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CALOPHYA LATIFORCEPS, A NEW SPECIES OF JUMPING PLANT LICE (HEMIPTERA: CALOPHYIDAE) ASSOCIATED WITH *SCHINUS TEREBINTHIFOLIUS* (ANACARDIACEAE) IN BRAZIL

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Abstract

Brazilian peppertree, *Schinus terebinthifolius* Raddi, is a perennial woody plant native to Brazil, Argentina, and Paraguay that is recognized as one of the most invasive weeds in Florida. A new species of leaflet galling psyllid was discovered attacking Brazilian peppertree in Bahia, Brazil in March 2010. In this paper, a formal morphological description of the new psyllid *Calophya latiforceps* **sp. nov.** (Hemiptera: Calophyidae) is presented along with molecular evidence confirming new species designation. This previously unknown natural enemy may have potential as a biological control agent for the Brazilian peppertree.

Key Words: *Calophya latiforceps* **sp. nov.**, natural enemy, Brazilian peppertree, leaflet gall former, weed biological control

Resumen

El pimentero brasileño (=Brazilian peppertree), *Schinus terebinthifolius* Raddi, es un arbusto perenne nativo de Brasil, Argentina y Paraguay, el cual es considerado una de las malezas más invasivas en Florida. Una nueva especie de psilido que forma agallas en las hojas fue descubierto atacando pimentero brasileño en Bahia, Brazil en Marzo del 2010. En este artículo se presenta la descripción morfológica de esta nueva especie de psilido *Calophya latiforceps* **sp. nov.** (Hemiptera: Calophyidae) además de estudios moleculares que confirman su designación como especie. Este enemigo natural previamente desconocido podría tener un potencial como agente de control biológico del pimentero brasileño.

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Sapindales: Anacardiaceae), a perennial woody plant native to Brazil, Argentina, and Paraguay (Barkley 1944, 1957), has become one of the most invasive weeds in Florida (Cuda et al. 2006). Originally introduced to Florida in the 1840s as an ornamental (Mack 1991), it escaped cultivation in the 1950s (Austin & Smith 1998), and invaded disturbed sites, natural communities, and environmentally sensitive areas such as the Everglades National Park (Ewel et al. 1982). In central and south Florida, Brazilian peppertree occupies more than 2,833 km^2 (Cuda et al. 2006), and is listed as a prohibited plant (FLDEP 1993), a noxious weed (FLDACS 2006), and a Category I invasive species (FLEPPC 2009).

Brazilian peppertree out competes native species by exhibiting fast growth, prolific seed production, and vigorous resprouting, and it tolerates a wide range of growing conditions including high salinity, moisture, and shade (Ewel 1979; Ewe and Sternberg 2005, 2007). This plant was recommended for classical biological control in Florida in the late 1970s because it is highly invasive, and there are no native *Schinus* spp. in North America (Barkley 1944, 1957; Kramer 1957; Delfosse 1979; Campbell et al. 1980). The lack of native congeners would minimize the risk of damage to non-target plants from introduced natural enemies (Pemberton 2000). Furthermore, biological control would provide a more cost effective and sustainable solution to the Brazilian peppertree problem when it is combined with conven-

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tional control methods (Cuda et al. 2006). Host specific natural enemies capable of selectively damaging Brazilian peppertree should be introduced to help control the plant (Bennett et al. 1990; McKay et al. 2009).

Several exploratory surveys for natural enemies of Brazilian peppertree have been conducted in South America (Krauss 1962, 1963; D'Araújo e Silva et al. 1968; Campbell et al. 1980; Bennett et al. 1990; Bennett & Habeck 1991; McKay et al. 2009; Cuda et al., unpubl.). One important natural enemy of Brazilian peppertree is the leaflet galling psyllid *Calophya terebinthifolii* Burckhardt & Basset (Hemiptera: Calophyidae), which is known to be widely distributed in Brazil, and has been found to be highly host specific (Burckhardt and Basset 2000, Barbieri 2004, Vitorino et al., submitted). *Calophya* Löw is a predominantly New World, Oriental and East Palaearctic genus of 59 described species which are mostly associated with Anacardiaceae and other Sapindales. Among the psyllids developing on *Schinus* there are 14 described *Calophya* species which all induce galls (Burckhardt & Basset 2000). These authors showed that several *Calophya* spp. are monophagous, i.e. restricted to a single host plant species (e.g. *C. rubra* (Blanchard) on *Schinus polygamus* (Cav.) Cabrera, *C. schini* Tuthill on *S. molle* L. or *C. therebinthifolii* on *S. therebinthifolius*, whereas others are oligophagous such as *Calophya mammifex* Burckhardt and Basset which induces nut-shaped galls on the leaves of *Schinus latifolius* (Gilles ex Lindl.) Engler, *S. polygamus* and *S. velutinus* (Turez.) Johnston. The *Calophya* spp. associated with *Schinus* are restricted to subtropical and temperate South America with the exception of *C. schini* which was introduced, along with its host, into North America, South Africa and New Zealand.

