

2014

Ficotylus laselvae n. sp. (Tylenchomorpha: Anguinidae) associated with *Ficus colubrinae* in Costa Rica

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Giblin-Davis, Robin M.; Kanzaki, Natsumi; Davies, Kerrie A.; Ye, Weimin; Zeng, Yongsan; Center, Barbara J.; Esquivel, Alejandro; and Powers, Thomas O., "*Ficotylus laselvae* n. sp. (Tylenchomorpha: Anguinidae) associated with *Ficus colubrinae* in Costa Rica" (2014). *Papers in Plant Pathology*. 551.
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***Ficotylus laselvae* n. sp. (Tylenchomorpha: Anguinidae) associated with *Ficus colubrinae* in Costa Rica**

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Abstract

Ficotylus laselvae n. sp. was recovered from under the bracts of figs (syconia) of *Ficus colubrinae* from La Selva, Costa Rica, during a survey of nematode rainforest biodiversity and is described herein. This is only the second report of an association between the nematode suborder Tylenchina and the sycones of figs. Previous reports of most nematode associates of the sycones of figs have been from the lumen and involved transmission by female fig wasp pollinators (Agaonidae) during pollination/oviposition (e.g., *Schistonchus* and *Parasitodiplogaster* spp.). The association between *F. laselvae* n. sp. and *Ficus colubrinae* may involve an invertebrate host, but none was recovered from dissections of the bracts during this study. It is also possible that this is a rainforest understory nematode that feeds ectoparasitically in protected areas on the aerial parts of *F. colubrinae*. Molecular analysis using near-full-length sequences of the small subunit (SSU) rRNA and D2-D3 expansion segments of the large subunit (LSU) rRNA genes of *Ficotylus laselvae* n. sp. suggests that it is a member of the suborder Tylenchina (infraorder: Tylenchomorpha; family: Anguinidae) and that the closest sequenced species is *F. congesta* from the lumen of sycones of *Ficus congesta* from Queensland, Australia. Although both nematode species are associated with figs, they are morphologically divergent, suggesting that the different micro-niches that they fill provide different selective pressures for evolution of differing morphological characters or they represent different life history morphotypes of a dicyclic genus.

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

Figs are amazing plants. Although their densities can be quite low per unit area, their species richness can be very high, especially in the lowland tropics (Harrison, 2005). Many *Ficus* are considered important pioneer species with a critical role in succession, and many vertebrates are heavily reliant upon them because of their habit of year-round fruiting (Harrison, 2005). There are over 700 species of *Ficus* with about half of the species being

monoecious, the figs (= sycones) possessing both male and female florets, whilst the other half are gynodioecious, different trees each having functionally female or male sycones (Berg, 1989). Most figs rely upon fig wasp pollinators (Agaonidae) to transport pollen from figs on one tree to the next in a classical pollination-for-food mutualism. All of the endemic fig species in the neotropics are monoecious and comprise 111 species from the section

Americana and 21 species from the section Pharmacosycea (Berg, 1989; Harrison, 2005). There are 40-50 different native species of *Ficus* in Costa Rica and Panama (Burger, 1977; Hammel, 1986), the region in which this study was conducted.

Martin *et al.* (1973) reported a variety of different nematode types associated with figs in tropical Africa, but provided few taxonomic details. Two nematode genera, *Schistonchus* Cobb, 1927 (Fuchs, 1937) (Aphelenchoididae) and *Parasitodiplogaster* Poinar, 1979 (Diplogastridae), are well known from inside the sycones of *Ficus* species worldwide (Gasparrini, 1864; Poinar, 1979; Kumari & Reddy, 1984; Reddy & Rao, 1984; Poinar & Herre, 1991; Giblin-Davis *et al.*, 1995, 2003, 2004, 2006; Lloyd & Davies, 1997; DeCrappeo & Giblin-Davis, 2001; Anand, 2002; Zeng *et al.*, 2007, 2010, 2011, 2013a, b, c; Bartholomaeus *et al.*, 2009, 2012; Davies *et al.*, 2009, 2010; Kanzaki *et al.*, 2010, 2012, 2013, 2014a; Wöhr *et al.*, 2014). *Schistonchus* is a polyphyletic plant-parasitic nematode grouping in the Aphelenchoididae that feeds and reproduces on the epidermis of florets inside the sycone and is an internal phoretic of its fig wasp pollinator host which transports it to the next suitable fig (Vovlas *et al.*, 1992, 1996; Giblin-Davis *et al.*, 1995; Center *et al.*, 1999; Davies *et al.*, 2010). This genus currently contains 25 nominal species (Zeng *et al.*, 2013a). *Parasitodiplogaster* is an apparently parasitic or possibly necromenic nematode associate that develops in its fig wasp pollinator host before leaving to mate and produce infective juveniles inside the fig that infect the next generation of wasps as they leave for a new fig (Poinar & Herre, 1991; Giblin-Davis *et al.*, 1995, 2006; Kanzaki *et al.*, 2010, 2012, 2013, 2014a). There are currently 15 described *Parasitodiplogaster* species (Kanzaki *et al.*, 2014a). In addition, the diplogastrids *Teratodiplogaster* Kanzaki, Giblin-Davis, Ye, Center & Thomas, 2009 (Kanzaki *et al.*, 2009, 2012) and *Rhabditolaimus* Fuchs, 1914 (= *Myctolaimus* and *Ceratosolenus*) have been reported from *Ficus racemosa* L. and figs of the subgenus *Sycomorus* (Anand, 2005, 2006; Kanzaki *et al.*, 2006), and the rhabditid *Caenorhabditis briggsae* (Dougherty & Nigon, 1949) Dougherty, 1953 has been reported from rotting figs (Kiontke *et al.*, 2011), while other undescribed species of the latter genus have also been observed in early phase figs (Kanzaki, unpubl. obs.). In addition, *Bursaphelenchus* has recently been found associated with *Ficus variegata* which belongs to the subgenus *Sycomorus* (Kanzaki *et al.*, 2014b). Lastly, Davies *et al.* (2009) reported the association between *Ficotylus* Davies *in* Davies, Ye, Giblin-Davis & Thomas,

