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# Complex of primary and secondary parasitoids (Hymenoptera: Encyrtidae and Signiphoridae) of *Hypogeococcus* spp. mealybugs (Hemiptera: Pseudococcidae) in the New World

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## Abstract

Parasitoids, both primary and secondary (hyperparasitoids), of *Hypogeococcus* spp. mealybugs (Hemiptera: Pseudococcidae) are reviewed to report results of the surveys in the New World conducted during 2009 to 2017 for prospective natural enemies of the *Harrisia* cactus mealybug, *Hypogeococcus* sp., which is devastating native cacti in Puerto Rico and threatening cacti in the adjacent Caribbean islands. Five species of Encyrtidae (Hymenoptera: Chalcidoidea) are recorded as primary parasitoids of *Hypogeococcus* spp., including the newly described *Leptomastidea hypogeococci* Triapitsyn **sp. n.**, which is the only species of the genus *Leptomastidea* García Mercet in the New World where the clava of the female antenna is contrastingly white. Genetic analysis of the individuals of *L. hypogeococci* from Argentina, Brazil, and Puerto Rico (USA) corroborates the morphological data that the same species occurs in South America, the Caribbean islands, and Florida (USA). A key to the New World species of *Leptomastidea* is given and taxonomic notes are provided on its other known species in the Neotropical region. *Leptomastidea antillicola* Dozier, **syn. n.** from Puerto Rico is synonymized under *L. abnormis* (Girault). Based on the presented molecular data, *Anagyrus ciomperliki* Triapitsyn **syn. n.** (Encyrtidae), originally described from Puerto Rico, is synonymized under *A. quilmes* Triapitsyn, Logarzo & Aguirre, where the known distributional range is expanded to also include Brazil. *Anagyrus cachamai* Triapitsyn, Logarzo & Aguirre, *A. lapachosus* Triapitsyn, Aguirre & Logarzo, and *A. quilmes* are newly recorded from Paraguay. The previously unknown male of *Prochiloneurus argentinensis* (De Santis) (Encyrtidae) is described from Misiones Province of Argentina, and that of *P. narendrani* Noyes & Triapitsyn is described from Mona Island, Puerto Rico. So far, *Anagyrus cachamai* and *A. lapachosus* are considered to be the primary target species for introduction from Argentina and Paraguay into Puerto Rico for the biological control of *Harrisia* cactus mealybug. The holotype of *Anagyrus tanystis* De Santis from Buenos Aires, Argentina, host associations are unknown, and is illustrated to facilitate its recognition from other congeneric species.

Key Words: Chalcidoidea; *Anagyrus*; *Leptomastidea*; new species; synonymy; biological control

## Resumen

Se informan los resultados de los relevamientos de los parasitoides primarios y secundarios (hiperparasitoides) de *Hypogeococcus* spp. (Hemiptera: Pseudococcidae) realizados en el Nuevo Mundo durante el período 2009 para 2017 para obtener enemigos naturales de la cochinilla harinosa de los cactus (*Harrisia* cactus mealybug) *Hypogeococcus* sp., que está devastando cactus nativos en Puerto Rico y amenaza a los cactus presentes en Islas del Caribe adyacentes. Se registraron cinco especies de Encyrtidae (Hymenoptera: Chalcidoidea) como parasitoides primarios de *Hypogeococcus* spp., incluyendo el recientemente descrito *Leptomastidea hypogeococci* Triapitsyn **sp. n.**, que es la única especie del género *Leptomastidea* García Mercet en el Nuevo Mundo cuya clava de la antena de la hembra es contrastantemente blanca. El análisis genético de los individuos de *L. hypogeococci* de Argentina, Brasil y Puerto Rico (EE. UU.) corrobora los datos morfológicos de que la misma especie se encuentra en América del Sur, las islas del Caribe y Florida (EE. UU.). Se proporciona una clave para las especies del Nuevo Mundo de *Leptomastidea*. *Leptomastidea antillicola* Dozier, **syn. n.** de Puerto Rico es sinonimizado bajo *L. abnormis* (Girault). Basado en los datos moleculares presentados, *Anagyrus ciomperliki* Triapitsyn **syn. n.** (Encyrtidae), originalmente descrito de Puerto Rico, es sinonimizado bajo *A. quilmes* Triapitsyn, Logarzo & Aguirre, cuyo rango de distribución conocido también se amplía para incluir a Brasil. *Anagyrus cachamai* Triapitsyn, Logarzo y Aguirre, *A. lapachosus* Triapitsyn, Aguirre y Logarzo y *A. quilmes* se registraron recientemente en Paraguay. Se describe el macho previamente desconocido de *Prochiloneurus argentinensis* (De Santis) (Encyrtidae) de la provincia de Misiones de Argentina, y el de *P. narendrani* Noyes & Triapitsyn de la Isla de Mona, Puerto Rico. Hasta aquí, *Anagyrus cachamai* y *A. lapachosus* se consideran como las principales especies para la introducción desde Argentina y Paraguay a Puerto Rico para el control biológico de la cochinilla harinosa de los cactus. El holotipo de *Anagyrus tanystis* De Santis de Buenos Aires, Argentina, cuyos hospederos asociados son desconocidos, se ilustra para facilitar su reconocimiento de otras especies congénicas.

