegoscapus* sp. from syconia of *Ficus laevigata* in successive developmental phases from Florida.

Syconial phase‡	Wasp stage	No. of syconia/wasps dissected	% infested wasps			No. of *Parasitodiplogaster* sp.† Stage			No. of *Schistonchus* sp.† Stage
			Parasito-diplogaster sp. only	*Schistonchus* sp. only	Both nematode species	J3	♂J4	♀J4	Entomogenous ♀
B	Live foundress§	18/31	19	19	0	5 ± 3‖	1 ± 1	1 ± 1	3 ± 3
B or early C	Dead foundress¶	31/42	60	0	2	1#	2 ± 1	2 ± 1	3
Late C	Pupa in gall	1/24‡	0	0	0	0	0	0	0
D	Female in gall	3/22ᴵ	0	50	0	0	0	0	3 ± 4
D	Live male	5/10°	40	0	0	8 ± 10	0	0	0
D	Live female newly emerged in syconia without any observed nematodes	2/20	0	0	0	0	0	0	0
D	Live female newly emerged in syconia with *Parasitodiplogaster* sp. only	11/85	85	0	0	4 ± 5	0	0	0
D	Live female newly emerged in syconia with *Schistonchus* sp. only	6/46	0	70	0	0	0	0	3 ± 5
D	Live female newly emerged in syconia with both nematode species	12/62	27	19	34	3 ± 2	0	0	5 ± 11

† Mean number of nematodes per infested wasp ± standard deviation.

‡ See introduction for a description of the developmental phases of monoecious figs.

§ Number of foundresses per syconium ranged from 1–8 (1.7 ± 1.8).

‖ Three of the six wasps infested with *Parasitodiplogaster* sp. had J3 only, two of the wasps had J4 only, and one wasp had both stages.

¶ Number of foundresses per syconium ranged from 1–3 (1.3 ± 0.6).

One of the 26 wasps infested with *Parasitodiplogaster* sp. had J3 only and 25 of the wasps had J4 only.

ᴵ All wasps from one syconium with *Schistonchus* sp. present.

ᴵ Eight wasps from two syconia with both nematode species present and 14 wasps from one syconia with only *Schistonchus* sp. present.

° Two wasps from one syconium with both nematode species present, three wasps from two syconia with *Parasitodiplogaster* sp. present only, and five wasps from two syconia with *Schistonchus* sp. present only.

the next generation of wasps would be highly unlikely, this is also a dead-end host. The respective mean burdens of J3 *Parasitodiplogaster* sp. per newly emerged *Pegoscapus* sp. were 4 (range 1–28) and 3 (range 1–10) from syconia with *Parasitodiplogaster* sp. only or both nematode species (Table 2). These burden numbers are lower than those observed in live pollinators from phase B syconia (Table 2), probably because the exposure time was shortened by the dissection. Third-stage juveniles of *Parasitodiplogaster* sp. infested 85% of newly emerged *Pegoscapus* sp. in syconia infested with *Parasitodiplogaster* sp. only and 27% of wasps in syconia infested with both nematode species. A similar trend was observed in the association between *Schistonchus* sp. and *Pegoscapus* sp. In syconia infested with *Schistonchus* sp. only, 70% of the fig wasps were infested with entomogenous females of the nematode, whereas only 19% of the wasps were infested in syconia with both species of nematodes (Table 2). These differences in infection levels may be due to interspecific competition or some other form of interference between *Parasitodiplogaster* sp. and *Schistonchus* sp. Further study is needed to clarify this phenomenom.