2009, a new monotypic genus of tylenchid from inside the figs of *Ficus congesta* from Queensland, Australia. In recent biodiversity survey work at La Selva Biological Station, Costa Rica, an unexpected association was found between an undescribed species of tylenchid nematode from under the bracts of aerial syconia of *Ficus colubrinae* Standl. (Giblin-Davis *et al.*, 2007). This nematode superficially looks like a member of the genus *Nothotylenchus* but has strong molecular phylogenetic affinities with *Ficotylus*. The nematode is described and illustrated herein as *Ficotylus laselvae* n. sp. and hypothesized to be an example of divergent morphological variation within a tylenchid genus in response to adaptation to different micro-niches associated with fig sycones or different life history morphotypes of a dicyclic genus.

Materials and methods

Nematode isolation and morphological observation

Phase B-D syconia were collected from *Ficus colubrinae* from the grounds of the La Selva Biological Station rain-forest reserve in Costa Rica (10°25'52.513"N Latitude, 84°00'12.959"W Longitude) in March of 2005, 2007 and 2008. They were kept cool until dissected (within 3 days). Each fig was opened or dissected with a flamed scalpel or new razor blade and placed in distilled water for 20 min to allow nematodes to emerge for collection and observation. Observations using modifications of this protocol with 95% ethanol as an external killing solution were done to help pinpoint the exact location of the different nematode species associated with sycones.

Nematodes for morphological and molecular studies were cleaned and collected on a Baermann funnel. They were then hand-picked alive into 100% un-denatured ethanol on ice to be shipped for DNA extraction, amplification, and sequencing attempts or collected, heat-killed and placed into 5% formalin for measurements by light microscopy and then processed into 100% glycerin for permanent mounts (Southey, 1970).

Drawings and measurements of nematodes were done with the aid of a camera lucida and a stage micrometer. Photomicrographs were taken with a Nikon Coolpix 4500 or an Olympus E-410 attached *via* an adapter through the phototube of an Olympus BH-2 microscope, and edited using Adobe Photoshop 7.0. The morphometric data was processed using Excel software (Ye, 1996).

Molecular characterization and phylogeny of *Ficotylus laselvae* n. sp.

Nematode samples that had been stored in ethanol were picked (ten or fewer) into distilled water before being placed into 50 μ l 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 D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and reverse primer D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Nunn, 1992). Primers for SSU amplification were forward primer 18SnF (5'-TGGATAACTGTGTAATTCTAGAGC-3'), and 18SnR (5'-TTACGACTTTTGCCCGTTC-3') (Kanzaki & Futai, 2002). The 25 μ l PCR was performed using AmpliTaq DNA polymerase (Applied Biosystems) 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 Montage™ PCR centrifugal filter devices (Millipore) according to the manufacturer's protocol. PCR products were sequenced by the Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida, using an Applied Biosystems 377 sequencing system.

Sequences of *F. laselvae* n. sp. were deposited in the GenBank database under the accession numbers of EU018050 for partial sequence of SSU and EU018048 for D2-D3 LSU regions of rRNA gene. Some outgroup species with voucher numbers in the figures were from our previous sequencing projects. Other species from GenBank (see the Figures for the accession numbers) were also used for phylogenetic analysis. DNA sequences were aligned using ClustalW (<http://workbench.sdsc.edu>). Separate alignments of SSU and LSU sequences were visually inspected and, in the rare situations where ambiguities existed, they were manually removed. The best fit models of base substitution in the SSU and LSU sets were evaluated using MODELTEST version 3.06 (Posada & Crandall, 1998). The Akaike-supported model, the log likelihood (ln L), the Akaike information criterion (AIC), 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 3.1.0 (Huelsenbeck & Ronquist, 2001), running the chain

for 10⁶ generations and setting the 'burn in' at 1000. We used MCMC (Markov Chain Monte Carlo) methods within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using 50% majority-rule. The maximum parsimony (MP) method was performed using the heuristic search with stepwise-addition options to determine the most parsimonious tree. Neighbor-joining (NJ) analysis (Saitou & Nei, 1987) was conducted using the HKY85 (Hasegawa *et al.*, 1985) distance option. Sites with missing data or gaps were treated as missing characters for all analyses. The robustness of the parsimony and NJ trees was tested using the bootstrap method (Felsenstein, 1985). All bootstrap values were based on 1000 pseudoreplicates.