Palabras Clave: Chalcidoidea; *Anagyrus*; *Leptomastidea*; especie nueva; sinonimia; control biológico

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The mealybug *Hypogeococcus* sp. (Hemiptera: Pseudococcidae) has become a serious pest of native and endemic cacti in Puerto Rico (USA) (Pérez Sandi y Cuen et al. 2006; Jenkins et al. 2014; Carrera-Martínez et al. 2015) since it was first intercepted on *Portulaca oleracea* (Portulacaceae) ornamentals in San Juan in 2000 and then sighted on cacti in the Guánica State Forest and Biosphere Reserve in 2005 (Segarra-Carmona et al. 2010; Zimmermann et al. 2010). The mealybug pest in Puerto Rico was reported as *Hypogeococcus pungens* Granara de Willink (Hemiptera: Pseudococcidae) (Segarra-Carmona et al. 2010; Zimmermann et al. 2010), the successful biological control agent released for introduced and invasive cactus in Australia and South Africa (McFadyen & Tomley 1978, 1981; White & Donnelly 1993; Paterson et al. 2011). *Hypogeococcus pungens* often is referred to as the Harrisia cactus mealybug due to the successful control of the initial primary target cactus weed in Australia, *Harrisia martinii* (Cactaceae). In Puerto Rico, the Harrisia cactus mealybug impacts 7 of 14 native cactus species occurring in dry forests, including 3 endemic and 2 endangered species in the subfamily Cactoideae of the family Cactaceae, by interfering with sexual reproduction and causing large tissue deformations (Fig. 1) that often leads to mortality (Carrera-Martínez et al. 2015). If the Harrisia cactus mealybug pest reported in Puerto Rico spreads beyond its current distribution range, species of cactus throughout the Caribbean region and North America, including Mexico, are at risk of being negatively affected by the insect (Pérez Sandi y Cuen et al. 2006).

The taxonomic identity of the Harrisia cactus mealybug is under question (Aguirre et al. 2016). As a biological control agent, the Harrisia cactus mealybug was collected originally on species of Cactoideae from western Argentina to central Paraguay and identified as *Hypogeococcus festerianus* (Lizer y Trelles) (Hemiptera: Pseudococcidae) (McFadyen & Tomley 1978, 1981). Based on morphological

characters of adult female mealybugs, the Harrisia cactus mealybug's identity was changed to *H. pungens*, a newly described species from Argentina; however, it was collected from a non-cactus host plant, *Alternanthera pungens* (Amaranthaceae) (Granara de Willink 1981). The host plants used by the Harrisia cactus mealybug introduced as a biological control agent into Australia and South Africa consist only of species in Cactoideae (Zimmermann & Pérez Sandi y Cuen 2010; McFadyen 2012; Aguirre et al. 2016). In addition to these 2 countries, the distribution of *H. pungens* has expanded, due to inadvertent introductions from its apparently native South America, to locations in Italy, Spain, US Virgin Islands, Barbados, the Dominican Republic, Puerto Rico, and the USA states of Florida, California, and Hawaii (Hosking et al. 1988; Halbert 1996; Mazzeo et al. 2008; Hodges & Hodges 2009; Zimmermann & Pérez Sandi y Cuen 2010; Beltrà & Soto 2011; German-Ramirez et al. 2014). The host range of the Harrisia cactus mealybug identified as *H. pungens* includes species of plants in the families Cactaceae, Amaranthaceae, and Portulacaceae (Ben-Dov 1994; Claps & de Haro 2001; Hodges & Hodges 2009). Based on biological and molecular genetics studies conducted in Argentina on *H. pungens* populations derived from Amaranthaceae and Cactaceae, it appears that *H. pungens* is a species complex (Aguirre et al. 2016). In general, Aguirre et al. (2016) propose the following: (1) the species introduced to Australia is an undescribed species, but is the same species that attacks Cactaceae in northern Argentina; (2) the species using Amaranthaceae in Argentina is *H. pungens* sensu stricto (s. str.), but it does not attack Cactaceae in the laboratory or the field; and (3) the Puerto Rico pest Harrisia cactus mealybug species appears to be an evolutionary lineage independent from *H. pungens* s. str.

A classical biological control program was initiated in Puerto Rico to search for natural enemies of the Harrisia cactus mealybug in what was



Fig. 1. A gall-like formation caused by the feeding of Harrisia cactus mealybug, *Hypogeococcus* sp., on *Stenocereus fimbriatus* columnar cactus in Caja de Muertos Island, Puerto Rico.

thought to be its South American native range. However, due to the revelation that the *Harrisia* cactus mealybug appears to be a species complex, the natural enemy selected to manage the Puerto Rico *Harrisia* cactus mealybug depends on the correct identification of the pest: a standard classical biological control program would be initiated when the pest can be identified and its origin known; and a new association biological control program would be initiated when the origin of the pest is unknown or the pest cannot be identified to species. Additional biological, taxonomic, and molecular studies are being conducted (currently in progress in collaboration with mealybug taxonomists and molecular biologists in Argentina) with populations of *H. pungens* on different host plants and from different locations, including Argentina, Paraguay, Brazil, Australia, Florida, and Caribbean Islands, to clarify the identities of *H. pungens* complex components, in particular the pest species of Puerto Rico. In addition, natural enemies, particularly parasitoids associated with the mealybugs, were reared from collections for identification of possible agents for biological control of the *Harrisia* cactus mealybug in Puerto Rico.

