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The great greenbriers gall mystery resolved? New species of *Aprostocetus* Westwood (Hymenoptera, Eulophidae) gall inducer and two new parasitoids (Hymenoptera, Eurytomidae) associated with *Smilax* L. in southern Florida, USA

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Abstract

Aprostocetus smilax Gates & Zhang, **sp. nov.**, is described from stem and leaf galls on *Smilax havanensis* Jacq. in southern Florida, USA. It is the third species of *Aprostocetus* Westwood known to induce plant galls. Two parasitoids of *A. smilax* are also described: *Phylloxeroxenus smilax* Gates & Zhang **sp. nov.** and *Sycophila smilax* Gates & Zhang, **sp. nov.** We conclude that *A. smilax* is the true gall inducer on *Smilax* L., and thus the host records of *Diastrophus smilacis* Ashmead and its inquiline *Periclistus smilacis* Ashmead, both from *Smilax*, are erroneous.

Keywords

Chalcidoidea, Cynipidae, *Diastrophus*, *Sycophila*, *Phylloxeroxenus*, *Periclistus*

Introduction

Gall induction in Chalcidoidea was summarized by La Salle (2005) wherein he noted its occurrence in six families, representing at least 15 independent origins. Within Eulophidae

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he reported 11 genera across two subfamilies, Opheliminae (two genera) and Tetrastichinae (nine genera), with documented gall induction behaviors. Since then, an additional six genera have been added to the list of gall inducers (Fisher et al. 2014; Kim et al. 2004; Kim and La Salle 2008; Kim et al. 2005; Mendel et al. 2004; Rasplus et al. 2011), including serious invasive pests of *Eucalyptus* L'Hér worldwide. In Tetrastichinae, the Neotropical gall associates and inducers tend to have heavier sclerotization and be larger in size than other members of the subfamily (La Salle 2005).

Aprostocetus Westwood is the largest genus within the subfamily Tetrastichinae, containing >800 species distributed worldwide that are most frequently associated with insect galls induced by four insect orders and Acari as parasitoids or inquilines (Graham 1987; La Salle 1994). Gall induction is somewhat rare in *Aprostocetus*, with only five documented cases worldwide: (1) *A. colliguayae* (Philippi) in flower buds of *Colliguaja* Molina (Euphorbiaceae) in Chile (Martinez et al. 1992); (2) *A. gallicolus* Nieves-Aldrey & Askew on stems of *Hedyarum boveanum* Bunge ex Basiner (Fabaceae) in Spain (Nieves-Aldrey and Askew 2011); (3) *Aprostocetus monacoi* Viggiani described from stem galls in *Melilotus indicus* L. (Fabaceae) from Italy; (4) *Aprostocetus* sp. on leaf midribs of *Corymbia citriodora* (Hook.) (Myrtaceae) reported from California and Hawaii (Beardsley and Perreira 2000); and (5) *Aprostocetus* sp. on stems of *Melilotus* Mill. infested with wound tumor virus *Aureogenus* Black in the US (Teitelbaum and Black 1954). In this paper we describe *Aprostocetus smilax*, sp. nov. (Hymenoptera: Eulophidae), a gall inducer on *Smilax havanensis* Jacq. and the second recorded case of gall induction for the genus in North America. We also describe two parasitoids of *A. smilax*, *Phylloxeroxenus smilax*, sp. nov., and *Sycophila smilax*, sp. nov. (Fig. 1).

Smilax L. are monocots in the family Smilaceae, with ~350 species found mostly in tropical and subtropical regions of the world (Ferrufino-Acosta 2014). A variety of gall midges (Diptera: Cecidomyiidae) and fungi in the genus *Synchytrium* de Bary & Woronin are known to induce galls on *Smilax* (Cook 1951; Uechi et al. 2012; Urso-Guimarães and Scareli-Santos 2006). The only record of a *Smilax* gall induced by Hymenoptera in North America is *Diastrophus smilacis* Ashmead (Cynipidae: Diastrophini), collected from Illinois and Florida (Ashmead 1896a). This host record is somewhat puzzling, as other members of *Diastrophus* Hartig exclusively induce galls on Rosaceae (*Fragaria* L., *Rubus* L., and *Potentilla* L.), and all other known cynipids have been recorded from dicots (Ronquist and Liljeblad 2001; Schick et al. 2003).

Methods

Dissection

Freshly collected stem and leaf galls of *S. havanensis* were dissected during field work in the Miami area in 2010 by MWG and MLB. A Nikon 20× Mini Field stereoscope, fine forceps, and GEM Blue Star Super Single Edge razors were used. Slices of galls were successively removed, gradually exposing individual locules. We dissected ~20

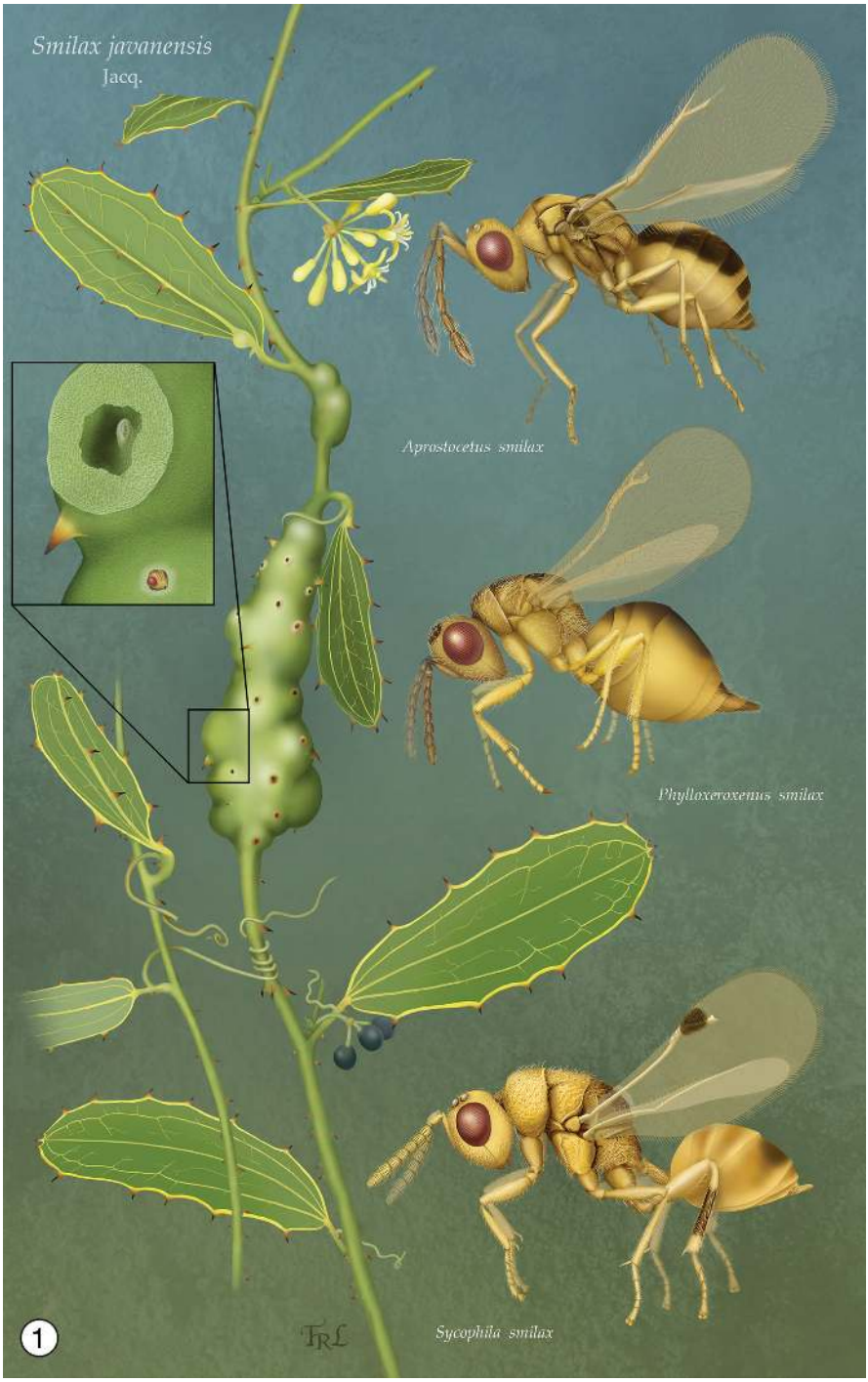

Figure 1. Illustration of the stem gall on *Smilax havanensis* induced by *Aprostocetus smilax* (top right), with the inset showing the internal structure and an egg. Two eurytomid parasitoids, *Phylloxeroxenus smilax* (middle right), and *Sycophila smilax* (bottom right) are included. Illustration by Taina Litwak.

multilocular galls and notes were made about the contents of each locule in terms of its condition and occupant prior to each occupant being assigned a code and preserved in 80% ethanol. We noted six ectoparasitoid specimens. Pertinent taxon-specific notes are included in results below.