Results

*Ficotylus laselvae** n. sp.

(Figs 1, 2)

Measurements

See Table 1.

Description

Males from figs

Body slender, cylindrical, arcuate, sometimes ventrally curved posteriorly when heat-killed, of medium length. Cuticle finely striated. Lateral fields distinct, with two lines. Deirids not observed, probably due to condition of specimens. Cephalic region low, flattened, continuous, not offset. Lips prominent, ratio of lip diam. to height = 2.0 ± 0.6 (1.3-3.0). Stylet short, with conus less than 50% of total length and rounded, well developed basal knobs. Orifice of dorsal pharyngeal gland close to stylet base. Pharynx subcylindroid, with a non-muscular, fusiform swelling, lacking valves, glandular basal bulb comprising a dorsal and smaller ventral lobes overlapping intestine. Nerve ring surrounding isthmus. Pharyngo-intestinal junction just posterior to nerve ring, cardia absent. Opening of excretory pore variable but posterior to nerve ring, hemizonid immediately anterior to excretory pore. Testis single, outstretched, on right of intestine. Spermatocytes arranged as single-double row(s). Posterior fifth of male gonad forming a simple tube-like *vas deferens*. *Vas deferens* fusing

* The species epithet for this nematode is derived from the collection location, La Selva, Costa Rica.

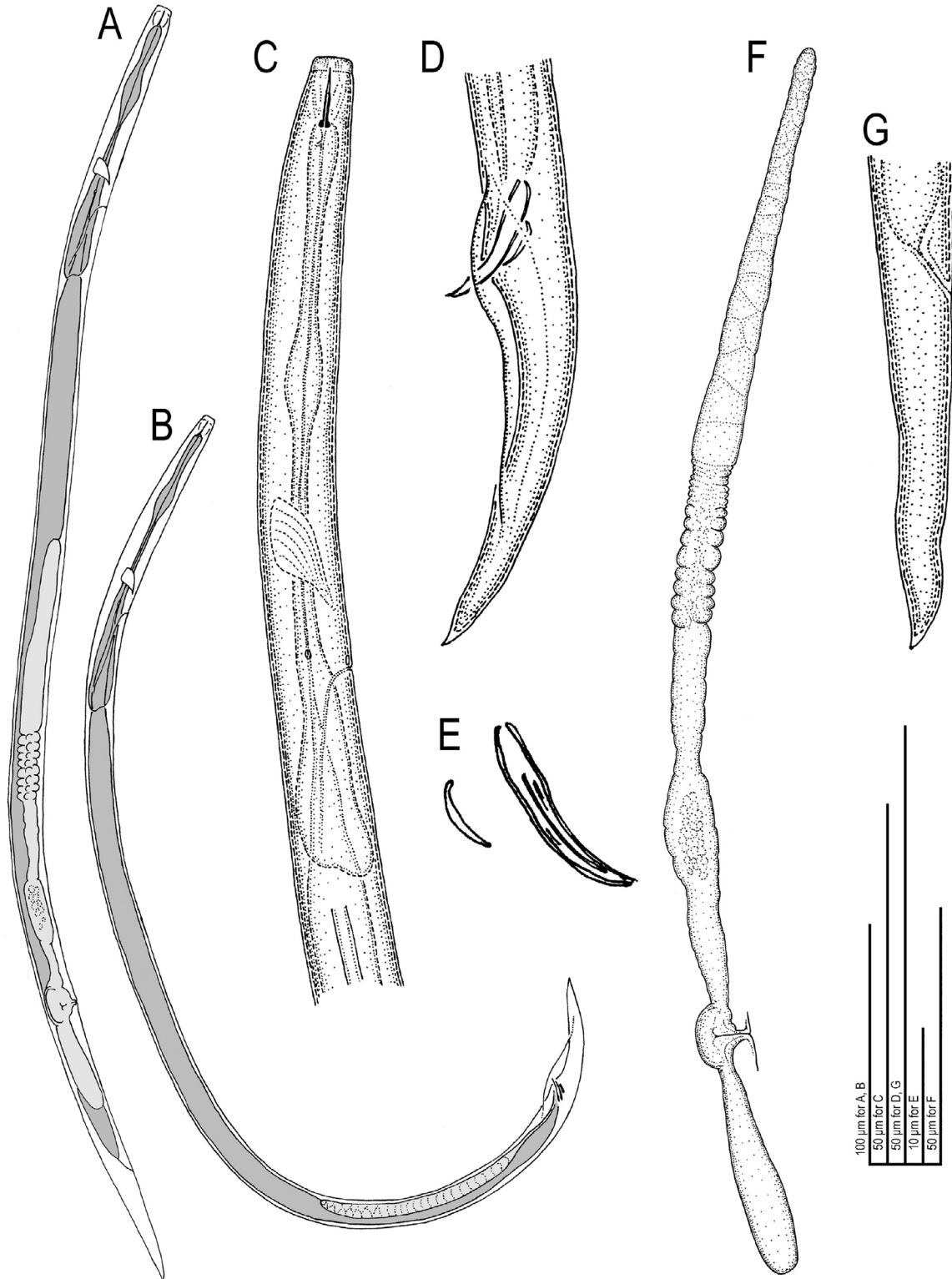


Fig. 1. Drawings of adult female and male of *Ficotylus laselvae* n. sp. in lateral view. A: Entire female; B: Entire male; C: Anterior body of male; D: Male tail; E: Gubernaculum and spicule; F: Female reproductive system; G: Female tail.