Several species of predators as well as 2 primary and 2 secondary parasitoid species, all belonging to the family Encyrtidae (Hymenoptera), were reared from the *Harrisia* cactus mealybug since 2009 when a brief survey of the *Harrisia* cactus mealybug was conducted in Puerto Rico and Barbados (M. A. Ciomperlik, unpublished data). One of the primary parasitoids, an undescribed species of *Leptomastidea* García Mercet (Encyrtidae), was collected from both Puerto Rico and Barbados; also, an undescribed species of *Anagyrus* Howard (Encyrtidae) was collected from Puerto Rico, and was subsequently described by Triapitsyn (2016) as *Anagyrus ciomperliki* Triapitsyn. Also, Segarra-Carmona et al. (2010) reported 4 species of predators associated with the *Harrisia* cactus mealybug on *Pilosocereus royenii* (Cactaceae) in Puerto Rico.

Since 2010, surveys for natural enemies of *H. pungens* and other congeneric mealybug species have been conducted in South America, the putative origin of the pest. Also, in Puerto Rico, persistent collections of parasitoids of the *Harrisia* cactus mealybug have been conducted since 2012. As a result of these surveys, several species of parasitoid wasps have been identified as natural enemies of the *Harrisia* cactus mealybug. Five primary and 3 secondary parasitoids in 2 families of Chalcidoidea (Hymenoptera), Encyrtidae and Signiphoridae, were identified, and 5 were described as new species (Triapitsyn et al. 2014a, 2014b, 2016; Triapitsyn 2016; Noyes & Triapitsyn 2018).

Here we present a review of the results of our surveys of the primary and secondary parasitoids of *Hypogeococcus* spp. mealybugs in the New World (i.e., Argentina, Barbados, Brazil, Puerto Rico, Paraguay, and USA [Florida]) conducted during 2009 to 2017. We also describe an additional primary parasitoid of *Hypogeococcus* spp. and present data and results of other, relevant taxonomic and genetic studies.

## Materials and Methods

### COLLECTING AND REARING OF MEALYBUGS AND THEIR PARASITIDS

Adult wasps emerged from mummies of the adult mealybugs of *Hypogeococcus* spp. infesting various Amaranthaceae, Cactaceae, and Portulacaceae in Argentina, Barbados, Brazil, Florida (USA), Paraguay, and Puerto Rico.

In Puerto Rico, gall-like malformations were collected from the localities of Guánica and Cabo Rojo monthly, and *Harrisia* cactus mealybug mummies were collected and placed in gelatin capsules for emer-

gence of parasitoids. In addition, galls also were placed in Berlese's funnels for collection of potential natural enemies. *Leptomastidea* sp., described here as a new taxon, was in all cases the most abundant parasitoid species associated with the Puerto Rican *Harrisia* cactus mealybug. Colonies of *Harrisia* cactus mealybug were obtained and established on *Alternanthera* sp. (Amaranthaceae), specific varieties of *Portulaca* spp. (Portulacaceae) (J. C. V. Rodrigues, unpublished data), and on *Leptocereus quadricostatus* (Cactaceae) (La Quay-Velázquez et al. 2015). *Harrisia* cactus mealybug colonies then were used for rearing the *Leptomastidea* sp. parasitoid for several generations in the laboratory at the University of Puerto Rico.

### TAXONOMIC STUDIES

Terms used for morphological features are those of Gibson (1997). Abbreviations used are the following: F = antennal funicle segment; mps = multiporous plate sensillum or sensilla on the antennal flagellar segments (= longitudinal sensillum or sensilla or sensory ridge(s) of other authors).

Most specimens were dried from ethanol using a critical point drier, then point-mounted and labeled. Selected specimens were dissected further and slide-mounted in Canada balsam. Slide mounts were examined under a Zeiss Axioskop 2 plus compound microscope (Carl Zeiss Microscopy, Thornwood, New York, New York, USA) and photographed using the Auto-Montage® system (Syncroscopy, Princeton, New Jersey, USA). Photographs were retouched where necessary using Adobe Photoshop® (Adobe Systems, Inc., San Jose, California, USA). Acronyms for depositories of specimens are: The Natural History Museum, London, England, UK [BMNH]; Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina [MACN]; Museum of Entomology and Tropical Biodiversity, University of Puerto Rico, Río Piedras, San Juan, Puerto Rico, USA [METB]; Museo de La Plata, La Plata, Buenos Aires, Argentina [MLPA]; Entomology Research Museum, University of California at Riverside, California, USA [UCRC]; and National Museum of Natural History, Washington, District of Columbia, USA [USNM].

### DNA EXTRACTION AND AMPLIFICATION

DNA was extracted from individual wasps using the "HotSHOT" method of Truett et al. (2000), in a total volume of 80 µL. This non-destructive method allowed for the recovery and slide-mounting of each specimen following extraction; each slide was then labeled with the assigned P. F. Rugman-Jones' primary molecular voucher PR number and UCRC database UCRC ENT number. The polymerase chain reaction (PCR) was used to amplify a section of the D2 domain of 28S nuclear ribosomal RNA (rRNA) using the primers 28sF3633 (5'-TACC-GTGAGGGAAAGTTGAAA-3') and 28sR4076 (5'-AGACTCCTGGTCCGT-GTTT-3') as described in Rugman-Jones et al. (2010). A second rRNA locus, the internal transcribed spacer 2 (ITS2), also was amplified using the primers 58SF (5'-GTGAACTGCAGGACACATGAAC-3'; Porter & Collins 1991) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al. 1990) as described in Morse et al. (2016). Finally, the "barcoding" region of the mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified using LCO1490 (5'-GGTCAACAATCATAAAGATATTGG-3') and HCO2198 (5'-TAACTTCAGGGTGACCAAAAAATCA-3'; Folmer et al. 1994) as described in Rugman-Jones et al. (2012). All reactions were performed in 25 µL volumes on a Mastercycler® ep gradient S thermocycler (Eppendorf North America Inc., New York, New York, USA). Amplification was confirmed by gel electrophoresis, and PCR products were cleaned using a DNA Clean & Concentrator™-5 kit (Zymo Research Corporation, Irvine, California, USA). Amplicons were direct sequenced