According to Hodkinson (1974), the larval feeding habits are detrimental to the plant whereas the damage by the adults is insignificant. Injury to the plant results from the injection of toxic saliva, which causes toxemia in the host. Psyllid damage may be localized and/or systemic and may manifest as necrosis, rolling and changes in leaf color, withering, and gall formation (Hodkinson 1974).

During a recent survey trip in March 2010, a new undescribed leaf galling psyllid in the genus *Calophya* was collected on Brazilian peppertree in northeastern Brazil (Salvador, Bahia). The description of this new species is presented here in addition to molecular analyses that compare this species to other closely related psyllids from Brazil.

Materials and Methods

Specimens were collected by V. Manrique, R. Diaz, and M. Vitorino beating the branches of Brazilian peppertrees and dislodging the psyllids from the foliage. Adults were aspirated from a collecting sheet and transferred to snap cap vials containing a fresh leaflet and filter paper. In addition, leaflets containing pit galls were collected and placed inside Ziploc® bags with a moist paper towel to keep them fresh. Samples were hand carried under permit to the University of Florida. The collecting sites are shown in Fig. 1.

Collecting data and type depositories of the type specimens described here are as follows. Comparative material of other *Calophya* species was examined from the collection of the Naturhistorisches Museum Basel.

Holotype Male, Brazil

Bahia, Feira de Santana, 23.III.2010, site #146, -12.32705 lat., -38.86293 long., (V. Manrique, R. Diaz, and M. Vitorino) (dry mounted, depository, Naturhistorisches Museum Basel). Paratypes. Brazil: 3 males, 8 females, 18 larvae, same data as holotype (slide mounted and in 70% ethanol).

The morphological terminology follows Ossiannilsson (1992), Burckhardt & Basset (2000) and Yang et al. (2009). Images of the adult female and fifth larval instar collected in Salvador were obtained using Automontage® software (Figs. 2 and 3). Photos of morphological details were taken with a Leica MZ12 stereo microscope and Leica DMLB compound microscope fitted with a Leica DFC320 digital camera and then further treated with the software CombineZM 1.0 (freely available from the web) (Figs. 4-18 and 28-42). The drawings where made from permanent mounts in Canada balsam of dissected specimens beforehand cleared in KOH and washed in H_2O using a drawing tube mounted on the Leica DMLB compound microscope (Figs. 19-27). Measurements were taken from slide mounted specimens (1 male, 2 females, 3 larvae) with a Leica MZ12 stereo microscope.

Twenty individuals of *C. terebinthifolii* collected in Curitiba, Paraná and Santa Catarina and 4 individuals of *C. latiforceps* collected in Salvador, Bahia were genetically characterized at the mitochondrial cytochrome oxidase I (COI) gene. Psyllids were crushed with a pestle in 100 µl of a 5% chelex solution with 5 µl Proteinase K (20 mg/ml) and incubated overnight at 55°C. The samples were then heated to 95°C for 15 min before amplification. A portion of COI was first amplified using primers *LCO1490* and *HCO2198* (Folmer et al. 1994) in a few individuals. To obtain more reliable amplifications, primers were redesigned specifically for *C. terebinthifolii*: *PsyCOIF* 5'-CGATCTGTTAAAAGTATTGTGATAGCC-3' and *PsyCOIR* 5'-TCCGAATGGAACTCAGACAA-3'. These were then used to amplify both *C. terebinthifolii* and *C. latiforceps*. Amplifications were performed

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Fig. 1. Location of sampling sites (indicated by squares) of *Calophya latiforceps* **sp. nov.** in Bahia, Brazil and number of psyllids collected at each site.

with 2 µl of the chelex solution, 1.5 mM $MgCl_2$, 1X PCR buffer, 0.2 U of Taq polymerase (Promega), 5 pmols of each primer, 2X BSA, and 0.2 mM dNTPs. Samples were amplified using the following PCR profile: 94°C for 2 min then 30 cycles of 94°C for 15 s, 50°C for 15 s, 72°C for 1 min, then a final extension at 72°C for 5 min. PCR products were enzymatically cleaned using *ExoI* and *Antarctic Phosphatase* (New England Biolabs) according to manufacturer protocols. PCR products were then sequenced in both forward and reverse directions using BigDye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems). Sequences were electrophoresed on an ABI 3130 Genetic Analyzer, trimmed and contiged using Sequencher v. 4.8, and aligned using Clustal W. The Kimura 2-parameter distance measure (Kimura 1980) was calculated between all unique haplotypes using MEGA v.4.1 (Kumar et al. 2004).

Results

Description of *Calophya latiforceps* ***sp. nov.*** Burckhardt

Adult Coloration (Fig. 2). Body dorsally yellow, ventrally light yellow. Eyes greyish, antennal flagellum dirty yellowish basally gradually getting

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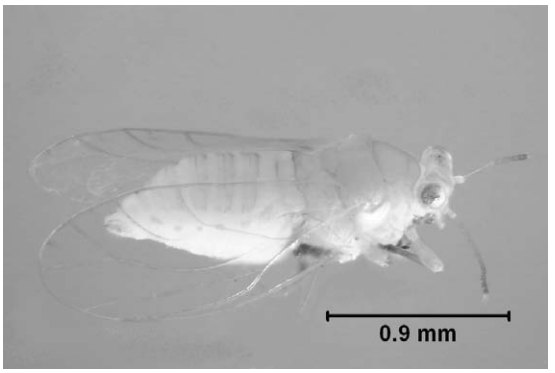


Fig. 2. *Calophya latiforceps* **sp. nov.**, adult female, lateral view.

darker towards apex. Tibiae and tarsi greyish brown. Forewing with yellow or light brown veins and greyish radular areas, membrane colorless, transparent.