Fig. 2. Photomicrographs of adult male and female of *Ficotylus laselvae* n. sp. in lateral view. A: Anterior body of male; B: Lateral field of mid-body of male; C: Vulva and post-uterine sac of female; D: Male tail. (Scale bars = 10 μ m.)

with posterior end of intestine (rectum) at level of spicule to form a simple cloacal tube. Spicules arcuate and weakly cephalated, gubernaculum short, simple, fixed. Tail elongate-conoid, 4.6 anal body diam. long. Tail tip sharp, sometimes finely bifurcate, no mucron. Bursa small, ad-anal and leptoderan, arising anteriorly *ca* one anal body diam. anterior to cloacal aperture and terminating *ca* one anal body diam. prior to tail tip.

Females from figs

Body, cuticle, lateral field, head (cephalic region), stylet, nerve ring, excretory pore, pharynx and intestine as in male. Ovary mono- and prodelphic, on right of intestine, organs arranged from anterior as ovary, oviduct, 'spermatheca', crustaformeria, uterus, uterine sac, vulva/ vagina and post-uterine sac. Ovary outstretched, with single-double rows of oocytes. Oviduct between ovary and 'spermatheca' composed of small cells. Putative 'spermatheca' quadricolumellar, composed of rather large and rounded cells. Uterus similar in length to ovary, with middle third composed of large and rounded cells, often containing a mass of sperm, and possibly serving as a 'spermatheca'.

Dorsal wall of vagina/uterus junction thickened, forming an oval-shaped *receptaculum seminis*-like expanded sac. Vulva at *ca* 75% of total body length, located more than five body diam. anterior to anus, vulval lips almost flat, vagina perpendicular to body wall. Postuterine sac long, *ca* four vulval body diam. long. Tail similar to male.

Type host and locality

Isolated from beneath bracts of aerial *Ficus colubrinae* sycones from the research station grounds at La Selva, Costa Rica, collected on March 20, 2005.

Type material

Holotype male and one paratype male and paratype female deposited at the University of California-Riverside Nematode Collection, Riverside, CA, USA. One paratype each of males and females (same data as holotype) deposited at the University of California, Davis, CA, USA, the USDA Nematode Collection, Beltsville, MD, USA, and the Universidad Nacional at Heredia, Costa Rica.

Table 1. Morphometrics of male holotype and male and female paratypes of *Ficotylus laselvae* n. sp. in glycerin mounts. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Male		Female
	Holotype	Paratypes	Paratypes
n	–	9	5
L	579	529 \pm 53.8 (450-600)	565 \pm 20.8 (549-600)
a	48.0	46.1 \pm 6.2 (37.5-57.0)	43.7 \pm 4.5 (39.2-50.0)
b'	4.4	4.4 \pm 0.4 (3.4-4.8)	3.7 \pm 0.5 (3.0-4.2)
c	13.1	12.4 \pm 0.9 (11.1-13.6)	12.4 \pm 1.1 (10.8-14.0)
c'	4.5	4.6 \pm 0.4 (4.0-4.9)	5.0 \pm 0.4 (4.3-5.5)
V	–	–	76.8 \pm 2.3 (74.2-78.7)
Body diam. (male = max.; female = at vulva: VBD)	12.1	11.6 \pm 1.0 (10.0-13.0)	13.0 \pm 1.0 (12.0-14.0)
Stylet length	8.6	9.1 \pm 0.5 (8.0-10.0)	9.1 \pm 0.2 (9.0-9.5)
DGO (dorsal gland orifice to basal knobs)	1.0	1.0 \pm 0.0 (1.0-1.0)	1.0 \pm 0.0 (1.0-1.0)
Pharynx length	130	121 \pm 11.2 (100-134)	155 \pm 19.3 (133-186)
Spicule length (line)	12.6	14.2 \pm 1.1 (12.0-15.0)	–
Spicule length (curve)	16.1	17.2 \pm 1.3 (15.0-18.0)	–
Gubernaculum length	4.1	4.5 \pm 0.5	– (4.0-5.5)
Nerve ring from anterior end	52	58 \pm 8.8 (45-70)	57 \pm 3.7 (54-62)
Post-uterine sac length (PUS)	–	–	55 \pm 7.9 (48-68)
Anal/cloacal body diam.	9.8	9.3 \pm 0.5 (9.0-10.0)	9.2 \pm 0.8 (8.0-10.0)
Tail length	44	43 \pm 3.3 (36-48)	46 \pm 3.2 (43-51)
Excretory pore from anterior end	89	85 \pm 9.8 (68-98)	87 \pm 3.3 (82-91)
Lip diam.	5.1	4.9 \pm 0.6 (4.0-6.0)	5.0 \pm 0.0 (5.0-5.0)
Lip height	1.7	2.7 \pm 0.8 (2.0-4.0)	2.7 \pm 0.8 (2.0-4.0)
PUS/VBD	–	–	4.2 \pm 0.7 (3.6-5.2)
PUS/vulva-anal distance	–	–	0.6 \pm 0.1 (0.6-0.7)
Lip diam./height	3.0	2.0 \pm 0.6 (1.3-3.0)	2.0 \pm 0.5 (1.3-2.5)