in both directions at the Institute for Integrative Genome Biology, University of California at Riverside. Direct sequencing was not possible for the COI amplicon of 4 specimens: PR17-247, PR17-248, PR17-249, and PR17-256. In each case, sequencing was facilitated by inserting the PCR product into a plasmid vector (pGEM-T Easy Vector System; Promega, Fitchburg, Wisconsin, USA). Plasmids were transformed in JM109 competent cells and insert-positive clones were identified by blue-white screening. At least 3 clones of the COI product were subsequently amplified and sequenced from each problematic specimen using M13 primers. A consensus of those clones was then taken for each problematic specimen to reduce the chance of incorporating PCR-induced errors into our analyses.

The parity of forward and reverse reads was checked using SEQUENCHER 4.9 (Gene Codes Corp., Ann Arbor, Michigan, USA) and priming regions were removed manually in BioEdit version 7.0.5.3 (Hall 1999). The online tool, EMBOSS Transeq (Rice et al. 2000), was used to translate all protein coding COI sequences into their amino acid chains, confirming the absence of indels and pseudogenes in the final dataset. All sequences were deposited in GenBank® (Benson et al. 2008).

## GENETIC ANALYSIS

Phylogenetic analysis of DNA sequence variation was restricted to the 28SD2 and COI data. Sequences of 3 “outgroup” Encyrtinae, *Encyrtus* spp., and those of 2 additional Tetracneminae (*Leptomastix epona* (Walker) and *Anagyrus* sp. near *pseudococci* (Girault)), were retrieved from GenBank and included in the analyses (see Fig. 42 for accession numbers). Sequences of 28S and COI were aligned separately using MAFFT version 7.050 (Kato & Standley 2013) utilizing the Q-INS-i algorithm with default settings, and subsequently concatenated. The resulting matrix contained 35 terminal taxa (including outgroups), each with 1,168 nucleotide positions (COI = 652 bp; 28SD2 = 516 bp). Phylogenetic reconstruction was performed by conducting a maximum likelihood (ML) analysis in RAxML (Stamatakis 2006), using the raxmlGUI v. 1.3 (Silvestro & Michalak 2012). The GTR +  $\Gamma$  model (identified as the best-fit using jModeltest 2.1.4; Darriba et al. 2012) was applied and the entire dataset was partitioned by locus and, for COI, also by third codon position. Node support was assessed from 1,000 rapid bootstrap replicates as implemented in raxmlGUI (according to Stamatakis et al. 2008). Phylogenetic inference from ITS2 is typically problematic due to large interspecific differences that make alignment of this region dif-

ficult and somewhat ambiguous. Therefore, ITS2 sequences, aligned using MAFFT, were simply compared “by eye” to determine if distinct groups of sequences were present.

ML analyses recovered 3 distinct clades/species (see Results) and genetic variation between and within those clades/species was subsequently estimated by calculating average pairwise uncorrected p-distances, based on COI, using MEGA6 (Tamura et al. 2013). All ambiguous positions were removed for each sequence pair. The UPGMA method was used to infer a tree based on the p-distances, again in MEGA6. Branch support was assessed by bootstrapping with 1000 replicates.

## Results and Discussion

### TAXONOMY

#### Genus *Leptomastidea* García Mercet, 1916 (Encyrtidae)

*Comments.* All known members of *Leptomastidea* (i.e., 22 species that are recognized currently as valid) are mealybug parasitoids, and at least 1 species, *L. abnormis* (Girault), commonly is used in biological control programs for various mealybugs worldwide (Triapitsyn 2015). A new species of *Leptomastidea* was identified attacking *Hypogeococcus* spp. in the New World; its individuals were collected from Argentina, Barbados, Florida (USA), and Puerto Rico (USA) (Triapitsyn et al. 2014a), as well as more recently from Brazil. Three named species of *Leptomastidea* have been known to occur in the New World: the almost cosmopolitan *L. abnormis* (Triapitsyn 2015); *L. antillicola* Dozier (Dozier 1937), which is known from a single male from Puerto Rico and shown here to be a junior synonym of *L. abnormis*; and *L. debachi* Trjapitzin & Ruíz Cancino from Mexico (Trjapitzin & Ruíz Cancino 2002; Triapitsyn 2015).

Noyes (2000) defined *Leptomastidea* as an Old World genus, although he also mentioned that it is likely synonymous with the cosmopolitan genus *Gyranoidea* Compere. Indeed, their limits are not well defined and the differences between them are minor and seem to be based on, at most, species group rather than generic level characters (Triapitsyn et al. 2014a; Triapitsyn 2015). However, a revision of this group of genera is well beyond the scope of this study, so the species keyed below are treated in *Leptomastidea*, even though 2 of them, *L. debachi* and the new species described herein, are without any doubt native to the New World.