Imaging

Ethanol-preserved specimens were dehydrated through increasing concentrations of ethanol, and transferred to hexamethyldisilazane (HMDS) (Heraty and Hawks 1998) before point-mounting. MWG identified parasitoids using a Leica M205C stereomicroscope with 10X oculars and a Leica LED ring light source for point-mounted specimen observation. We took scanning electron microscope (SEM) images with a Hitachi TM3000 (Tungsten source). Body parts of disarticulated specimens were adhered to a 12.7 × 3.2 mm Leica/Cambridge aluminum SEM stub by a carbon adhesive tab (Electron Microscopy Sciences, #77825-12). Stub-mounted specimens were sputter coated with gold-palladium using a Cressington Scientific 108 Auto from multiple angles to ensure complete coverage (~20–30 nm coating). Habitus images were obtained using a Visionary Digital imaging system. The system consists of a Canon EOS 5D Mark II digital SLR camera with a 65 mm macro lens. A Dynalite MP8 power pack and lights provided illumination. Image capture software was Visionary Digital's proprietary application with images saved as TIF with the RAW conversion occurring in Canon Digital Photo Professional software. Image stacks were montaged with Helicon Focus 6.2.2. Image editing was done in Adobe Photoshop and plate layout in Adobe Illustrator. The painting was made from pinned and live insect specimens, plant herbarium sheets and photographs. Additional structural details of the insects were obtained from SEM photographs. The final image was painted using Adobe Photoshop.

We used several species keys to determine whether our material belonged to any described species (Balduf 1932; Graham 1987) with details below under each specific treatment. Where possible, all species identifications were corroborated by comparison with authoritatively identified specimens in the Smithsonian National Museum of Natural History.

Terminologies used for surface sculptures follow Harris (1979), while the morphology follows Gibson (1997), La Salle (1994), Lotfalizadeh et al. (2007), and Gates and Pérez-Lachaud (2012). Abbreviations for museums are: ABS, Archbold Biological Station, Archbold, FL, USA; FSCA, Florida State Collection of Arthropods; USNM, United States National Museum of Natural History, Washington, D.C., USA.

Molecular protocol

Specimens were extracted, amplified, and sequenced at USDA Beltsville Agricultural Research Center (BARC) using the DNeasyTM Tissue Kit protocol (Qiagen, Valencia, CA, USA). Specimens were digested for circa three hours using 20 µL of 20 mg/

mL Proteinase K at 55 °C. The DNA was resuspended with 150 µL of Qiagen elution buffer. Fragments of mtDNA *COI* (620 bp) were amplified using LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). Amplifications for rDNA *28S* (820 bp) used 28S_D1F 5'-ACCCGCTGAATTTAAGCATAT-3' (Harry et al. 1996) and 28S_D2R 5'-TTGGTCCGTGTTTCAAGACGGG-3' (Campbell et al. 1994). All PCRs were performed using approximately 2 µL DNA extract, 1.25 µL 10× Buffer, 1 µL dNTP, 1 µL of each primer, 1 unit of Taq DNA polymerase (TaKaRa Bio, Mountain View, CA, USA), and purified water for a final volume of 25 µL. Amplicons of *COI* were generated with an initial denaturation of 1 min at 95 °C, followed by 35 cycles of 95 °C for 15 s, 49 °C for 15 s and 72 °C for 45 s, and a final elongation period of 4 min at 72 °C. The thermocycler setting for *28S* is similar to *COI*, with the exception of annealing temperature being at 55 °C. Sequencing was conducted using a ABI 3730xl DNA sequencer following manufacturer's instructions. Contigs were assembled and edited using Sequencher version 4.5 (Gene Codes). DNA sequences were then compared with available sequences in the Barcode of Life Database (BOLD, Ratnasingham and Hebert 2007) and the Basic Local Alignment Search Tool (BLAST) for nucleotides in GenBank. All sequences are uploaded onto GenBank (see Table 1).

Phylogenetic analysis

COI was aligned using default MAFFT v7.45 settings (Katoh et al. 2002) and checked by eye, for *28S* the Q-INS-I strategy (Katoh and Toh 2008) was implemented to account for secondary RNA structures. Each gene was analyzed separately, and concatenated using SequenceMatrix (Vaidya et al. 2011) in IQ-TREE v2.0.5 (Minh et al. 2020). Best models of evolution were determined using ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE, with 1000 ultrafast bootstrap pseudoreplicate support (Hoang et al. 2017). The output trees were visualized in R v4.0 (R Core Team 2020) using the packages ggtree v2.2.0 (Yu et al. 2017) and treeio v1.12.0 (Wang et al. 2020).

Results

Taxonomy

Eulophidae

***Aprostocetus smilax* Gates & Zhang, sp. nov.**
http://zoobank.org/D29A7AF7-E6D5-488D-996A-1228BD8F320F
Figs 2–18

Diagnosis. This species keys to *Aprostocetus* subgenus *Aprostocetus*, couplet 103 in Schauff et al. (1997) and 53 in La Salle (1997). This is the most biologically diverse

Table 1. Voucher identification and associated GenBank accession numbers.

ID	Voucher	COI	*28S*
Aprostocetus smilax	G0006A	MT576085	MT560740
	G0008A	N/A	MT560741
	G0008B	MT576086	MT560742
	G0009	MT576087	MT560743
	G0010A	MT576088	MT560744
	G0010C	MT576089	MT560745
	G0010E	MT576090	MT560746
	G0010F	MT576091	MT560747
	G0013A	MT576092	MT560748
	G0014	MT576093	MT560749
Phylloxeroxenus smilax	G0015	MT576094	MT560750
Aprostocetus smilax	G0016	MT576095	N/A
	G0017	MT576096	MT560751
	G0018	MT576097	MT560752
	G0019	MT576098	MT560753
	G0021	MT576099	MT560754
	G0022	MT576100	MT560755
	G0023	MT576101	MT560756
	G0024	MT576102	MT560757
	G0025	MT576103	MT560758
	G0026	MT576104	MT560759
	G0030	MT576105	MT560760
	G0031	MT576106	MT560761
	G0034	MT576107	MT560762
	G0036	MT576108	MT560763
	G0041	MT576109	MT560764
Tetrastichinae sp.	G0042	MT576110	MT560765
Aprostocetus smilax	G0043	MT576111	MT560766
	G0044	MT576112	MT560767
Sycophila smilax	G0045	N/A	MT560768
Aprostocetus smilax	G0046	MT576113	MT560769
	G0047	MT576114	MT560770
	G0049	MT576115	MT560771
	G0050	MT576116	N/A
Phylloxeroxenus smilax	G0051	N/A	MT560772
Brasema sp.	G0052	MT576117	MT560773
Sycophila smilax	G0053	N/A	MT560774
Phylloxeroxenus smilax	G0054	MT576118	MT560775
Sycophila smilax	G0055	N/A	MT560776
	G0056	MT576119	MT560777
Phylloxeroxenus smilax	G0057	N/A	MT560778
	G0058	N/A	MT560779
Aprostocetus smilax	G0059	MT576120	MT560780
	G0061	MT576121	MT560781
	G0063	MT576122	MT560782
	G0064	MT576123	MT560783
	G0065	N/A	MT560784
	G0066	MT576124	MT560785
	G0070	MT576125	MT560786
Phylloxeroxenus smilax	G0071	N/A	MT560787
Sycophila smilax	G0076	MT576126	MT560788
	G0077	MT576127	MT560789
Aprostocetus smilax	G0078	MT576128	MT560790
	G0080	MT576129	MT560791
Phylloxeroxenus smilax	G0082	N/A	MT560792

and speciose of the five *Aprostocetus* subgenera, often associated with insects inhabiting plant galls such as Diptera (Cecidomyiidae), Hymenoptera (Cynipoidea), Hemiptera (Coccoidea), Coleoptera, and eriophyid mites (La Salle 2005). Burks (1967) published a key to 13 North American species, which is dated, and a comparative diagnosis of all 58 species is beyond the scope of this paper. Nevertheless, this species keys to couplet 2 of Burks' key, and differs from the two species with coriaceous mesoscutum (*A. coelioxydis* Burks and *A. granulatus* Ashmead) which are both metallic blue/black in coloration. Recent phylogenomic study of Eulophidae has shown *Aprostocetus* to be paraphyletic (Rasplus et al. 2020), and some of these subgenera might be elevated to genus level in the future.

Material examined. ***Holotype***, female: USA • **FL:** Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 19.Dec.2001, C. Rodriguez & T. Smith leg.; USNMENT01735185 (deposited at USNM). Paratypes (4♀, 7♂): Same information as holotype; USNMENT01735186, 01735187 (1♀, 1♂, USNM). **FL:** Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 18.Apr. 2010; M. Gates & M. Buffington leg.; USNMENT01735188–01735196 (3♀, 6♂, USNM).

Description. Female. Body length 1.7 mm (Fig. 2).

Color. Mostly whitish-yellow, pedicel, flagellomeres, clava, axillula, and marginal vein, sides of gastral tergites brown. Fore and midlegs white (Fig. 2).

Head. Squareish in dorsal view, 1.2× as wide as long in dorsal view (Fig. 4). Lower face coriaceous, clypeus bilobed, mandible tridentate (Fig. 5). Malar sulcus present, malar space 0.7× eye height. Genal carina absent. Toruli positioned slightly below median of compound eyes, diameter of torulus equal that of the intertorular space. Frons striate, scrobal depression converging towards the clypeus with a row of setae along depression (Fig. 4). Vertex coriaceous, ratios of POL:OOL:LOL equal to 2.8:2.1:1 (Fig. 6). Ratio of scape (minus radicle):pedicel:A1:A2: F1:F2: F3:club as 72:33:3:1:53:40:35:68; pedicel conical expanding distally; funicle cylindrical; anellus two segmented, funicular segments with single row of longitudinal sensilla and one whorl of setae, shorter than its bearing segment; clava trisegmented (Fig. 8). Head posteriorly coriaceous with a ring of setae around the outer edge, smooth with two setae. Postgenal bridge ornamentation narrow. Postgenal sulci, postgenal groove, and hypostomal bridge absent (Fig. 7). Labium square-shaped.