Structure. Anterior portion of vertex covered in very short, inconspicuous setae; genal processes long, slender, pointed apically, contiguous in the middle (Fig. 7). Forewing (Fig. 4) oblong-oval, widest in the middle, rounded apically; surface spinules present in all cells, leaving broad, spinule-free stripes along the veins, absent from basal three quarters of cell c+sc and basal two thirds of cell r_1. Terminalia as in Figs. 10-12 and 19-21. Male subgenital plate short, subglobular. Male proctiger moderately slender. Paramere much shorter than proctiger, subrectangular, with short digitiform antero-apical strongly sclerotized process, and broad postero-apical strongly sclerotized tooth; covered in long setae in distal

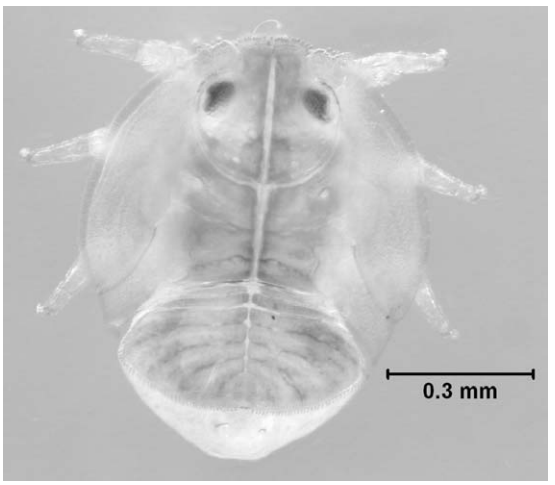
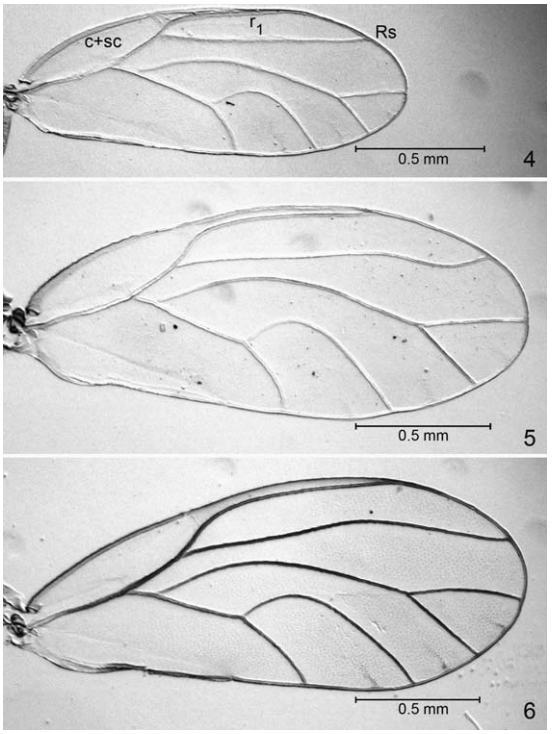


Fig. 3. *Calophya latiforceps* **sp. nov.**, fifth instar, dorsal view.