Diagnosis and relationships

Ficotylus laselvae n. sp. is characterized by having both males and females forming a free-living generation under the bracts of aerial sycones of *Ficus colubrinae* in La Selva, Costa Rica. It is morphologically characterized as a slender nematode with head continuous with the body, lateral field with two lines, a weak fusiform pharyngeal bulb lacking apparent musculature and valves, pharyngeal glands overlapping the intestine, excretory pore posterior to the nerve ring, female with a mono-prodelphic gonad and post-uterine sac about four vulval body diam. long, male with slender, weakly cephalated spicules, a simple and small gubernaculum, and small adanal leptoderan bursa.

Morphologically, *F. laselvae* n. sp. is unspectacular relative to its putative sister *F. congestae* Davies *in* Davies, Ye, Giblin-Davis & Thomas, 2009 and has the overall general appearance of members of the genus *Nothotylenchus* Thorne, 1941. It can be separated from all species of *Nothotylenchus* based upon the presence of two incisures in the lateral field vs four or more. *Ficotylus laselvae* n. sp. is separated from *F. congestae* by a shorter stylet (8-10 vs 29-39 μm), stylet knobs that are not bifurcate, possession of a ventral lobe that is smaller than the dorsal lobe of the pharyngeal gland (it is the opposite in *F. congestae*), number of incisures in the lateral field (two vs four), and by having a small adanal leptoderan bursa vs a characteristic peloderan bursa supported by a pair of papilla-like structures (= homologue of phasmids?) at its widest point and then narrowing rapidly to just envelop the tail tip.

It can be separated from all species of *Ditylenchus* based upon the absence of a muscled and valved median pharyngeal bulb and can be differentiated from *D. angustus* (Butler, 1913) Filipjev, 1936 based upon the lack of musculature and a valve in the median pharyngeal bulb, number of incisures in the lateral field (two vs four), and shorter length of the bursa in males.

Molecular characterization and phylogeny of *Ficotylus laselvae* n. sp.

The trees inferred by Bayesian MP and NJ analysis did not show significant conflicts in branching order. Thus, the Bayesian trees are shown in Figures 3 and 4. For SSU, Bayesian analysis strongly tied *F. laselvae* n. sp., *F. congestae*, *D. angustus* and another *Ditylenchus* sp. (AY284637) together but did not resolve a deeper connection with a

well-resolved clade comprising *Anguina tritici* (Steinbuch, 1799), Chitwood, 1935, *Subanguina radicola* (Greef, 1872) Paramonov, 1967 and *D. dipsaci* (Kühn, 1857) Filipjev, 1936 (Fig. 3). The underlying molecular phylogenetic inference is similar to that reported by van Megen *et al.* (2009) and Holtermann *et al.* (2009) for the relative placement of *D. angustus* and *Sphaerularia bombi* Dufour, 1837 as common ancestors for the two *Ficotylus* species.

Unfortunately, all of the taxa that were compared for SSU were not available for analysis using LSU D2-D3 expansion segment sequence data. However, Bayesian analysis again grouped *F. laselvae* n. sp. strongly with *F. congestae* and a *Neotylenchus* sp. (DQ328725) that was not available for the SSU tree. Overall, there was support for the *Ficotylus* clade being monophyletic with an 'Anguinidae' clade that also included *D. destructor* Thorne, 1945, *D. halictus* Giblin-Davis, Erteld, Kanzaki, Zeng, Ye & Center, 2010 and the bumble bee-parasitic *S. bombi*.

Biology

Ficotylus laselvae n. sp. was discovered during early dissections of aerial figs from small trees of *Ficus colubrinae* in the grounds of La Selva, Biological Station in Costa Rica (Giblin-Davis *et al.*, 2007). Originally, it was unclear whether its origin was internal or external to the fig fruits. The localization of *F. laselvae* n. sp. under bracts was confirmed by excision of bracts, which were soaked individually in a drop of water for 20 min. Nematodes were then observed, suggesting that they were ectoparasitic under the fruit bract. Forty-two randomly selected sycones were surface-disinfested by soaking in 95% ethanol for 30 min and none was positive for nematodes upon subsequent removal of the bracts in water. However, when another subset of 56 randomly selected figs were soaked in water for 30 min and then dissected to open and expose the underside of the bract, 391 *F. laselvae* n. sp. were recovered. In a subsample of eight figs that were individually dissected to excise and expose the underside of the bracts in water without opening the sycone, 88% had *F. laselvae* n. sp. under the bracts with 5-23 males, females and juveniles, but no eggs per fig. Some nematodes appeared to be in an anhydrobiotic state and rehydrated within 20 min after water was added. These same eight figs were then externally soaked in ethanol for 30 s, dried, and dissected open in water to reveal 63% with *Schistonchus* and 25% with *Parasitodiplogaster* and *Schistonchus*. *Ficotylus laselvae* n. sp. was not found internally in these sycones which