Nineteen percent of the *Pegoscapus* sp. foundresses that were dissected alive from phase B syconia were infested with an average of about seven *Parasitodiplogaster* sp. per wasp (Table 2). About 30% of the phase B and C syconia of *F. laevigata* were infested with *Parasitodiplogaster* sp. only, and an additional 5–7% of the syconia were infested with both *Parasitodiplogaster* sp. and *Schistonchus* sp. (Table 1). The number of developing J4 and male *Parasitodiplogaster* sp. per phase B syconium was about eight, or five nematodes per wasp (Table 1). Poinar and Herre (21) reported that adults of the Panamanian *Parasitodiplogaster* spp. emerge from their fig wasp hosts. The Floridian *Parasitodiplogaster* sp. usually emerged in the J4 stage and occasionally emerged as males (Tables 1,2). Burdens of *Parasitodiplogaster* spp. were 6–7 per fig wasp in Panama (14).

It would be maladaptive for a parasite to affect the fitness of its fig wasp host negatively before or during its difficult journey locating and entering a phase B syconium. Thus, any increase in parasite-induced virulence due to increased horizontal transmission of parasites as proposed by Herre (14) should be manifested after fig wasp entry into the syconium. This is apparently what occurs in the Floridian *Parasitodiplogaster* sp. Live *Pegoscapus* sp. foundresses that were observed ovipositing when dissected from phase B syconia had normal-sized J3 and (or) small J4 *Parasitodiplogaster* sp. in the hemocoel (*n* = three fig wasps observed), and small J4 were usually observed in live foundress wasps dissected from phase B syconia (Table 2). Thus, *Parasitodiplogaster* sp. may be slow to develop or may delay development until some physiological or physical stimulus associated with successful entry of its wasp host into the syconium occurs.

None of the attempts to culture *Parasitodiplogaster* sp. or *Schistonchus* sp. were successful. This suggests that *Parasitodiplogaster* sp. is not a commensal saprophyte that feeds on its dead host, or a facultative parasite, like some members of the Diplogasteridae (10). This also suggests that *Schistonchus* sp. is not a facultative fungal-plant parasite like some other members of the Aphelenchoididae (11) and is obligatorily bound to the fig for growth and reproduction.

Almost all of the *Pegoscapus* sp. placed on 1% water agar in the study on *Parasitodiplogaster* sp. development died within 72 hours. None of the *Parasitodiplogaster* sp. developed or remained within the host cadavers. At 72 hours, the J3 of *Parasitodiplogaster* sp. were moving around on the plate; within 1 week, they had died. The conditions on the water agar plate were not optimal for the fig wasps because their wings stuck to the agar or to condensation droplets, rendering them immobile. However, this preliminary experiment and the culture attempts on TSB suggest that *Parasitodiplogaster* sp. is not a commensal saprophyte waiting for the fig wasp to die

in order to complete its life cycle. *Parasito-diplogaster* spp. studied in Panama were hypothesized to have similar life histories, with J3 maturing to the adult or the J4 (preadult stage) at the time of fig wasp oviposition (21).

If *Parasitodiplogaster* require nutrients from the hemocoel of a live wasp for growth and development, it would be adaptive for it to feed only after the wasp foundress has entered the syconium. Intense interspecific and intraspecific (contingent upon the sex ratio and number of parasites) competition may ensue because of the short life of ovipositing fig wasps and of the duration of phase B syconia (both <72 hours; see references 2–4). Increased virulence reported by Herre (14) in species of *Parasitodiplogaster* might be caused by more efficient timing or faster growth and development by the nematode(s) once the fig wasp host is safely inside the syconium. Decreased virulence may be due to increased parasitic efficiency (control of numbers in parasite burden or delayed timing for the onset of consumption of host resources until after the host has laid eggs) or a lack of parasitism (commensalism). Examination of the life history in timed studies could help elucidate the correlation of increased virulence in *Ficus* spp. with decreased proportions of single foundress broods (14).

Biology of Schistonchus sp. and Histopathology of Schistonchus sp.-infected syconia: The life history of *Schistonchus* sp. associated with *Pegoscapus* sp. and *F. laevigata* in Florida is summarized in Fig. 1. *Schistonchus* sp. females are transported in the hemocoel of the thorax and abdomen of *Pegoscapus* sp. females (Fig. 3C) to the cavity of a phase B syconium (Figs. 1,2A; Table 2). The average burden of *Schistonchus* sp. per infested *Pegoscapus* sp. was about three entomogenous females (range 1–56) (Table 2). No apparent pathology was noted in comparisons of histological sections of healthy and heavily infested wasps. Whether *Schistonchus* sp. is a parasite or an internal phoretic animal when inside *Pegoscapus* sp. was not determined.