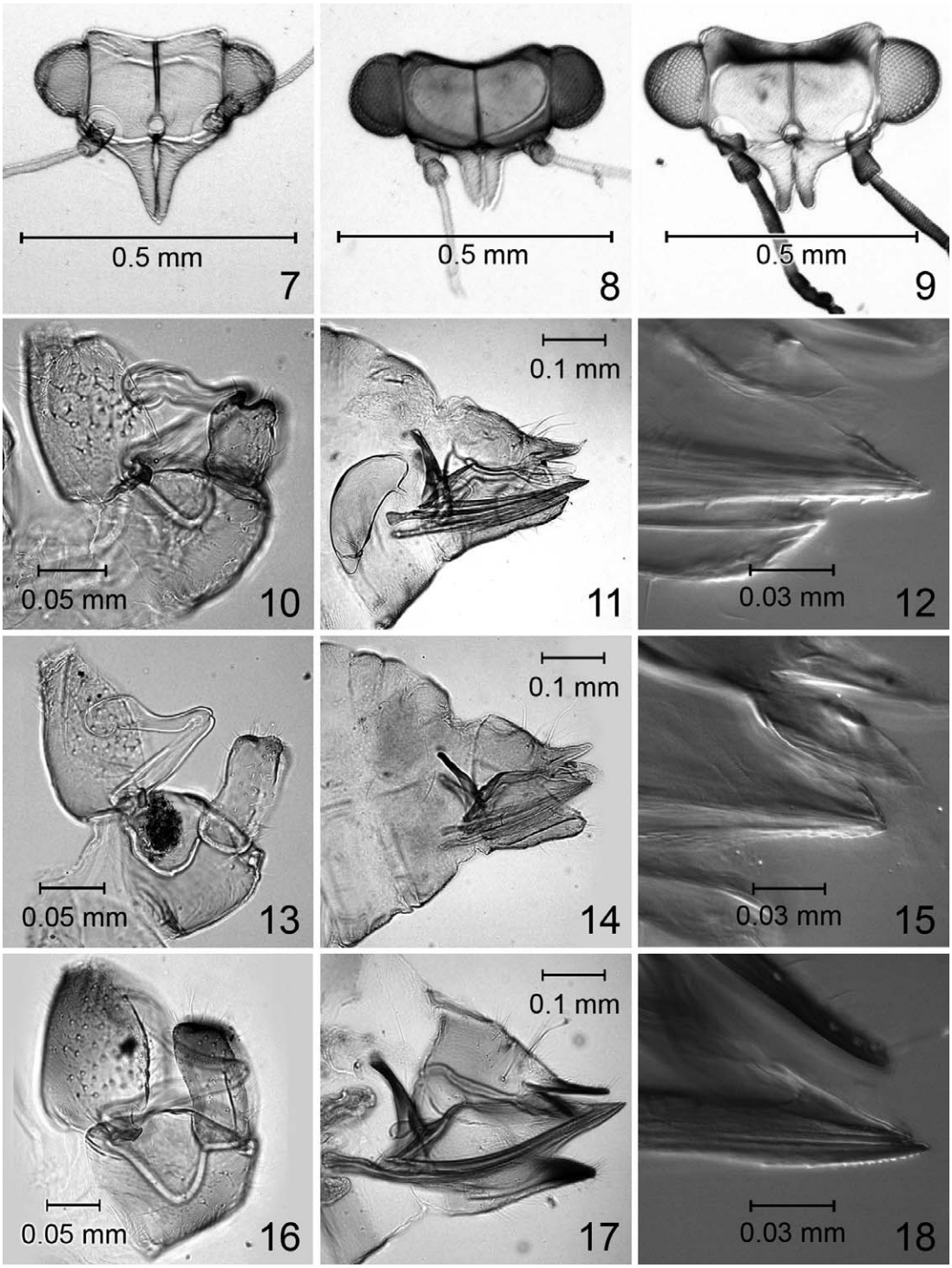
Figs. 4-6. 4. *Calophya latiforceps* **sp.nov.**, forewing; 5. *Calophya terebinthifolii*, forewing; 6. *Calophya schini*, forewing.

and posterior half on the outer face, and more or less evenly on the entire surface on the inner surface. Distal portion of aedeagus short with irregularly inflated apical half; sclerotized end tube of ductus ejaculatorius short, S-shaped. Female proctiger short, cuneate in profile, dorsal margin weakly sinuous. Subgenital plate elongate, truncate apically. Valvula ventralis coarsely serrate ventrally. Measurements in mm (1 male, 2 females). Head width 0.44; antenna length 0.46-0.50; forewing length 1.40-1.50; male proctiger length 0.12; female proctiger length 0.22-0.26.

Fifth Instar Larva (Figs. 3 and 28). Coloration. Head, thorax and body light brown with irregular grey infuscation. Eyes brown. Membranes yellowish. Ventral body surface and legs white.

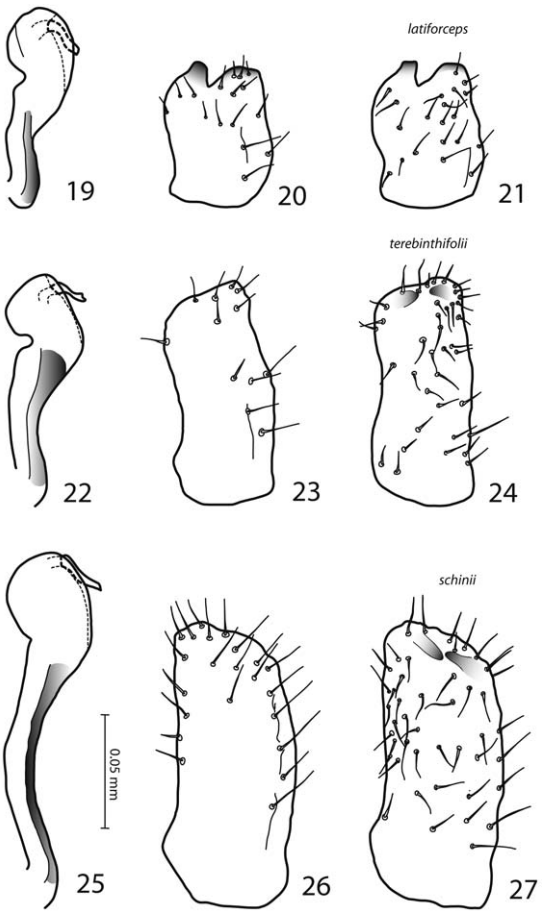
Anterior margin of head forming 2 large lobes. Antenna (Fig. 29) with large irregular base and digitiform apex; flagellum with 1-3 diamond-shaped setae and 3 rhinaria. Tarsi with small claws about as long as arolium which is almost circular (Fig. 31). Anterior margin of humeral lobes ending distal to anterior eye margin, subacute. Abdominal dorsum without median row of horns. Caudal plate ratio 0.33-42. Circumanal ring near posterior abdominal margin, oval, consisting of a single row of oval pores; on each side