Forewing. Three setae on submarginal vein, 7 setae on marginal vein. Ratio of marginal vein:postmarginal vein:stigmal vein as 22.5:1:6.

Mesosoma. Mesosoma coriaceous, 1.14× as long as broad (Fig. 9); notauli complete, shallow. With 2 adnotaular seta on the midlobe of mesoscutum, and two setae on the lateral lobes (Fig. 10). Scutellum with two setae on each side, submedian groove deep, complete. Lateral panel of axilla strigate, axillula coriaceous dorsally and strigate ventrally. Prepectus coriaceous. Mesopleuron coriaceous, dorsally delimited from femoral depression raised ridge. Epicnemium flat and ventral shelf not projected forward (Fig. 11). Propodeum coriaceous and divided by median carina that diverges into

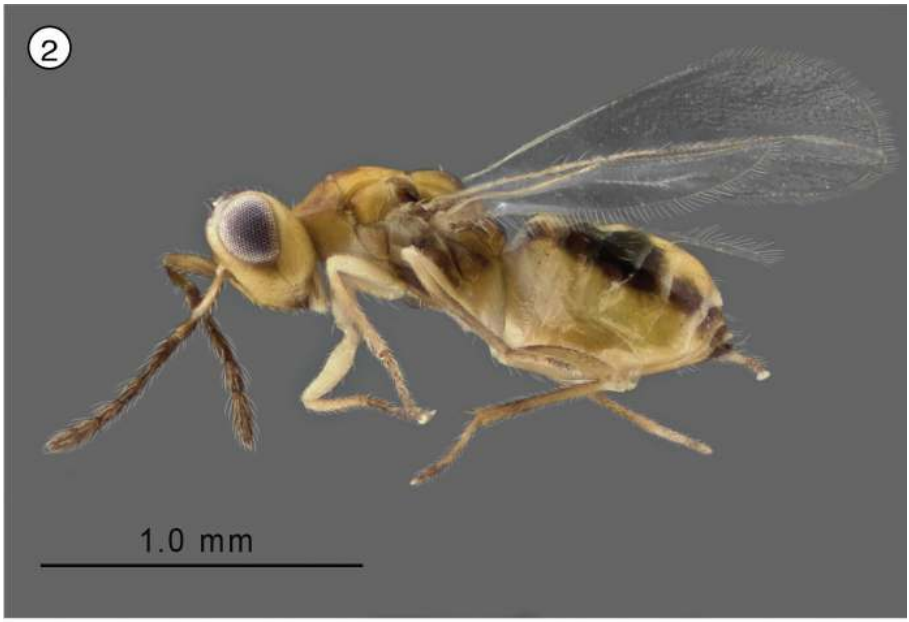

Figure 2–3. *Aprostocetus smilax* **2** female habitus **3** male habitus.

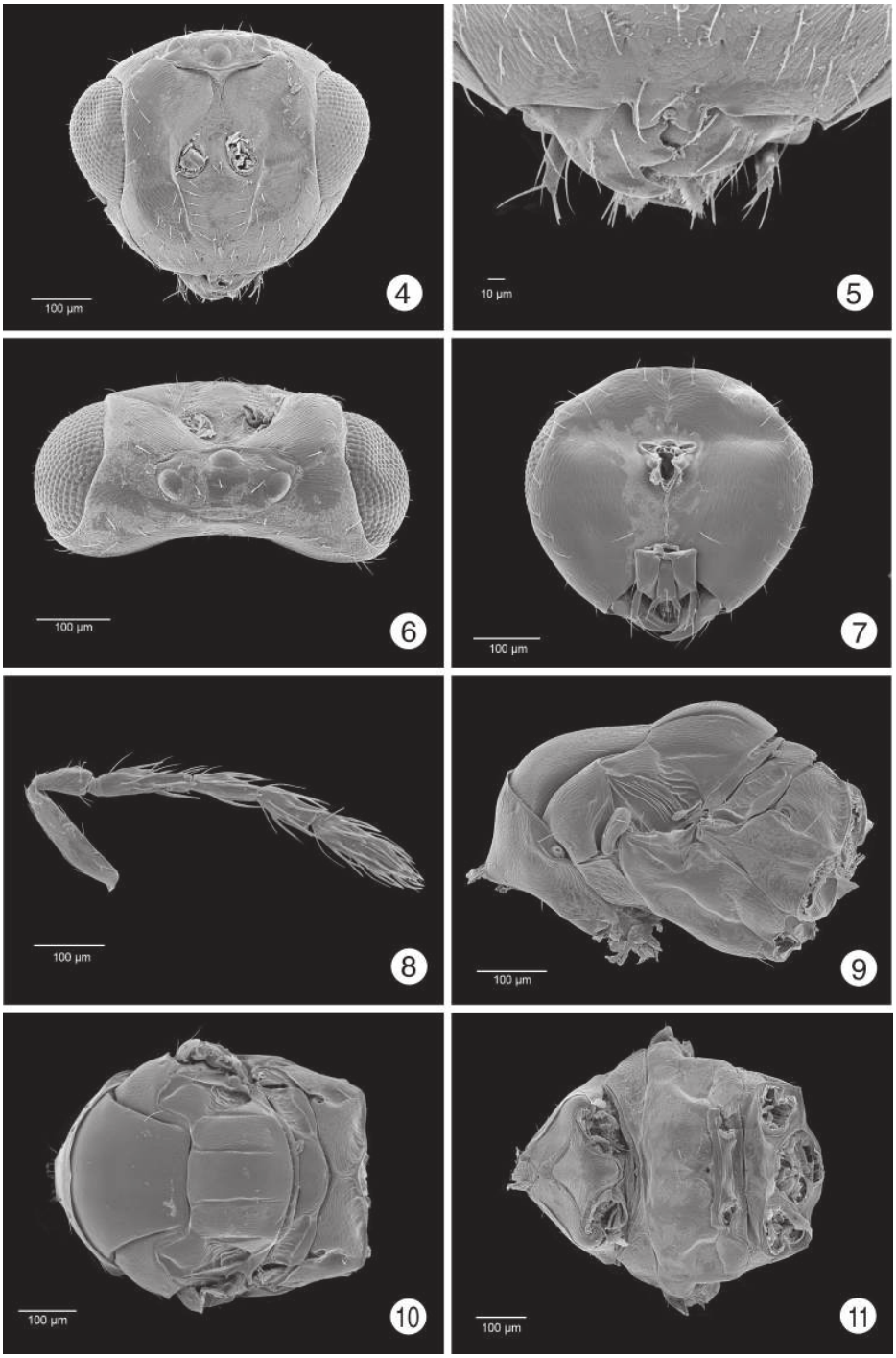

Figure 4–11. *Aprostocetus smilax* **4** frontal view of head **5** frontal view of lower face **6** dorsal view of head **7** posterior view of head **8** female antenna **9** lateral view of mesosoma **10** dorsal view of mesosoma **11** ventral view of mesosoma.