Entomogenous females of *Schistonchus* sp. exit the ovipositing wasp (Table 1) and invade immature male florets in a phase B syconium (Figs. 1,2B). Both entomogenous and larger propagating females were recovered from phase B syconia (Table 1). Phytophagous stages of *Schistonchus* sp. feed ectoparasitically in the space between the anther, anther filament, and perianth lobes (Figs. 4,5). One, or perhaps two, generations of *Schistonchus* sp. exist in the male floret before the end of phase C, when slender entomogenous females appear (Fig. 1). In *Schistonchus*-infested phase C syconia, more than 70% had propagating females and juvenile nematodes, and more than 40% had males and eggs (Table 1). The female:male ratio was about 2:1 in phase C and D syconia (Table 1) but was closer to 4:1 in early phase C syconia.

Florets ($n = 262$) were examined from serial sections of four *Schistonchus*-infested phase C syconia of *F. laevigata* (when last instar or pupae of *Pegoscapus* sp. were present in galls). Nineteen percent of the florets examined were male; 46% of these were infested with *Schistonchus* sp. Estimates of the number of *Schistonchus* per male floret were made by counting the highest number of nematodes observed in one section from the series for the specific floret. This method was used because of the difficulty in determining where nematodes come from in a freshly dissected syconium. There were 124 ± 77 (mean $\pm$ standard deviation; range = 20–275) *Schistonchus* sp. per male floret. None of the female florets examined ($n = 114$ wasp galls, $n = 75$ fig embryos, and $n = 23$ aborted fig embryos) were infested with *Schistonchus* sp. These data strongly suggest that *Schistonchus* sp. search out and parasitize only immature male florets in *F. laevigata.*