#### Key to the New World species of *Leptomastidea*, both sexes

- |       |   |  |
|-------|---|--|
| 1     | Flagellar segments with short setae; clava 3-segmented (Figs. 2, 6, 11, 18) females                             | 2  |
| 1'.   | Flagellar segments with very long setae; clava entire (Figs. 5, 14, 20) males                                   | 4  |
| 2 (1) | Clava white, contrasting strikingly with dark brown to black funicle (Figs. 8, 11, 18)                          | <i>L. hypogeococci</i> Triapitsyn, <b>sp. n.</b> |
| 2'.   | Clava light brown, more or less concolorous with funicle or at most slightly lighter (Figs. 2, 6)               | 3  |
| 3 (2) | Scape with a conspicuous transverse black band in the middle (Fig. 6); fore wing as in Fig. 7                   | <i>L. debachi</i> Trjapitzin & Ruíz Cancino      |
| 3'.   | Scape without a transverse black band in the middle, brown along dorsal margin (Fig. 2); fore wing as in Fig. 3 | <i>L. abnormis</i> (Girault)                     |
| 4 (1) | Fore wing hyaline (Fig. 5, p. 13 in Triapitsyn 2015)  | <i>L. debachi</i> Trjapitzin & Ruíz Cancino      |
| 4'.   | Fore wing with at least 2 brown bands (Figs. 5, 16, 21)   | 5  |
| 5 (4) | Fore wing with proximal brown band (behind submarginal vein) transverse, more or less straight (Figs. 16, 21)   | <i>L. hypogeococci</i> Triapitsyn, <b>sp. n.</b> |
| 5'.   | Fore wing with proximal brown band (behind submarginal vein) oblique (Fig. 5)                                   | <i>L. abnormis</i> (Girault)                     |

*Leptomastidea abnormis* (Girault, 1915)

(Figs. 2–5)

*Paraleptomastix abnormis* Girault 1915: 184. Type locality: an unspecified locality in Sicily, Italy; apparently either [Contrada] Salina or Mondello in Palermo Province (Triapitsyn 2015). Lectotype female [USNM], examined (Triapitsyn 2015).

*Leptomastidea abnormis* (Girault): Noyes 2000: 137–138 (taxonomic history, list of synonyms, diagnosis, host associations, use in bio-control, distribution, comments), 301–302, 347 (illustrations); Trjapitzin & Ruíz Cancino 2002: 31–32 (mentioned), 34 (illustration); Trjapitzin et al. 2004: 90–91 (catalog); Triapitsyn 2015: 10–11 (type information, lectotype designation, distribution), 13 (illustrations).

*Leptomastidea antillicola* Dozier 1937: 121–122. Type locality: coffee grove of the University of Puerto Rico Demonstration Farm, San Sebastián, Puerto Rico (USA). Subsequent references: De Santis 1979: 191 (catalog); Noyes 1980: 206 (list, a comment that it may be correctly placed in *Leptomastidea*); Noyes & Hayat 1994: 439 (list); Trjapitzin & Ruíz Cancino 2002: 31 (mentioned); Trjapitzin et al. 2004: 91 (catalog). **Syn. n.**

**Type Material Examined.** Holotype male of *L. antillicola* [USNM] on slide (Fig. 4) labeled: 1. “*Leptomastidea antillicola* Doz. ♂ Reared from *Pseudo-coccus virgatus* on leaves of “Guaba,” *Inga inga* at Univ. o [sic] P.R. Dem. Farm San Sebastián, P.R. May 12-1936, M. R. Smith”; 2. [red] “*Leptomastidea antillicola* Dozier Type ♂ Type No. 51762 U.S.N.M.”. The holotype (Fig. 5) is uncleared, complete, mounted dorsoventrally.

Measurements of the holotype (mm, as length or length:width). Body: 1.476; head: 0.351; mesosoma: 0.572; gaster: 0.646; genitalia: 0.209. Antenna: radicle: 0.061; scape: 0.215; pedicel: 0.057; F1: 0.172;

F2: 0.133; F3: 0.16; F4: 0.136; F5: 0.139; F6: 0.133; clava: 0.227. Fore wing: 1.095:0.443; longest marginal seta: 0.03; hind wing: 0.707:0.14; longest marginal seta: 0.06.

**Material Examined.** ARGENTINA: San Juan, Departamento Albardón, Campo Afuera, 1 Feb 1997, S. V. Triapitsyn, K. M. Daane, D. González, P. Fidalgo, P. Schliserman (on table grapes in a private yard); emerged 2 Apr 1997 from *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) in University of California, Riverside Entomology Quarantine Laboratory (USA: California, Riverside Co., Riverside), K. C. McGiffen, S&R #97-03-06 [6 females, ~150 males, UCRC]. Tucumán, San Miguel de Tucumán, May 1976, M. Rose (from “*Pseudococcus* sp.” [rather likely a *Planococcus* sp.] on citrus) [4 females, 3 males, UCRC].

**Comments.** *Leptomastidea abnormis* is a widespread, common mealybug parasitoid that is probably native to the Mediterranean and has been introduced, accidentally or otherwise, to the New World and elsewhere (Noyes 2000; Trjapitzin & Ruíz Cancino 2000). The host of its junior synonym *L. antillicola* in Puerto Rico was *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae) on foliage of guaba, *Inga edulis* [as *Inga inga*] (Dozier 1937); this mealybug is among the known hosts of *L. abnormis*, as listed by Noyes (2000).