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Figs. 7-18. 7. *Calophya latiforceps* **sp. nov.**, genal processes, dorsal view; 8. *Calophya terebinthifolii*, genal processes, dorsal view; 9. *Calophya schini*, genal processes, dorsal view; 10. *Calophya latiforceps* **sp. nov.**, paramere, lateral view; 11. *Calophya latiforceps* **sp. nov.**, female proctiger, lateral view; 12. *Calophya latiforceps* **sp. nov.**, valvula 2, lateral view; 13. *Calophya terebinthifolii*, paramere, lateral view; 14. *Calophya terebinthifolii*, female proctiger, lateral view; 15. *Calophya terebinthifolii*, valvula 2, lateral view; 16. *Calophya schini*, paramere, lateral view; 17. *Calophya schini*, female proctiger, lateral view; 18. *Calophya schini*, valvula 2, lateral view.

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Figs. 19-27. 19. *Calophya latiforceps* **sp. nov.**, aedeagus, lateral view; 20. *Calophya latiforceps* **sp. nov.**, paramere, lateral view; 21. *Calophya latiforceps* **sp. nov.**, paramere, lateral view; 22. *Calophya terebinthifolii*, aedeagus, lateral view; 23. *Calophya terebinthifolii*, paramere, lateral view; 24. *Calophya terebinthifolii*, paramere, lateral view; 25. *Calophya schini*, aedeagus, lateral view; 26. *Calophya schini*, paramere, lateral view; 27. *Calophya schini*, paramere, lateral view.

of circumanal ring with 1 very long simple seta and with a pair of short normal setae between posterior margins of circumanal ring and caudal plate. Marginal setae as follows: head (Fig. 29) with densely spaced short diamond-shaped setae, forewing bud (Fig. 30) with small widely spaced stiff setae, and caudal plate (Fig. 32) densely spaced short diamond-shaped setae.Measurements in mm (3 larvae). Body length 0.82-0.82; body width 0.78-0.80.

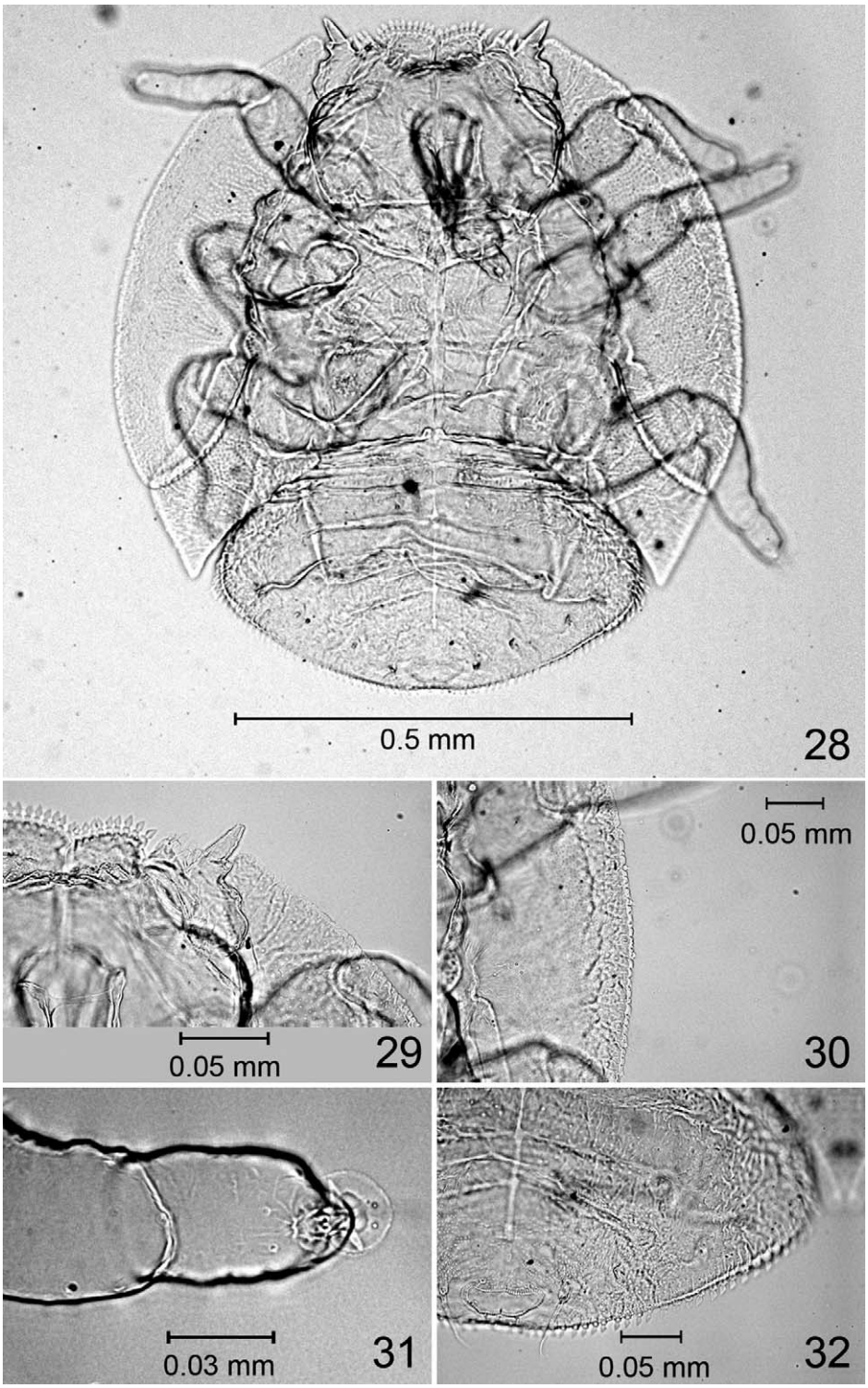
Etymology. From Latin latus = broad, and forceps = paramere, referring to the broad paramere of *Calophya latiforceps* compared to that of the closely related *C. schini* and *terebinthifolii*.