Histological examination of sections of *Schistonchus*-infested male florets ($n = 23$) in phase C syconia revealed that the nematodes usually cause the formation of hypertrophied uninucleate epidermal cells of the anther filaments and anthers (Figs. 4C–E; 5C,D). Hypertrophied epidermal
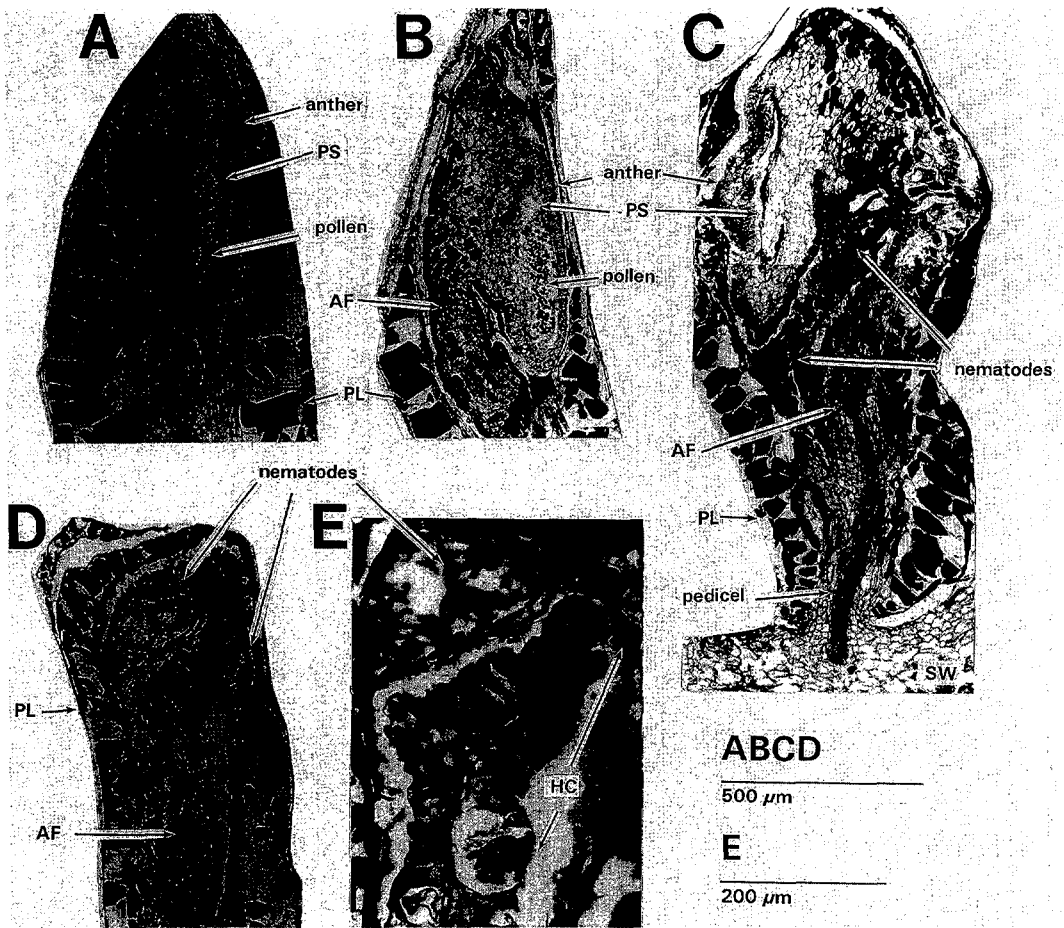
FIG. 4. Photomicrographs of longitudinal sections through male florets from interfloral phase C syconia of *Ficus laevigata*. A,B) Uninfested. C,D) Infested with *Schistonchus* sp. E) Higher magnification of D. AF = anther filament, HC = hypertrophied epidermal cells, PL = perianth lobe, PS = pollen sac, SW = syconial wall.

cells of the anther filaments were enlarged (×2–5), were stained light purple, had more granulated cytoplasm, and had an enlarged nucleus and prominent nucleolus relative to the dark purple-staining epidermal cells observed in uninfested male florets (Figs. 4,5). In several sections, the cells appeared to be affected more than one cell layer (up to three cells deep). Epidermal cell hypertrophy was often extensive on the anther filament, except for the narrow line of violet-staining tanniferous cells of the dorsal midrib, which was usually not affected. Hypertrophy of the epidermal and endothecial cells of the anther was not as extensive as observed for cells of the anther filament, but these uninucleate cells also showed increased granulation and en-