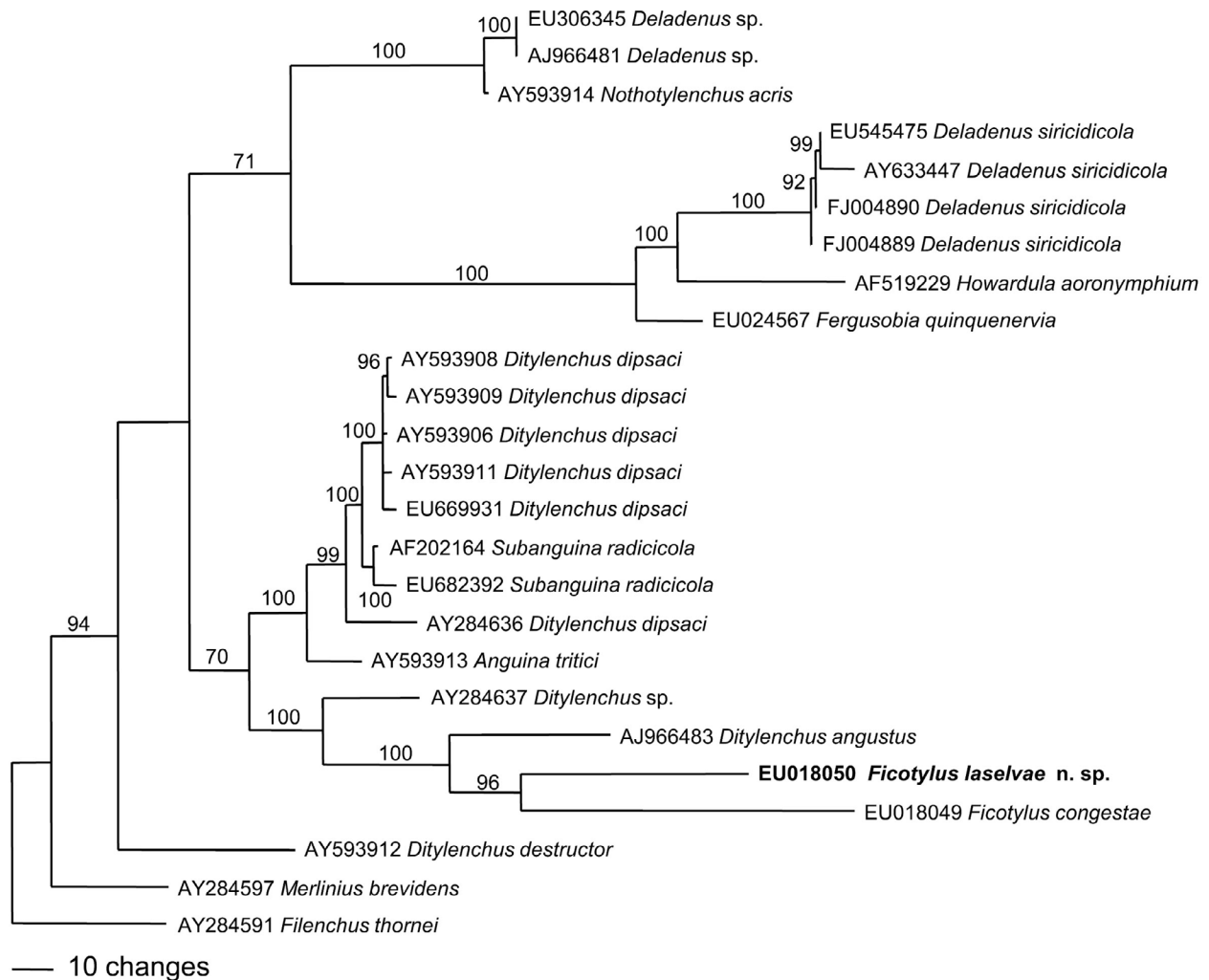


Fig. 3. The 10001st Bayesian tree inferred from DNA sequences of SSU rRNA gene under GTR + I + G model ($-\ln L = 6117.8779$; $\text{freqA} = 0.2564$; $\text{freqC} = 0.2062$; $\text{freqG} = 0.2631$; $\text{freqT} = 0.2743$; $R(a) = 1.3654$; $R(b) = 3.4602$; $R(c) = 1.7878$; $R(d) = 0.3458$; $R(e) = 7.4277$; $R(f) = 1$; $\text{Pinvar} = 0.4525$; $\text{Shape} = 0.6303$). Posterior probability values exceeding 50% are given on appropriate clades.

are protected by tightly sealed florets of the ostiole (entrance to the fig syconium). No other invertebrates were found under the bracts during these observations, suggesting that the nematodes might be aerial ecto-plant-parasitic nematodes that associate with accessible, but protected, areas of tender growth, such as the underside of bracts of sycones on *Ficus colubrinae*. Conditions in the rainforest may allow such associations to flourish. Powers *et al.* (2009) reported that as much as 62% of the overall nematode genetic diversity, measured by a SSU ribosomal barcode, existed in understory and litter habitats and not in soil. Further work is needed to elucidate the transmission and association of *F. laselvae* n. sp. with *Ficus colubrinae* and possibly other rainforest plants in the area.