Comparison of *C. latiforceps* **sp. nov.** to other species

Morphological Differences. *Calophya latiforceps* is a member of the *Calophya hermicitae* group based on the short female proctiger and the larval antenna which bears a broad base with a slender apical process (Burckhardt & Basset 2000), even though it lacks the strongly contrasted yellow and black body color. It is probably closely related to *C. terebinthifolii*, which develops on the same host plant species. Using the key of Burckhardt & Basset (2000) *C. latiforceps* keys out with *C. schini*, which is associated with *Schinus molle* L. *C. latiforceps* differs from *C. terebinthifolii* and *C. schini* in the smaller body dimensions and the light body color. The forewing shape in *C. latiforceps* (Fig. 4) is oval, as in *C. terebinthifolii* (Fig. 5), but not as in *C. schini* (Fig. 5) where it is ovoid; vein Rs is relatively straight in *C. latiforceps* but sinuate in the other two. The genal processes are long, very slender, subacute and contiguous in the middle in *C. latiforceps* (Fig. 7), slightly shorter in *C. terebinthifolii* (Fig. 8) and apically weakly diverging in *C. schini* (Fig. 9). The paramere in *C. latiforceps* (Figs. 10, 20, 21) is shorter than in the other 2 species (*C. terebinthifolii*: Figs. 13, 23, 24; *C. schini*: Figs. 16, 26, 27) as is the distal portion of the aedeagus (*C. latiforceps*: Fig. 19; *C. terebinthifolii*: Fig. 22; *C. schini*: Fig. 25). The female proctiger is short in *C. latiforceps* and *C. terebinthifolii* but long in *C. schini* which has also a subacute, apically strongly sclerotized subgenital plate contrary to the other 2 species where it is truncate and weakly sclerotized apically (*C. latiforceps*: Fig. 11; *C. terebinthifolii*: Fig. 14; *C. schini*: Fig. 17). The ventral margin of valvula 2 is coarsely serrate in *C. latiforceps* and *C. terebinthifolii* but finely serrate in *C. schini* (*C. latiforceps*: Fig. 12; *C. terebinthifolii*: Fig. 15; *C. schini*: Fig. 18). The last instar larva of *C. latiforceps* (Figs. 28-32) is characterized by the antennal shape and the very short marginal diamond-shaped setae on head and caudal plate. In *C. schini* (Figs. 38-42) marginal diamond-shaped setae are also developed on the forewing pad. *C. latiforceps* larvae differ from *C. terebinthifolii* (Figs. 33-37) in the very light color of the dorsal body surface.