largement of the cell, nucleus, and nucleolus relative to healthy cells (Fig. 5C,D). These affected cells were often restricted to one layer and were stained light purple to pinkish-purple (Fig. 5C,D), relative to the light-blue staining cells in healthy anthers (Fig. 5A,B). In heavily infested anthers, the cells of the endothecium were affected over a depth of several cell layers, and cell integrity was compromised. In one male floret, *Schistonchus* sp. were associated with hypertrophied epidermal cells of the perianth lobes (three lobes per male floret in *F. laevigata*) and the syconial wall facing the syconial cavity near the infested male floret. These hypertrophied syconial cells were purple staining, compared with the green of healthy cells.
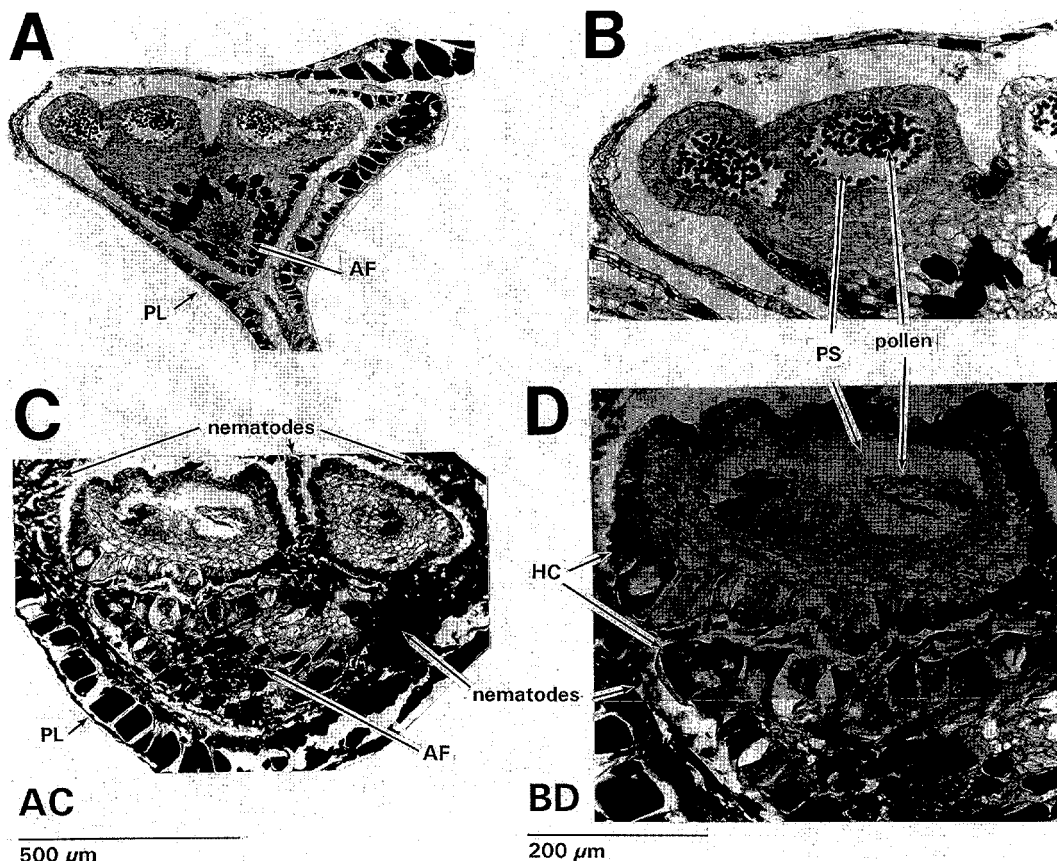
FIG. 5. Photomicrographs of transverse sections through male florets from interfloral phase C syconia of *Ficus laevigata.* A) Uninfested. B) Higher magnification of A). C) Infested with *Schistonchus* sp. D) Higher magnification of C. AF = anther filament, HC = hypertrophied epidermal cells, PL = perianth lobe, PS = pollen sac.

Schistonchus sp. feeding damage also caused a safranin-rich (blood red) exudate and aberrations of the shape of the anther filament, anthers, and pollen (Figs. 4,5). The source of the exudate appeared to be from damaged pink-staining epidermal and red-staining tanniferous cells lining the inner surface of the perianth lobes that surround the unpresented anther and anther filament. Initiation of cell hypertrophy may be due directly to mechanical feeding damage or secretions from the nematodes or indirectly to chemicals released from the observed exudate. Pollen production often appeared normal in one or more pollen sacs of lightly infested male florets. However, in heavily infested male florets, the four pollen sacs were com-pletely deformed and little or no normal pollen was observed. The tapetum was sometimes necrotic and (or) disorganized in nematode-infested anthers.

The pathology caused by *Schistonchus* sp. in *F. laevigata* male florets could cause male sterility or significant reductions in pollen availability for emerging fig wasps in a heavily nematode-infested syconium. Of the four partial syconia that were examined in sections, $45 \pm 24\%$ (range; 26–79%) of male florets per syconium were infested with *Schistonchus* sp. Damage to pollen production could negatively affect the fig wasp, with negative consequences to the nematodes and the fig. The fig wasp must have sufficient pollen to pollinate the florets in the new syconium in order to re-

produce (unpollinated syconia are aborted and poorly pollinated syconia may be less nutritive for wasps [8]).