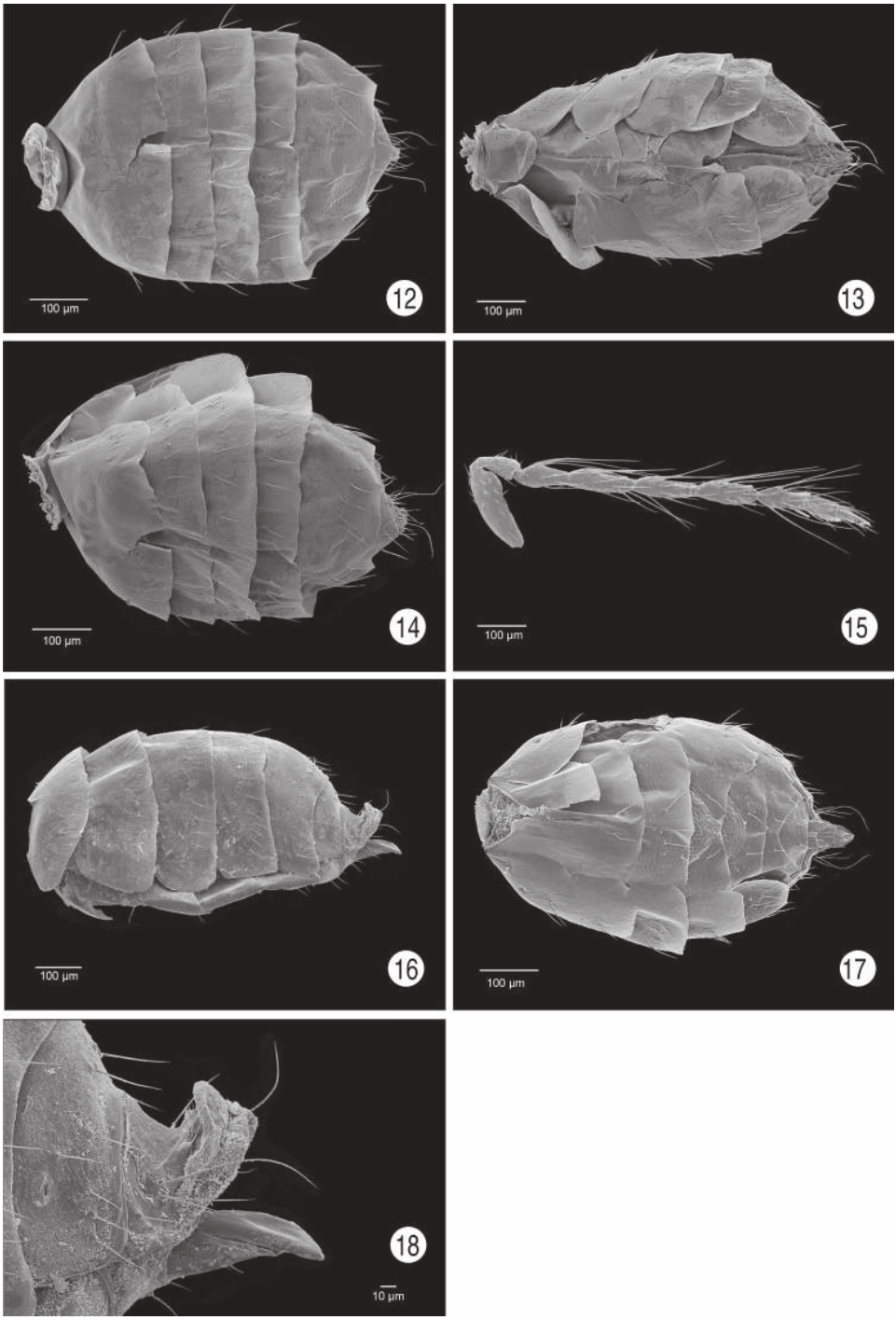

Figure 12–18. *Aprostocetus smilax* **12** dorsal view of female metasoma **13** ventral view of female metasoma **14** lateral view of female metasoma **15** male antenna **16** lateral view of male metasoma **17** ventral view of male metasoma **18** closeup of male genital opening.

raised, scalloped ridges posteriorly. Spiracle within a depression. Callus with a single seta, raised and partly overhanging outer rim of conspicuous spiracle (Fig. 10).

Metasoma. Metasoma smooth, Gt1 and Gt2 dorsally glabrous (Fig. 12), subsequent tergites each with a ring of setae (Fig. 13). Cercus with 1 seta distinctly longer (>1.5×) than others (Fig. 14).

Male. 1.1 mm. Color and sculpture as described for female (Fig. 3). Antennae with setae >1.5× as long as width of segment (Fig. 15). Gt7 curves up to form genital opening (Figs 16, 17), with a pair of long and three pairs of shorter cercal setae (Fig. 18).

Variation. Size ranges from 1.6–1.8 mm for females, and 1.1–1.2 mm for males. The number of setae on marginal vein ranges from 6–8.

Biology. It induces round galls on the stems of *Smilax havanensis*, often coalescing to form irregularly rounded, polythalamous swellings. Individual galls can also be found on the edge of leaves. Green when fresh and of a pithy structure (Figs 1, 63 inset), tissues around the emergence hole often form a black ring.

Distribution. Southern Florida, USA.

Eurytomidae

Phylloxeroxenus smilax Gates & Zhang, sp. nov.

http://zoobank.org/6D5262E0-7A91-4A3A-9E1A-C219C1F5EBA3
Figs 19–34

Diagnosis. *Phylloxeroxenus smilax* can be easily distinguished from the only other known North American species, *Phylloxeroxenus phylloxerae* (Ashmead), which is suspected to be a parasitoid of the cecidomyiid inquiline within *Phylloxera* Boyer de Fonscolombe galls on hickory (*Carya* Nutt.) (Ashmead 1881). The lower face is strigose and the ventral half of the body is yellow in *P. smilax*, while in *P. phylloxerae* the lower face is striate and the body is completely black. There are at least 50 undescribed species in at least three species groups for the Neotropical region that exhibit a range of variation in diagnostic generic characters such as the propodeum in lateral view forming a 90° angle with mesosoma; long/short petiole and resultant effect on striate part of S1 (Fig. 30), with the striae on S1 being a reliable diagnostic though expressed to varying degrees; and lower face with/without striae (Gates, unpublished data).

Material examined. ***Holotype***, female: USA • **FL:** Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 18.Apr. 2010; M. Gates & M. Buffington leg.; USNMENT01735174 (deposited at USNM). Paratypes (5♀, 6♂): Same information as holotype; USNMENT01735175–01735178 (3♀, 1♂, USNM). **FL:** Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 19.Dec.2001, C. Rodriguez & T. Smith leg.; USNMENT01735179–01735184 (2♀, 5♂, USNM). Additional material: **FL:** Dade Co.: Coral Gables, Deering Estate Pineland, Ex *Smilax havanensis* stem gall; 23.Feb.1995, G. Melika leg.; (3♀, 4♂, ABS).

Description. Female. Body length 1.88 mm (Fig. 19).

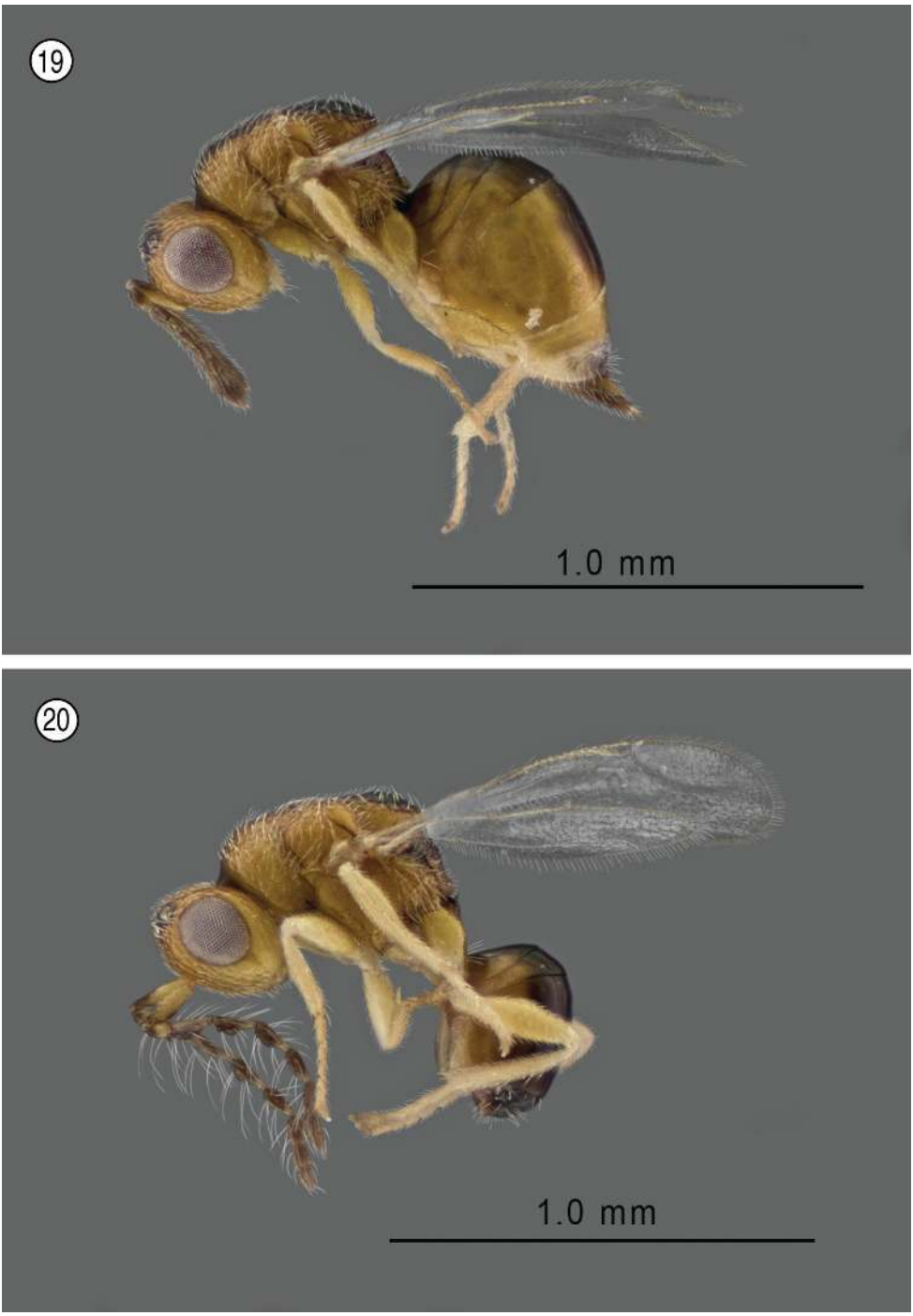

Figure 19–20. *Phylloxeroxenus smilax* **19** female habitus **20** male habitus.

Color. Orange-yellow; antennal segments light brown; edges of ocelli, scutellum, metasoma mediodorsally with black band, eyes pinkish red (Fig. 19).