*Leptomastidea debachi* Trjapitzin & Ruíz Cancino, 2002

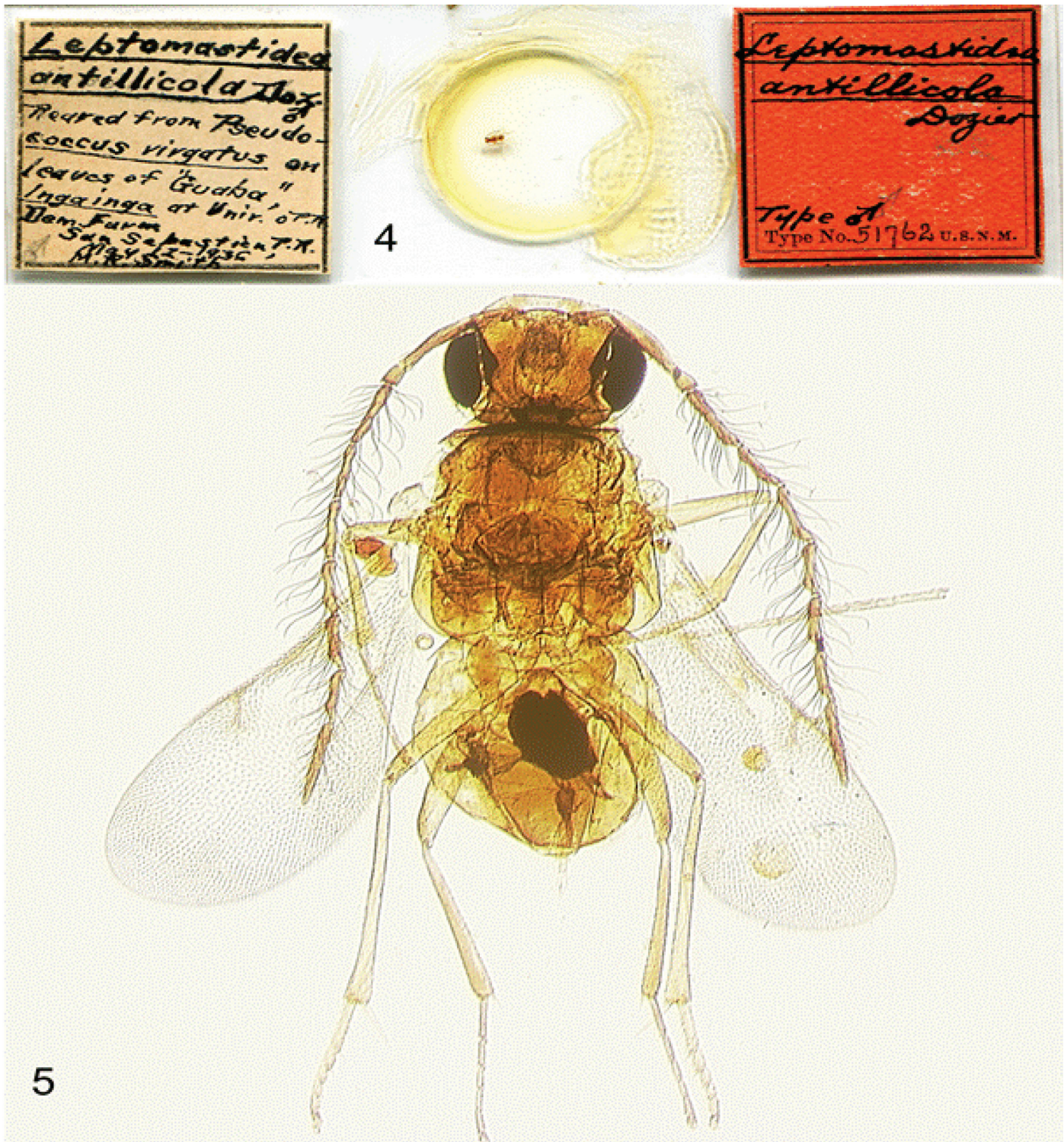
(Figs. 6–7)

*Leptomastidea debachi* Trjapitzin & Ruíz Cancino 2002: 32–35. Type locality: Las Barracas (about 30 km E of Santiago, 23.4672°N, 109.4502°W, 50 masl), Baja California Sur, Mexico. Holotype female [USNM], examined (Triapitsyn 2015).

*Leptomastidea debachi* Trjapitzin & Ruíz Cancino: Triapitsyn 2015: 9–10 (type information, description of the male, taxonomic comments), 12–13 (illustrations).