The enlarged cell size, granular cytoplasm, and hypertrophied nucleus and nucleolus in *Schistonchus*-infested epidermal cells suggest increased metabolic activity and a specialized function. These cells may be analogous to "feeding cells," or uninucleate nurse cells, which are stimulated by secretions of some phytoparasitic nematodes in the order Tylenchida. In all other associations between aphelenchoidids and plants that we are aware of, the nematodes cause cell death and necrotic lesions, whether they are ecto- or endoparasitic. This is the second *Schistonchus*–fig association to be studied in detail, but it suggests a highly specialized life history relative to what has been reported for *S. caprifici* and the fig *F. carica* (27). In that association, both male and female florets of *F. carica* are parasitized by *S. caprifici*, and the pathology is typical for aphelenchoidids, with production of necrotic cavities but no specialized "feeding cells" (27).

Schistonchus sp. were not observed in dissections of pupae of *Pegoscapus* sp. in a heavily infested phase C syconium (Table 2), but were observed in the hemocoels of 50% of the live females before their emergence from their galls (Table 2). This strongly suggests that entomogenous females of *Schistonchus* sp. infect *Pegoscapus* sp. only after male wasps chew a hole in each female's gall to mate in the phase D syconium. None of the male *Pegoscapus* sp. were observed with *Schistonchus* sp. (Table 2), contrary to our previous report (12). Up to about 1,500 entomogenous females (339 ± 538 per syconium) were observed concurrently with emerging female wasps in phase D syconia (Table 1). The entomogenous females are more slender and become straight when they die compared with the propagating females, which are broader and c-shaped in death. The vulva of entomogenous females is difficult to see, and the postuterine sac is filled with what appears to be sperm. Abundance of all nematode stages dropped dramatically in phase E syconia (Table 1).

Engomogenous females of another *Schistonchus* sp. were recovered from females of *P. mexicanus*, and propagating nematodes were recovered from the syconia of *F. aurea* in Broward and Lee Counties. The association is hypothesized to be similar in most Neotropical *Schistonchus* species, that is, in *Schistonchus* sp. from *P. mexicanus* and *F. aurea* from Florida, another *Schistonchus* species from *Pegoscapus* sp. from *F. pertusa* L. in Turrialba, Costa Rica (Giblin-Davis, unpubl. obs.), and two *Schistonchus* species from *F. hemsleyana* and *F. jimenezii* Standley in San Jose, Costa Rica (Giblin-Davis and Ramírez B., unpubl. obs.). In contrast, *S. caprifici* is associated throughout its life cycle in the hemocoel of its fig wasp host, where it continues to reproduce (27).

Morphology is quite divergent between the *Parasitodiplogaster* species from Panama (21), South Africa (20), and Florida. In contrast, morphology of *Schistonchus* species associated with *F. laevigata* and *F. aurea* in Florida, *F. pertusa* in Turrialba, Costa Rica (Giblin-Davis, unpub. obs.), and *F. hemsleyana* and *F. jimenezii* in San Jose, Costa Rica (Giblin-Davis and Ramírez B., unpubl. obs.) is very similar, suggesting that they may be sibling species. No *Schistonchus* has been reported for any other New World *Ficus*, but we predict that many, if not most, of the 700 species of *Ficus* in the world are associated with a species-specific *Schistonchus*. The association between *Schistonchus* and agaonids is probably ancient because it occurs in Europe (27), Asia (24), and the Americas (this study,11,12), if not elsewhere. It is likely that *Schistonchus* species were among the more than 20 unnamed species of nematodes reported from indigenous African figs (18). Gene flow is presumably controlled by the fig wasp pollinator in these tritrophic associations. Thus, when speciation of a *Ficus*–agaonid complex is complete, gene flow for the associated nematodes is curtailed, causing functional allo-

patry and potential for nematode speciation.

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