Head. Rounded in dorsal view, 1.3× as wide as long in dorsal view, umbilicate with appressed setae (Fig. 21). Lower face strigose, clypeus emarginate, mandible tridentate and step-like, supraclypeal area smooth, glabrous, slightly raised, and extending to the toruli (Fig. 22). Malar sulcus present, malar space 0.7× eye height. Genal carina present. Toruli positioned slightly above lower ocular line, diameter of torulus 4.4× that of the intertorular space. Scrobal depression carinate laterally, fading apically. Vertex imbricate, ratios of POL:OOL:LOL equal to 2.5:1:1 (Fig. 23). Ratio of scape (minus radicle):pedicel:anellus: F1:F2: F3:F4:F5:club as 19:7.3:1:7:6.6:6.6:6.4:6:18; pedicel chalice-shaped; funicle fusiform; funicular segments with single row of longitudinal sensilla and one whorl of setae, as long as its bearing segment; clava bisegmented (Fig. 25). Head posteriorly lacking postgenal lamina, postgenal groove straight and not converging in their lower part, extending ventrally to lower margin of eyes. Postgenal bridge ornamentation narrow and delicate (Fig. 24). Postgenal sulci small.

Forewing. Eight submarginal setae, 3 on parastigma. Ratio of marginal vein:postmarginal vein:stigmal vein as 2:1:1.

Mesosoma. Mesosoma umbilicate, 1.45× as long as broad; notauli complete, shallow (Fig. 27); lateral surface of prepectus triangular, smooth, ventral surface of prepectus without median tooth, subventral carina diverging strongly (Fig. 26). Mesopleuron reticulate ventrally, dorsally delimited from femoral depression by fine carina. Epicnemium flat and ventral shelf not projected forward. Propodeum in lateral view forming a 90° angle with mesosoma, broadly flattened and apically arcuate, with numerous carinae forming irregular asetose cells, these bordered laterally by setose cells; cluster of setae anterolaterad nucha (Fig. 29). Metaplural-precoxal carina complete (Fig. 28).

Metasoma. Metasoma smooth, Gt4–syntergum setose, Gt6 and syntergum microreticulate; petiole 0.78× as long as broad in dorsal view, with ventral anterior groove and carina (Fig. 31); gaster S-shaped in lateral view, ovipositor angled at about 30° dorsad horizontal axis (Fig. 30); Gt4 emarginate posteriorly in dorsal view.

Male. 1.51 mm. Color and sculpture as described for female (Fig. 20). Antennal with funicular segments pedicellate, each with 2 or more rows of erect setae and about 1.5× as long as width of segment. Four funicular segments and a trisegmented clava (Fig. 32). Gastral petiole in lateral view cylindrical with projecting lateral teeth and mediodorsal prong (Fig. 34), in dorsal view length about 2.5× as long as greatest width, 1.6× as long as the length to metacoxa; evenly reticulate dorsally and ventrally (Fig. 33), smooth laterally.

Variation. Size ranges from 1.76–1.91 mm for females, and 1.45–1.52 mm for males. The coloration on the body can range from almost completely yellow, to mostly black on the dorsolateral surfaces, particularly in males.

Biology. Associated with galls of *Aprostocetus smilax*, likely a parasitoid of the gall inducer.

Distribution. Southern Florida, USA.

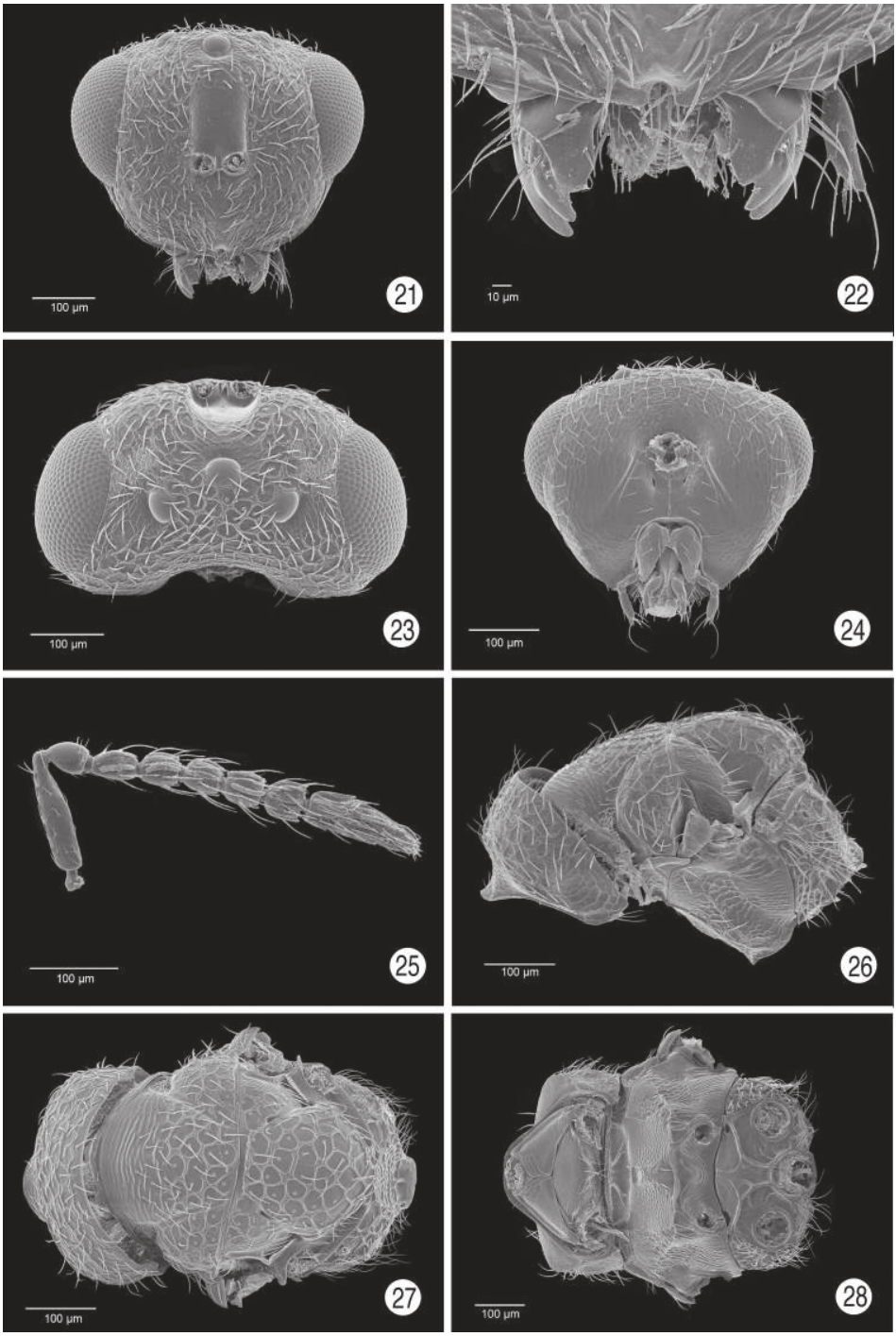

Figure 21–28. *Phylloxeroxenus smilax* **21** frontal view of head **22** frontal view of lower face **23** dorsal view of head **24** posterior view of head **25** female antenna **26** lateral view of mesosoma **27** dorsal view of mesosoma **28** ventral view of mesosoma.