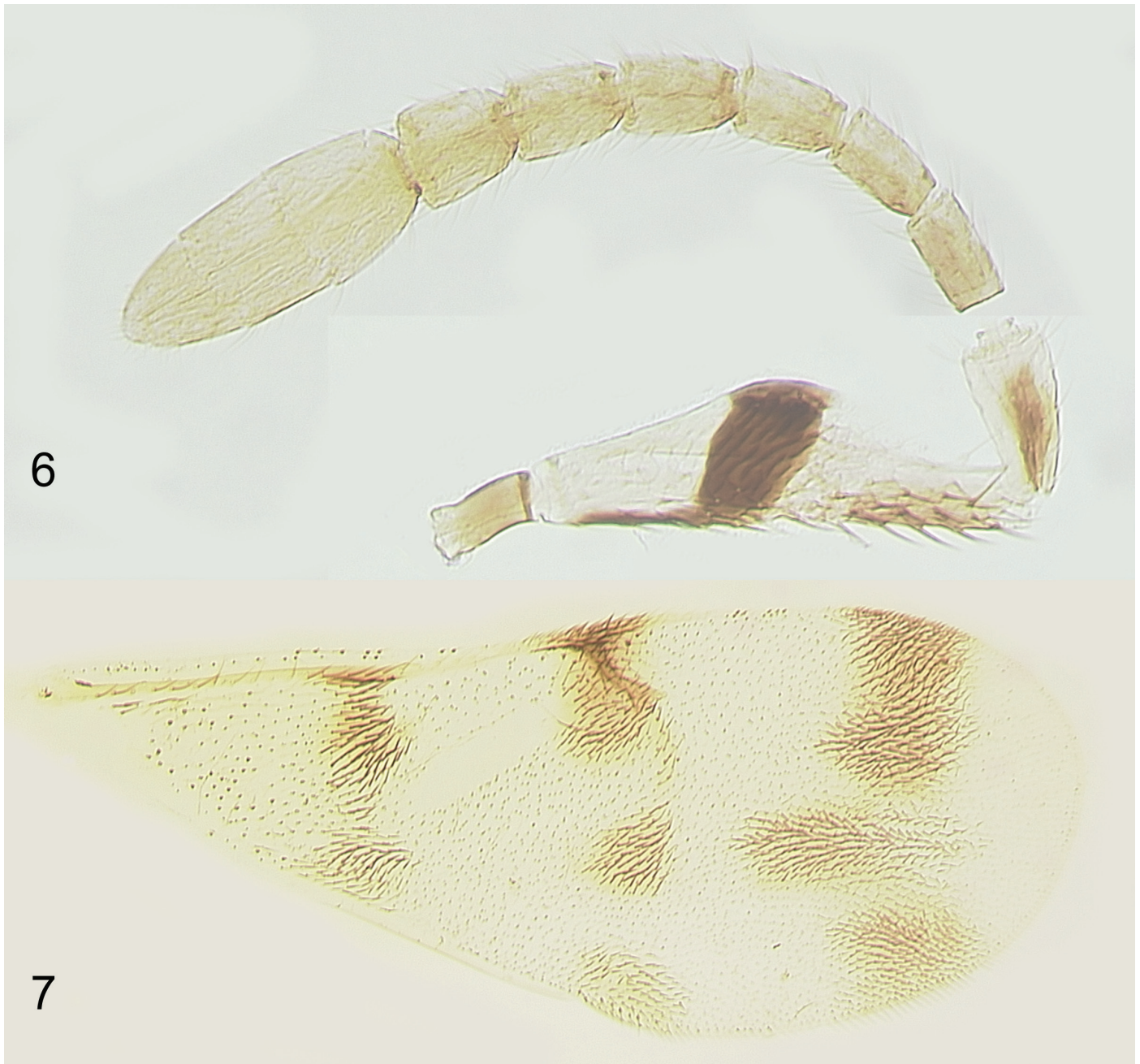
Figs. 2–3. *Leptomastidea abnormis* female (San Miguel de Tucumán, Tucumán, Argentina): 2, antenna; 3, fore wing.



Figs. 4–5. *Leptomastidea abnormis* male (holotype of *L. antillicola*): 4, slide; 5, habitus.

**Type Material Examined.** Paratype female [UCRC] on point labeled: 1. “Mex. Baja Cal. Sur Las Barracas 18-V-1985”; 2. “Coll. P. DeBach Pan trap”; 3. “*Leptomastidea* ♀ Det. V. Trjapitzin May 1997”; 4. [red] “Paratyus *Leptomastidea* ♀ *debachi* Trjapitzin et Ruíz Cancino”; 5. “Praep. micr. 7M”; 6. [bar code] “UCRC\_ENT 00408131” (an antenna was detached from this specimen; it is mounted on a slide labeled: 1. “*Leptomastidea debachi* ♀ Trjapitzin et Ruíz Cancino México: Baja California Sur Las Barracas. Pan trap 18.V.1985 (Coll. P. DeBach) 7M Antena”; 2.

[red] “Paratyus *Leptomastidea* ♀ *debachi* Trjapitzin et Ruíz Cancino”. Also 3 other female paratypes [UCRC] from the same locality, all collected by P. DeBach: 1 (lacking the head) with the same data as above except “Praep. micr. 6M” and “UCRC\_ENT 00408128” (a detached fore wing from this specimen is mounted on the slide number 6M with the identical label as above except “6M Ala ant.”); and 2 other females collected 5 May 1985 (UCRC\_ENT 00408129) and 10 May 1985 (UCRC\_ENT 00408130).



Figs. 6–7. *Leptomastidea debachi* female (paratypes): 6, antenna; 7, fore wing.

**Comments.** *Leptomastidea debachi* is known only from the type locality in Baja California Sur, Mexico, and is native to the region (Trjapitzin & Ruíz Cancino 2002; Triapitsyn 2015). Triapitsyn (2015) also specified its palpal formula and noted that it might fit better in *Gyranusoidea*, as defined by Noyes (2000). However, otherwise it has most of the rather vague defining features of *Leptomastidea*, of which *Gyranusoidea* quite likely would be eventually recognized as a junior synonym, so its transfer to the latter genus would not make much sense.

#### PRIMARY PARASITIDS OF *HYPOGEOCOCCUS* SPP.

*Leptomastidea hypogeococci* Triapitsyn, **sp. n.**

(Figs. 8–21)

*Leptomastidea* sp.: Triapitsyn et al. 2014a: 173, 176–179 (distribution, host associations, taxonomic comments, brief diagnosis, illustrations); Noyes & Triapitsyn 2018: 20 (mentioned from Puerto Rico as a host of *Prochiloneurus narendrani* Noyes & Triapitsyn, a hyperparasitoid of *Harrisia* cactus mealybug).