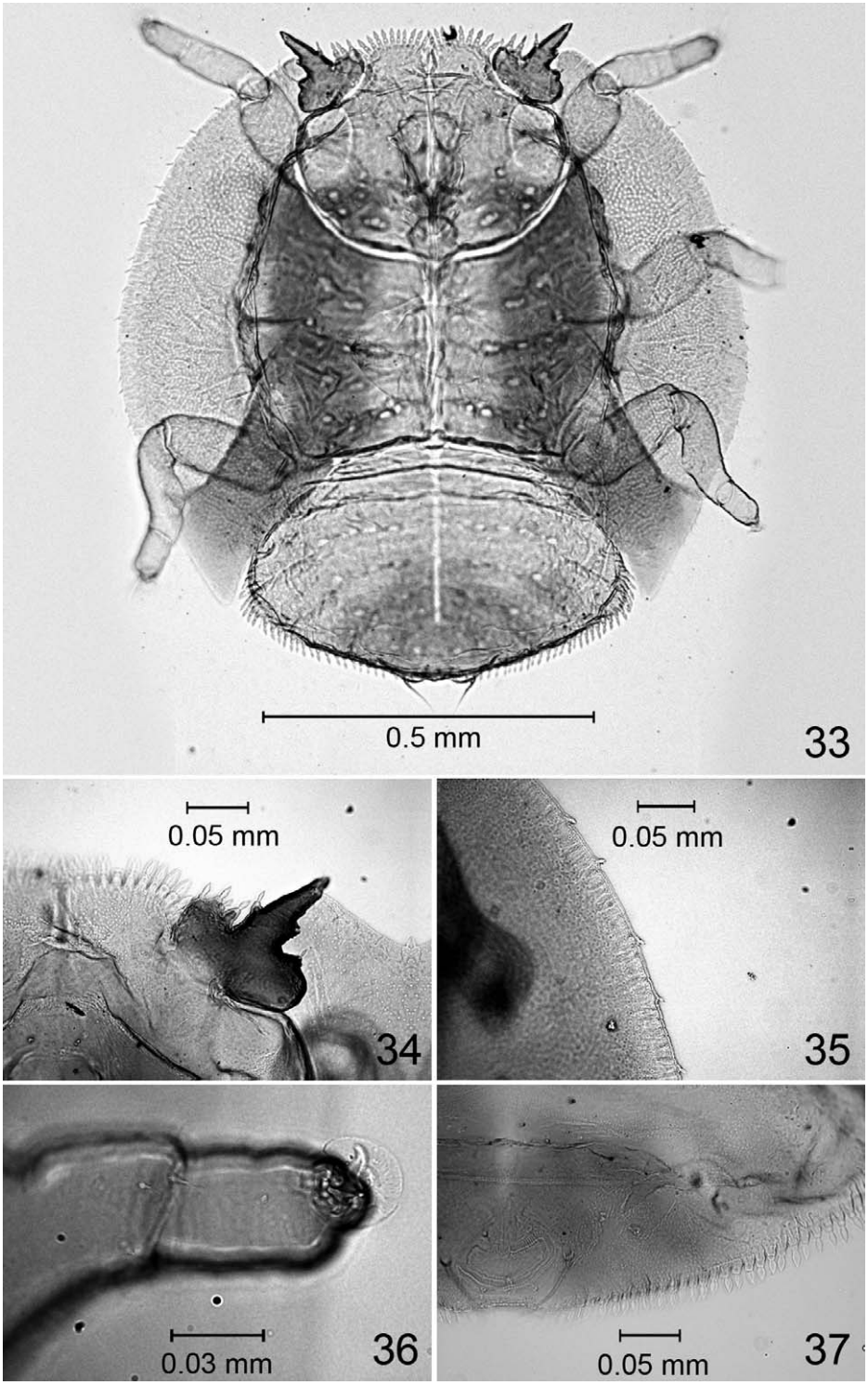
Molecular Differences. We sequenced 461 bp for each individual. The sequences did not contain indels or stop codons suggesting they were of mitochondrial origin and not nuclear pseudogenes. There was 0% sequence divergence between *C. terebinthifolii* samples collected in Curitiba and Santa Catarina and only 0.2-0.7% divergence between psyllids from Salvador. In contrast, a 13.2-13.5% sequence divergence was observed between the Salvador psyllids (*C. latiforceps*) and the psyllids from southern Brazil (*C. terebinthifolii*) supporting the designation of these psyllids as separate species.