Discussion

Remarks on the generic definition of *Ficotylus*

Davies *et al.* (2009) proposed the new monotypic genus *Ficotylus* to accommodate the first tylenchid nematode recovered from inside the sycones of *Ficus congesta* from Queensland, Australia. Molecularly, it had affinities with the Infraorder Anguinata whereas morphologically it was aligned with the superfamily Sphaerularioidea and the family Neotylenchidae (classification according to Siddiqi, 2000). The discovery of *F. laselvae* n. sp., which appears to be the putative sister to *F. congestae* based upon SSU and LSU D2-D3 inferences (Figs. 3, 4), supports congeneric

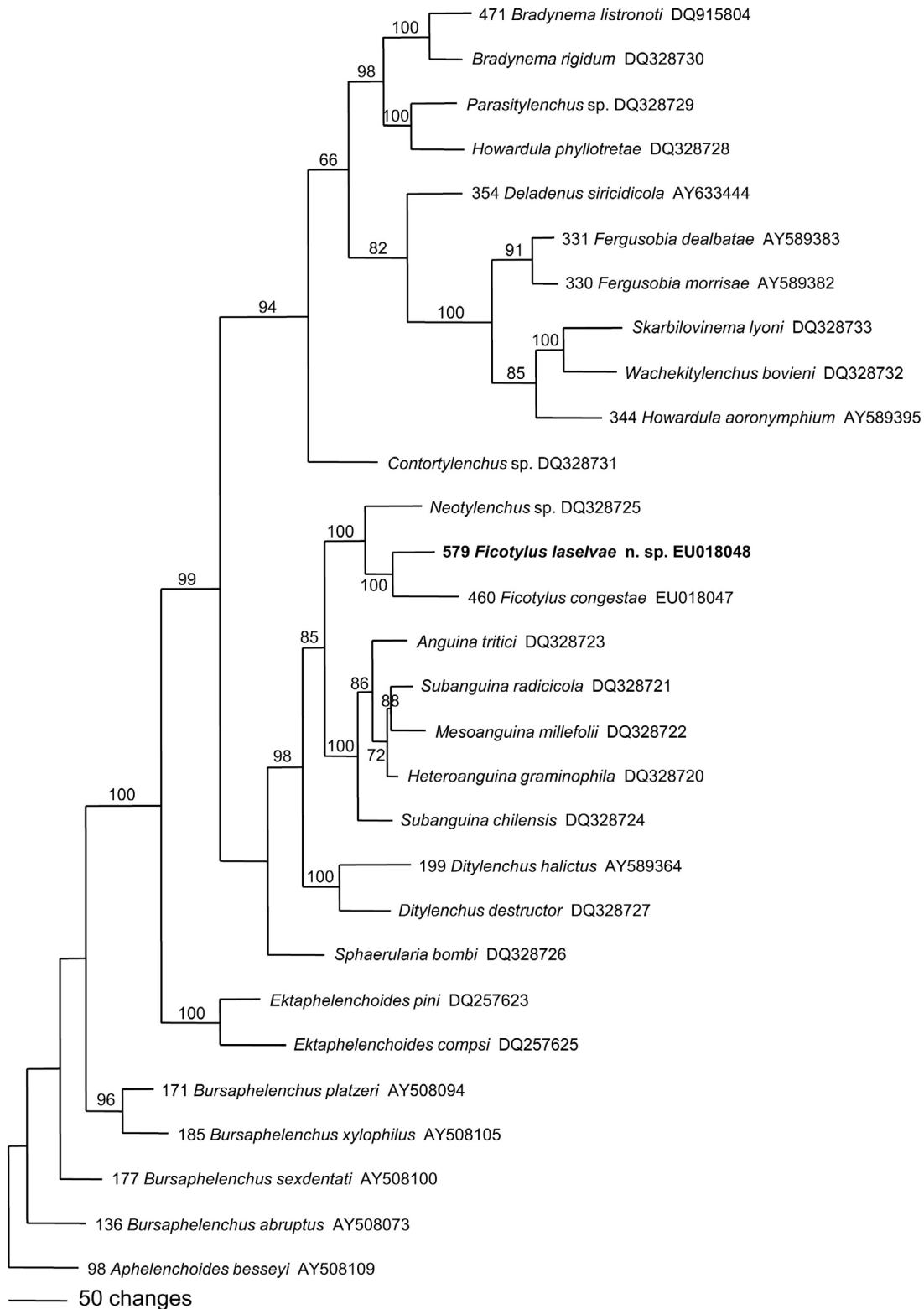


Fig. 4. The 10001st Bayesian maximum likelihood tree inferred from DNA sequences of D2-D3 expansion segments of LSU rRNA gene under GTR + I + G model ($-\ln L = 9975.5264$; $\text{freqA} = 0.2428$; $\text{freqC} = 0.1839$; $\text{freqG} = 0.2739$; $\text{freqT} = 0.2994$; $R(a) = 1.1819$; $R(b) = 10.8952$; $R(c) = 1.4396$; $R(d) = 0.5965$; $R(e) = 13.9854$; $R(f) = 1$; $\text{Pinvar} = 0.1997$; $\text{Shape} = 1.8225$). Posterior probability values exceeding 50% are given on appropriate clades.