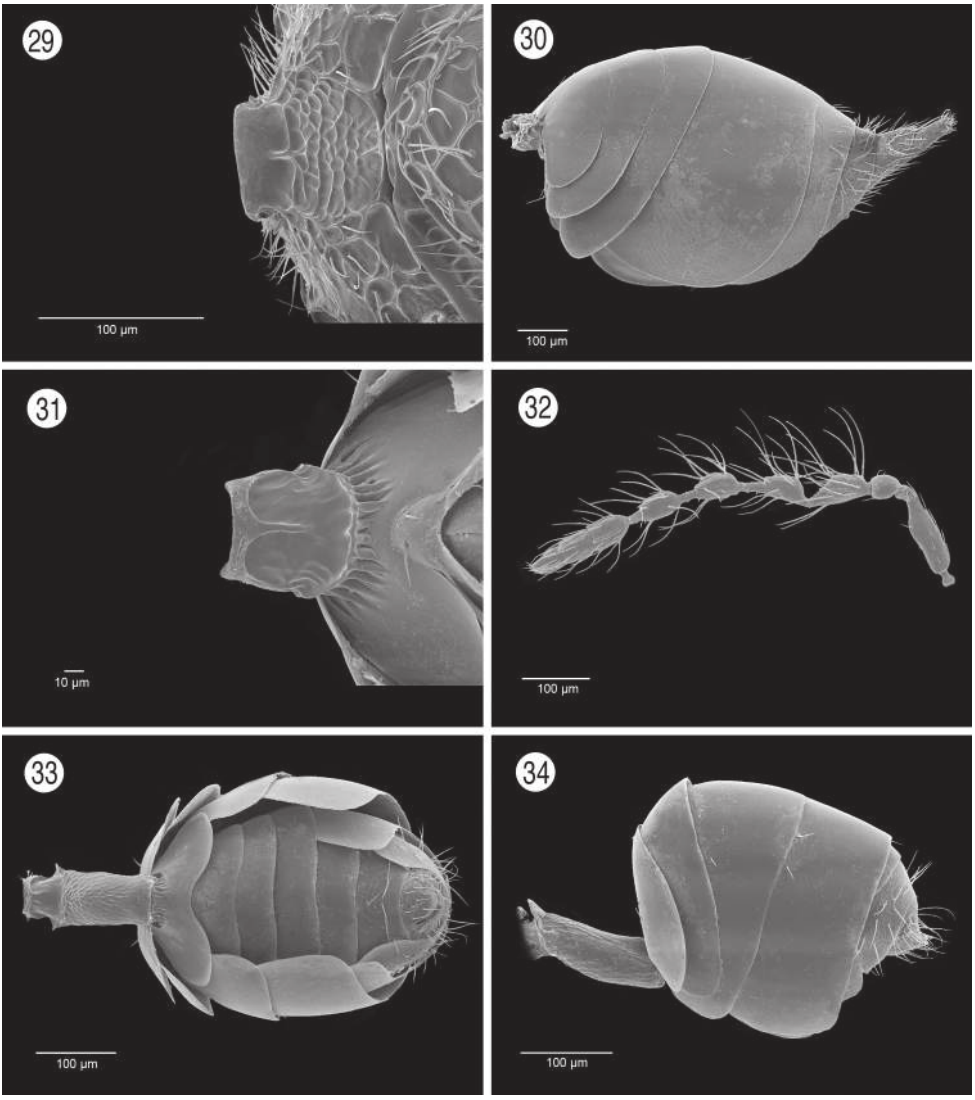

Figure 29–34. *Phylloxeroxenus smilax* **29** dorsal view of propodeum **30** lateral view of female metasoma **31** ventral view of female petiole **32** male antenna **33** ventral view of male metasoma **34** lateral view of male metasoma.

Sycophila smilax Gates & Zhang, sp. nov.

http://zoobank.org/AC0B37C6-F069-4E19-91EF-946EE0466F1B

Figs 35–53

Diagnosis. This species is recognized by its small size, pale yellow coloration and small/faint stigmal band. It keys to couplet 9 of Balduf (1932)'s key of North American *Sycophila*, but differs from the other mostly yellow species *Sycophila mimosae* Balduf

by the lack of a constricted marginal vein. The Central and South American *Sycophila* fauna is poorly known, and no current key exists.

Material examined. ***Holotype***, female: **FL:** Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 19.Dec.2001, C. Rodriguez & T. Smith leg.; USNMENT01735197 (deposited at USNM). Paratypes (36♀, 8♂): Same information as holotype; USNMENT01735198–01735206 (6♀, 2♂, USNM). **FL:** Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 18.Apr. 2010; M. Gates & M. Buffington leg.; USNMENT01735207–01735238 (27♀, 5♂, USNM). **FL:** Dade Co.: South Miami, Quail Roost Pineland, Em 1.VI.2000 from galls of *Smilax* sp.; 8.V.2000; USNMENT01735239–01735242 (3♀, 1♂, USNM). Additional material: **FL:** Dade Co.: Coral Gables, Deering Estate Pineland, Ex *Smilax havanensis* stem gall; 23.Feb.1995, G. Melika leg. (3♀, 2♂, ABS). **FL:** Dade Co.: Coral Gables, Ex. *Diastrophus smilacis* on *Smilax havanensis*; 8.Nov.1977, R. Schimmel leg. (1♀, 1♂, FSCA).

Description. Female. Body length 1.8 mm (Fig. 35).

Color. Mostly pale yellow; antennal segments dark yellow; edges of ocelli, scutellum, hindtibia laterally, tarsal claw, tip of ovipositor black, pterostigma dark brown, wing band light brown, eyes pinkish red (Fig. 35).

Head. Rounded in dorsal view, 1.22× as wide as long in dorsal view, umbilicate with appressed setae (Fig. 37). Lower face strigose, clypeus bilobate, mandible tridentate with supraclypeal area smooth, glabrous, slightly raised and extending to toruli (Fig. 38). Malar sulcus present, malar space 0.59× eye height. Genal carina absent. Toruli positioned on lower ocular line, diameter of torulus 1.2× that of the intertorular space. Interantennal projection narrow, 1.5× that of the diameter of torulus. Scrobal depression carinate laterally, slightly diverging basally. Vertex imbricate, ratios of POL:OOL:LOL equal to 2.7:1:1 (Fig. 39). Ratio of scape (minus radicle):pedicel:anellus: F1:F2: F3:F4:F5:club as 17:6.7:1:5:5:4.7:4.7:4.7:13; pedicel chalice-shaped; funicle fusiform; funicular segments with single row of longitudinal sensilla and two whorls of setae, as long as its bearing segment; clava bisegmented (Fig. 41). Head posteriorly lacking postgenal lamina, postgenal groove faint, straight and not converging in their lower part, extending ventrally to ⅘ the lower margin of eyes (Fig. 40). Postgenal sulci small.

Forewing. Dark brown band on the wing about the same width as pterostigma and does not reach uncus, faint, reaching about ½ down the wing width, 8 submarginal setae, 3 on parastigma, 1 in basal cell, surrounded by basal and costal setal lines. Pterostigma covering marginal, postmarginal, and stigmal vein.

Mesosoma. Mesosoma umbilicate, 1.52× as long as broad; notauli complete, shallow (Fig. 43); lateral surface of prepectus triangular, smooth, ventral surface of prepectus without median tooth (Fig. 42). Mesopleuron reticulate ventrally, dorsally delimited from femoral depression by fine carina. Epicnemium flat and ventral shelf not projected forward. Propodeum with median furrow bordered mediolaterally by numerous carinae forming irregular asetose cells, these bordered laterally by setose cells (Fig. 44). Metaplural-precoxal carina interrupted by rugose carinae (Fig. 45).

Figure 35–36. *Sycophila smilax* **35** female habitus **36** male habitus.

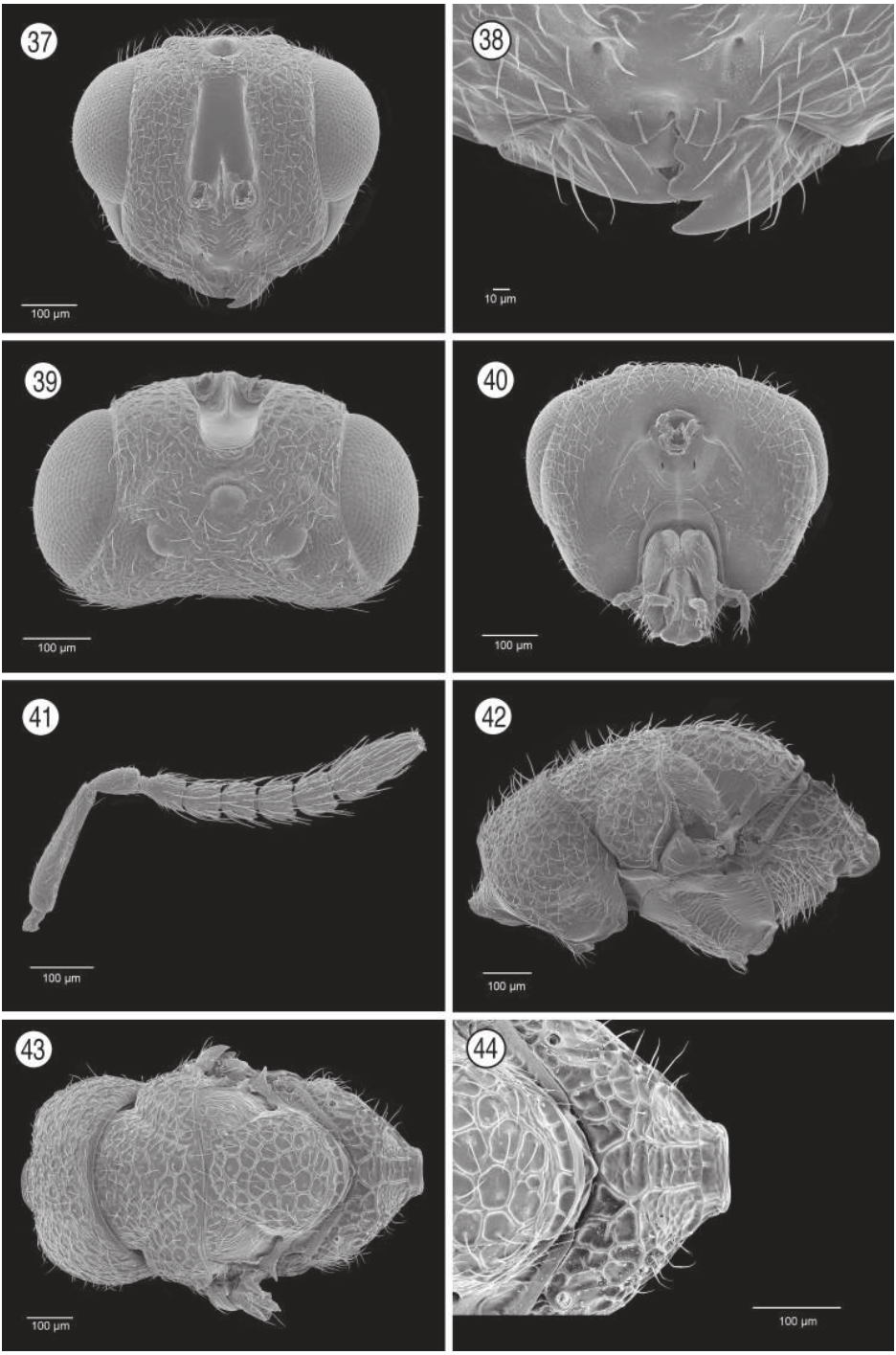

Figure 37–44. *Sycophila smilax* **37** frontal view of head **38** frontal view of lower face **39** dorsal view of head **40** posterior view of head **41** female antenna **42** lateral view of mesosoma **43** dorsal view of mesosoma **44** dorsal view of propodeum.