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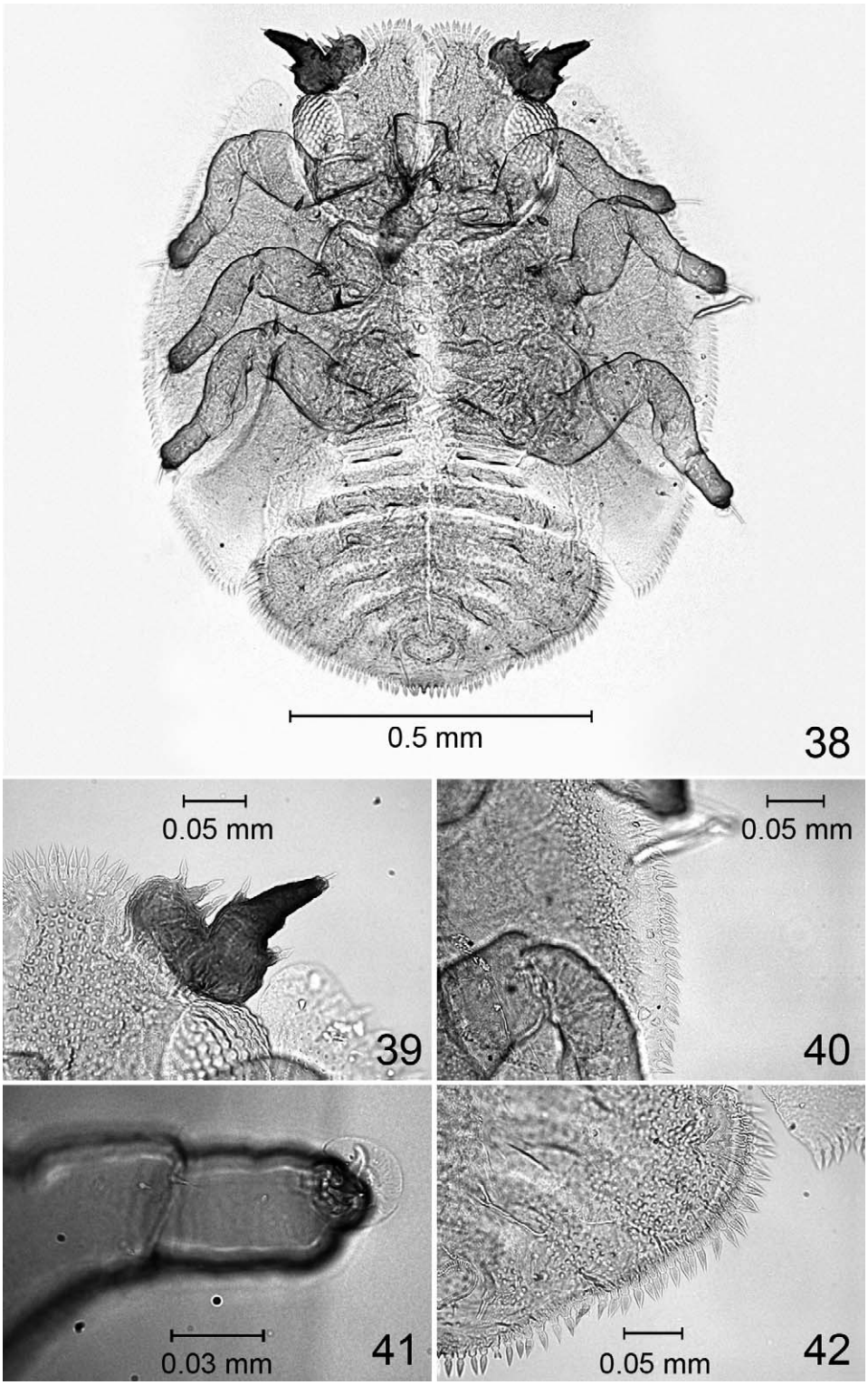
Figs. 28-32. 28. *Calophya latiforceps* **sp. nov.**, fifth instar, dorsal view; 29. *Calophya latiforceps* **sp. nov.**, fifth instar antenna and marginal setae, dorsal view; 30. *Calophya latiforceps* **sp. nov.**, fifth instar forewing bud and marginal setae, dorsal view; 31. *Calophya latiforceps* **sp. nov.**, fifth instar tarsus, dorsal view; 32. *Calophya latiforceps* **sp. nov.**, fifth instar caudal plate and marginal setae, dorsal view.

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Figs. 33-37. 33. *Calophya terebinthifolii*, fifth instar, dorsal view; 34. *Calophya terebinthifolii*, fifth instar antenna and marginal setae, dorsal view; 35. *Calophya terebinthifolii*, fifth instar forewing bud and marginal setae, dorsal view; 36. *Calophya terebinthifolii*, fifth instar tarsus, dorsal view; 37. *Calophya terebinthifolii*, fifth instar caudal plate and marginal setae, dorsal view.

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Figs. 38-42. 38. *Calophya schini*, fifth instar, dorsal view; 39. *Calophya schini*, fifth instar antenna and marginal setae, dorsal view; 40. *Calophya schini*, fifth instar forewing bud and marginal setae, dorsal view; 41. *Calophya schini*, fifth instar tarsus, dorsal view; 42. *Calophya schini*, fifth instar caudal plate and marginal setae, dorsal view.

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Discussion

A new potential biological control agent of Brazilian peppertree is described in this paper. *Calophya latiforceps* is part of the *Calophya hermicitae* group and is closely related to *C. terebinthifolii*. It also resembles morphologically *C. schini*. It differs from both species in the smaller body dimensions and the light body color in the adult, as well as, in the male, in the short and broad paramere and the relatively short distal portion of the aedeagus. The female terminalia are similar in *C. latiforceps* and *terebinthifolii* but differ from those of *C. schini* in the shorter proctiger and the apically truncate subgenital plate as well as the coarsely serrate ventral margin of valvula 2. The last instar larva of *C. latiforceps* is characterized by the characteristic antennal shape and the very short marginal diamond-shaped setae on head and caudal plate. According to molecular analyses, 13.4% sequence divergence was observed between *C. latiforceps* and *C. terebinthifolii*.

A complex of leaflet galling psyllids (*Calophya terebinthifolii* and the newly discovered *C. latiforceps*) may be good candidates for biological control of Brazilian peppertree. The rationale for using *Calophya* spp. as biological control agents is based on a similar, although unintentional, introduction of the closely related *C. schini* into California (Downer et al. 1988) which produces a similar type of leaflet damage (pit galls) on Peruvian or California Peppertree, *Schinus molle*. Downer et al. (1988) reported that *C. schini* attacked only Peruvian peppertree in California, causing extensive damage to these popular ornamental plants. Without endemic natural enemies to control the psyllid during the initial outbreaks, the pitting and larval development by *C. schini* on infested trees caused extensive defoliation in California (Downer et al. 1988). Assuming the Brazilian peppertree complex of *Calophya* spp. would respond similarly if they were introduced into Florida, they could contribute to the biological control of Brazilian peppertree. Further research on the biologies and host preferences of these 2 psyllids will determine if they are sufficiently host specific to release in Florida.

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