status for these two fig-associated nematodes from very different geographical regions and from different parts of the figs that they inhabit. However, the morphology of *F. laselvae* n. sp. is widely divergent in several key characters from *F. congestae* making it difficult to define the genus based on morphological synapomorphies. Generally, *F. laselvae* n. sp. looks like a *Nothotylenchus*, which was classified in the Infraorder Anguinata according to Siddiqi (2000). By contrast, *F. congestae* is highly derived with a long and robust stylet (29–39 μm) with bifurcate stylet knobs, a different relative pattern of ventral to dorsal pharyngeal gland lobe elongation, and possesses a specialized male tail. These morphological manifestations may be due to specializations that have occurred within each lineage and their fig hosts, e.g., *F. laselvae* n. sp. occurs under the bracts of a fig sycone, which is similar to the niches occupied by other seed and leaf-galling anguinids, whereas *F. congestae* occurs inside the sycone of fluid-filled figs. Recent evidence from a newly discovered *Bursaphelenchus* from inside the sycones of *Ficus variegata* in Japan has demonstrated morphological convergence with the multiple lineages currently ascribed to the genus '*Schistonchus*' that evolved several times from different aphelenchoidid ancestors (Kanzaki *et al.*, 2014b). These fig lumen-inhabiting and plant-parasitic aphelenchoidids all possess robust and long stylets with enlarged knobs relative to their mycophagous sister taxa that do not occur in fig sycones (Kanzaki *et al.*, 2014b). This suggests that the fig sycone is a protected ecosystem with micro-niches available for exploitation that favor adaptations such as long and robust stylets with pronounced basal knobs.

The relative molecular distances between *F. laselvae* n. sp. and *F. congestae* compared with other nominal genera in the Infraorder Tylenchomorpha *sensu* De Ley & Blaxter (2004) also challenges whether these should be congeners, or whether *F. laselvae* n. sp. belongs to an entirely new genus. For the purpose of this paper, and given the current state of paraphyly in the closely related '*Ditylenchus*' (*sensu lato*) due to convergence in general morphology, we have decided to assign this nematode to the genus *Ficotylus* until more representatives have been found and studied to further assess the classification relative to morphology, development, and ecology. Ultimately, the two species share an association with fig sycones, albeit different associations (inside the sycone vs under the bracts of the sycone), and molecular sequence similarities suggestive of recent shared ancestry.

Davies *et al.* (2009) concluded that *F. congestae* was an unusual anguinid based upon molecular phylogenetic inferences, but an unusual neotylenchid based upon

morphology, especially in terms of the similarly bifid stylet knobs that resemble *Hexatylus* Goodey, 1926 (see Siddiqi, 2000). The discovery of *F. laselvae* n. sp. as a putative congener based upon molecular phylogeny, which retains the more characteristic and presumed pleisomorphic anguinid morphology, suggests that *F. congestae* is a highly derived species due to the selective pressures of adapting to life in the lumen of sycones of *Ficus congesta*. *Ficotylus laselvae* n. sp., on the other hand, more closely resembles the morphological description and ecology of *D. angustus*, an anguinid ectoparasitic on the terminal buds and newly formed tissues of rice plants (Seshadri & Dasgupta, 1975). In mature rice at the time of flowering and seed set, these nematodes are associated with protected and growing above-ground regions of the stem, sheath, and deformed rice inflorescences (Seshadri & Dasgupta, 1975). The reproduction and movement of the nematodes is facilitated in times of sufficient moisture availability and does not appear to involve an invertebrate transfer host. Future work should evaluate whether *F. laselvae* n. sp. also infects the fig bracts and possibly other protected aerial parts of *Ficus colubrinae* on its own during times of high moisture or whether this nematode is possibly dicyclic like other neotylenchids such as *Fergusobia*, *Hexatylus*, or *Deladenus* which have free-living mycetophagous or phytophagous and entomoparasitic generations with correspondingly distinct and divergent morphologies (Siddiqi, 2000; Nelson *et al.*, 2014). Confirmation of the latter would require discovering the invertebrate transfer host and associated entomoparasitic stages.

Acknowledgments — The authors thank Kris Powers and Orlando Vargas for technical assistance. This study was supported by a visiting professor fellowship from Zhongkai University of Agriculture and Technology to Y. Zeng and in part by National Science Foundation (NSF) Biotic Surveys and Inventories projects (DEB-0450537; DEB-0640807) to R.G.-D. and T.P., and a USDA Tropical and Subtropical Agriculture grant CRSR-99-34135-8478 to R.G.-D. In addition, this work was supported in part by the USDA National Institute of Food and Agriculture, Hatch project FLA-FTL-005296. We also thank the CR-USA Foundation for funding (A.E.), and the Organization for Tropical Studies (OTS) for access to the La Selva Biological Station for sampling, Material Transfer Agreement c-1-0620-0955 for transfer of biological materials from María de los Angeles Mora López, holder of collecting permit 001-2005-OT-CONAGEBIO and the Centro de Investigación en Biología Celular y Molecular (CIBCM) at the Universidad Nacional of Costa Rica. Universidad Nacional of Costa Rica and Universidad Nacional at Heredia provided logistical and facilities support. We are also grateful to Deedra McClearn for providing facilities at the La Selva Biological Station.

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