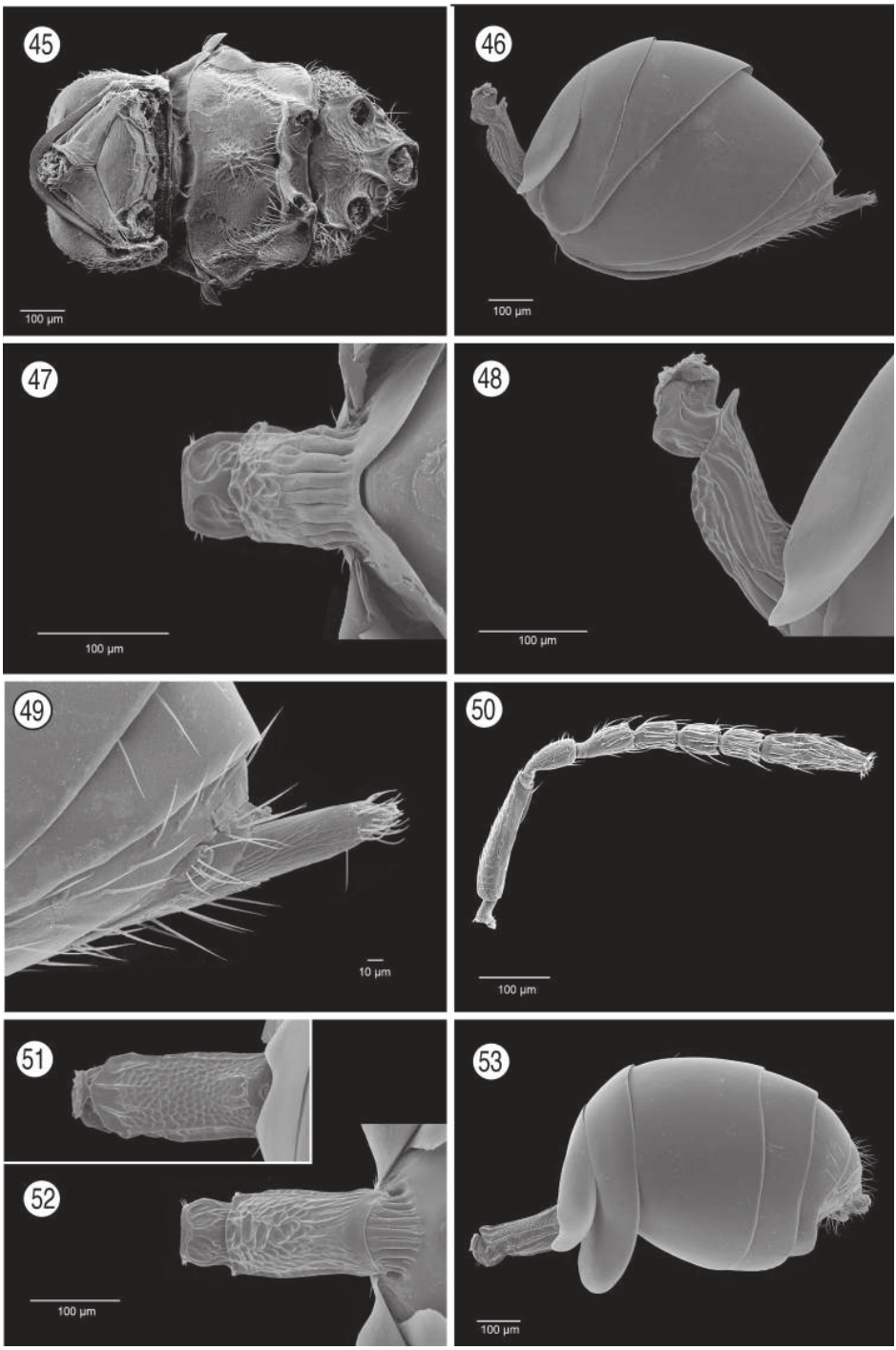

Figure 45–53. *Sycophila smilax* **45** ventral view of mesosoma **46** lateral view of female metasoma **47** ventral view of female petiole **48** lateral view of female petiole **49** closeup of female ovipositor **50** male antenna **51** dorsal view of male petiole **52** ventral view of male petiole **53** lateral view of male metasoma.

Metasoma. Metasoma smooth, ovipositor sheath microreticulate (Figs 46, 49); petiole 2.3× as long as broad in dorsal view, with ventral anterior groove, carina, and mediodorsal prong (Figs 47, 48); gaster teardrop-shaped in lateral view, ovipositor angled at about 30° dorsad horizontal axis (Fig. 49).

Male. 1.88 mm. Mediodorsal of Gt3–5 black, wing band very faint. Otherwise color and sculpture as described for female (Fig. 36). Antenna with four funicular segments (Fig. 50). Gaster cylindrical, petiole 3× as long as wide (Figs 51, 52). Gt4 emarginate posteriorly in dorsal view (Fig. 53).

Variation. Body ranges 1.7–1.8 mm for females, 1.65–1.88 mm for males. The wing band can range from very faint, mesosoma and metasoma dorsally can be yellow or with a tinge of black.

Biology. Associated with galls of *Aprostocetus smilax*, likely a parasitoid of the gall inducer.

Distribution. Southern Florida, USA.

Molecular analyses

A total of 55 individuals had both or at least one of the two genes sequenced. BLAST and BOLD search results confirmed the family and sometimes genus level identification, but did not return any hits at the species level. This *Smilax* gall contains 3 different families of chalcidoids: the majority of the gall inhabitants consisted of the suspected gall inducer *Aprostocetus smilax* (n = 40), and two eurytomid parasitoids *Phylloxeroxenus smilax* (n = 7) and *Sycophila smilax* (n = 6) (Fig. 63). Specimen G0042 was identified as an unknown tetrastichine eulophid that was destructively sampled, while G0052 was identified as *Brasema* Cameron (Eupelmidae) (Fig. 63). This *Brasema* specimen was never reared as an adult from this system, we noted it encircling another larva, presumably the gall inducer, and characterized by large mandibles and erect setae.

Validity of Cynipidae associated with *Smilax*

As the result of this study, the validity of *Diastrophus smilacis* (Figs 54, 55) inducing galls on *Smilax* was also investigated. The resulting fieldwork revealed *Aprostocetus smilax* is the true gall inducer in Florida, after some 400 galls never yielded any cynipids. Further, dissections of the galls from which the type specimen of *D. smilacis* was reared from (collected in Illinois) revealed vascular tissue patterns consistent with dicots and not monocots (Fig. 56). As no additional material of *D. smilacis* has been found since its original description, despite extensive searches in Illinois (Zhiwei Liu, pers. comm.) and other parts of North America (Weld 1959), we can safely conclude *Smilax* is not the host of *Diastrophus smilacis*.

Working with the type material of both *D. smilacis* and *Periclistus smilacis* Ashmead (Figs 59, 62, the putative inquilline of *D. smilacis*) revealed additional curiosities that require mentioning here. Ashmead (1896a) reports specimens of *D. smilacis* were apparently sent to C.V. Riley from Chicago, Illinois (Figs 57, 58), and that Ashmead

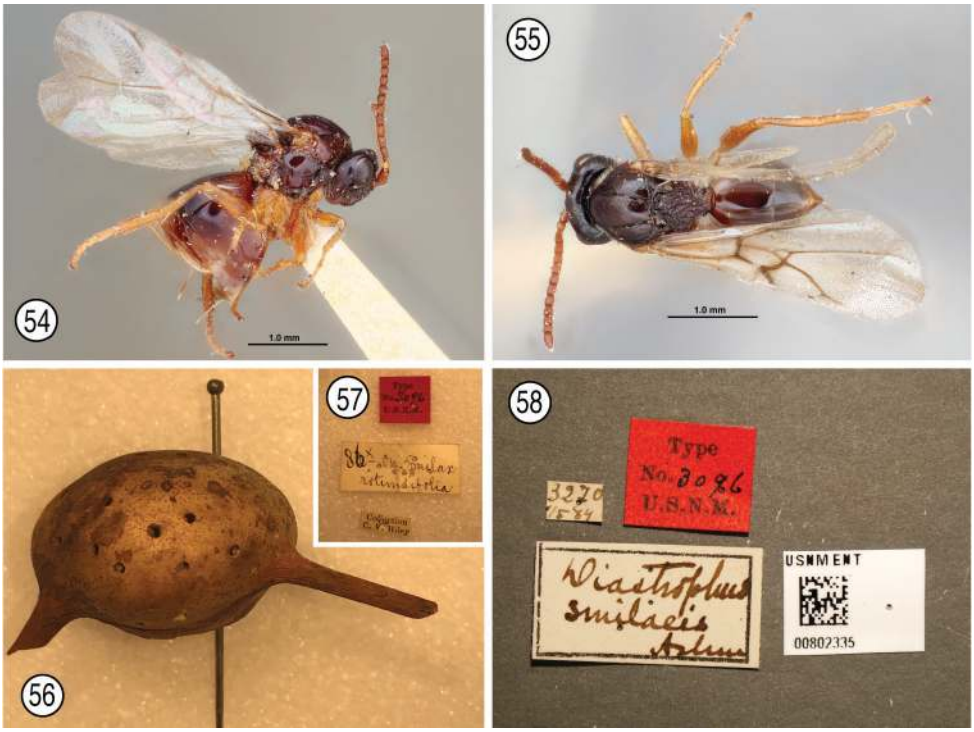

Figure 54–58. *Diastrophus smilacis* **54** lateral habitus of holotype **55** dorsal habitus of holotype **56** gall of holotype **57** label of holotype **58** label of other specimens collected by C.V. Riley.

intended to describe them but the publication of the manuscript was delayed due to C.V. Riley's untimely death. Ergo, time passed, and in the same year (1896), in two different publications, we find the descriptions of *D. smilacis* (Ashmead 1896a) and *P. smilacis* (Ashmead 1896b). While this is not an entirely foreign set of circumstances, the specimens referred to in these two publications are quite confusing.

Ashmead (1896a) reports 13 specimens (females) for the description of *D. smilacis*, but the taxon is only known from the type specimen in the USNM and there is no record of additional specimens being loaned out; one cotype of this taxon is in AMNH, for a total of two specimens. The gall with the same type specimen number as the holotype wasp in the USNM (No. 3096, Fig. 57) has the label '86x' affixed to the pin, and it is mentioned in Ashmead (1896a) that a gall was collected for this species in Florida, but no wasp. Hence, Illinois is the origin of all material associated with *D. smilacis*. Ashmead (1896b) describes *P. smilacis* from 17 specimens and goes on to say the collection data for 13 specimens (same number as *D. smilacis*, above) is labeled 'No. 864, reared April 28, 1871 and four numbered 1010, reared February 4, 1884, from *Diastrophus smilacis*'. However, the type specimen of *P. smilacis* (Fig. 61) in the USNM has label data consistent with the label data of *D. smilacis*, suggesting Ashmead (1896b) erroneously read '86x' as '864' and that the same gall that yielded the type

Figure 59–62. *Periclistus smilacis* **59** lateral habitus of lectotype **60** series of specimens and gall of lectotype **61** label of lectotype **62** dorsal habitus of lectotype.

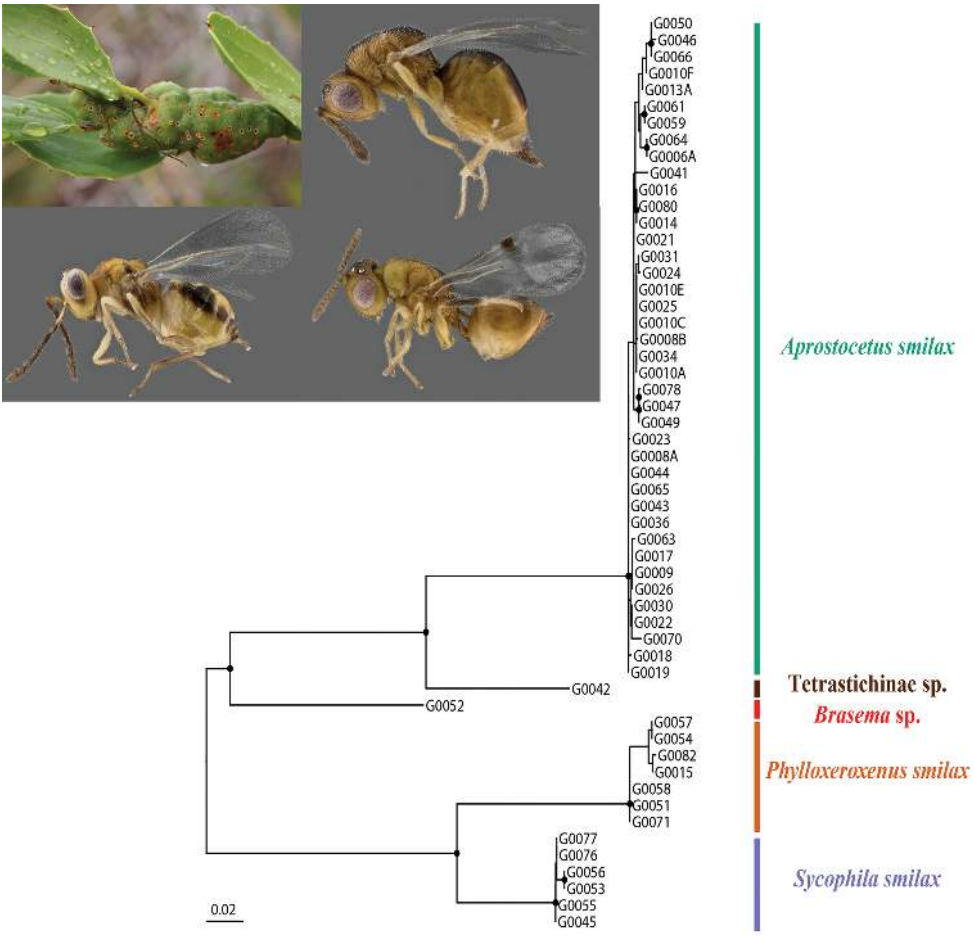

Figure 63. Concatenated *28S* and *COI* phylogram of the *Smilax* gall inhabitants estimated using Maximum Likelihood framework in IQ-TREE2. Black dots at the nodes indicate ≥90% ultrafast bootstrap support. Inset images in counterclockwise order: Stem gall induced by *Aprostocetus smilax* on *Smilax havanensis*, with emergence holes (photo by MWG); *A. smilax*, *Sycophila smilax*, *Phylloxeroxenus smilax*.

specimens of *D. smilacis* yielded the type specimens for *P. smilacis*; there is no date on the '86x' specimens and it is not clear how the collection date in Ashmead (1896b) was obtained. The four specimens labeled '1010' cannot be located and are presumed either lost or in another, unreported museum.

Adding to this confusing picture is that it appears A. Ritchie intended to include *P. smilacis* in his dissertation work on *Periclistus* in 1984, and even went so far as to designate a lectotype for this species (Fig. 61). The series of specimens seen in Fig. 60 is the source of the specimen that Ritchie intended as the lectotype, making the total number of specimens for *P. smilacis*, in the USNM, 11 specimens. When we consider *D. smilacis* is represented by two specimens (holotype in USNM, cotype in AMNH), we have

a grand total of 13 specimens. Our conclusion from all of this is that the original 13 specimens mentioned in Ashmead (1896a) for *D. smilacis* turned out to be a mixture of gall inducer and inquilline, and further, the host plant for this gall was mis-identified in the field as *Smilax rotundifolia* L. The US Forest Service Fire Effects Information System indicates *S. rotundifolia* and *Rubus* spp. co-occur in old fields throughout the range of *Smilax* and it is possible that the galls of *Diastrophus smilacis* are actually collected from a *Rubus*, and the two host plants were confused when the original collection was made.

The original collections made in Florida in 2010 that led to the chalcidoids described herein were also focused on the (now) erroneous records of *D. smilacis* on *Smilax havanensis* mentioned in Beutenmüller (1909) collected around Miami by Dr. E. Bessey. When looking closely at the *D. smilacis* gall figured in Beutenmüller (1909), it is clear that gall matches exactly what was collected in this project and illustrated in Fig. 1. No gall material from *S. havanensis* is in the cynipid gall collection, and indeed, there are no galls in this collection that look like the one figured in Beutenmüller (1909). As no cynipids apparently emerged from the Miami gall reported and figured in Beutenmüller (1909), we consider this an erroneous host record as well.

Lastly, the USNM has a specimen labeled as lectotype for *Periclistus smilacis*, yet this taxon lacks a published lectotype designation. We presume the team of Ritchie and Shorthouse, whose names appear on the purported lectotype labels, planned to publish these designations (as mentioned above), but were not able to. In order to stabilize the name of *Periclistus smilacis*, we hereby designate USNMENT00802336, type number 3287, as lectotype of this taxon, deposited in the USNM (Figs 59, 61, 62).

Conclusion

Here we describe the new eulophid species *Aprostocetus smilax*, the second recorded case of gall induction by *Aprostocetus* in North America. This new species is the true gall inducer on *Smilax*, and previous records of cynipid species *Diastrophus smilacis* and the inquiline *Periclistus smilacis* associated with this host plant are erroneous. Additionally, we described two eurytomid parasitoids associated with this *Smilax* gall. The distribution of all three new species is on the southern tip of mainland USA, but it is likely that they are also found in the Caribbean region in which the host plant *S. havanensis* is found (Ferrufino-Acosta 2014). A comprehensive taxonomic revision of these incredibly diverse but understudied minute wasps will undoubtedly reveal additional ecological associations and new species.

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staff from Miami-Dade County (C. Rodriguez and T. Smith) provided specimens and access to Rockdale Pineland for gall collecting. We would also like to thank Gary Oullette for performing all DNA extractions and amplifications, Taina Litwak for the illustration and image editing, and Zhiwei Liu for discussion on the validity of *Diastrophus smilacis*. Finally, we would like to thank Paul Hanson and an anonymous reviewer that have provided comments that improved the manuscript. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

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