**Type Material.** Holotype female [UCRC] on slide (Fig. 9) labeled: 1. "PUERTO RICO (USA): Cabo Rojo, 17.9794°N, 67.1700°W, vi.2012, A. Galindo, from *Hypogeococcus* sp. on cactus, *Pilosocereus royenii*, Berlese sample, code: CR-P6"; 2. "Mounted by V. V. Berezovskiy 2012 in Canada balsam"; 3. [magenta] "*Leptomastidea hypogeococci* S. Triapitsyn HOLOTYPE ♀"; 4. [bar code] "UCRC ENT 311025". The holotype is in excellent condition, complete, dissected under 4 coverslips. Paratypes: BARBADOS, St. Philip Parish, Mangrove, 31 Mar 2011, W.

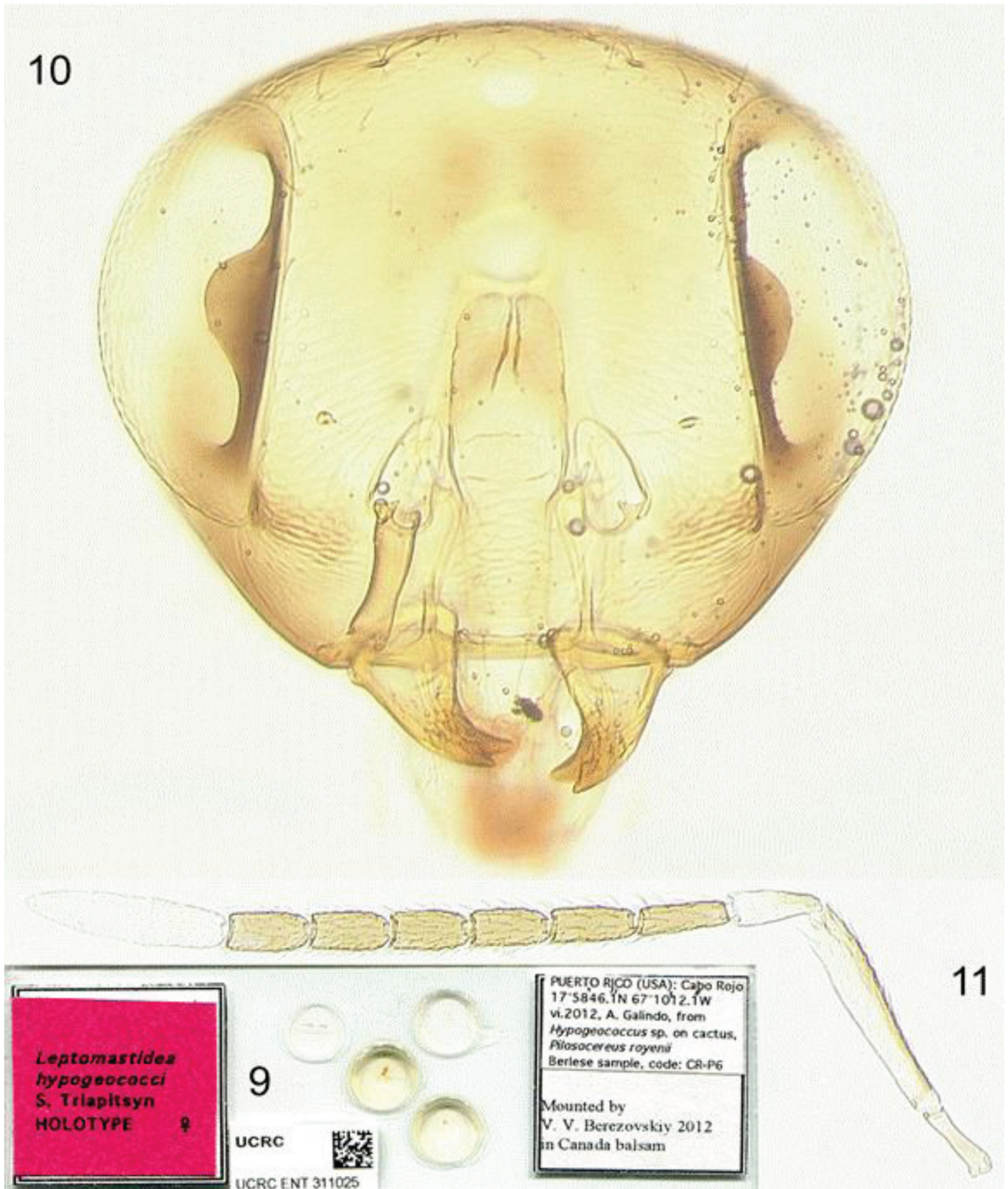




Fig. 8. *Leptomastidea hypogeococci* sp. n. female, photographed live (from colony, San Juan, Puerto Rico, USA): habitus.

McQuilkin (from *Hypogeococcus* sp. on *Alternanthera* sp.) [3 females, 3 males on points, UCRC]. PUERTO RICO (USA): Cabo Rojo, 17.9794°N, 67.1700°W: Jun 2012, A. Galindo-Cardona (from *Hypogeococcus* sp. on *P. royenii*, Berlese sample) [1 female on point, 1 male on point and 1 male on slide, UCRC]; date unknown, E. Vélez (from *Hypogeococcus* sp. on *P. royenii*, encapsulations) [6 females on points, UCRC]. Guánica, 17.9561°N, 66.8722°W: Jun 2012, A. Galindo-Cardona (from *Hypogeococcus* sp. on *P. royenii*, Berlese sample) [2 females, 1 male on points,

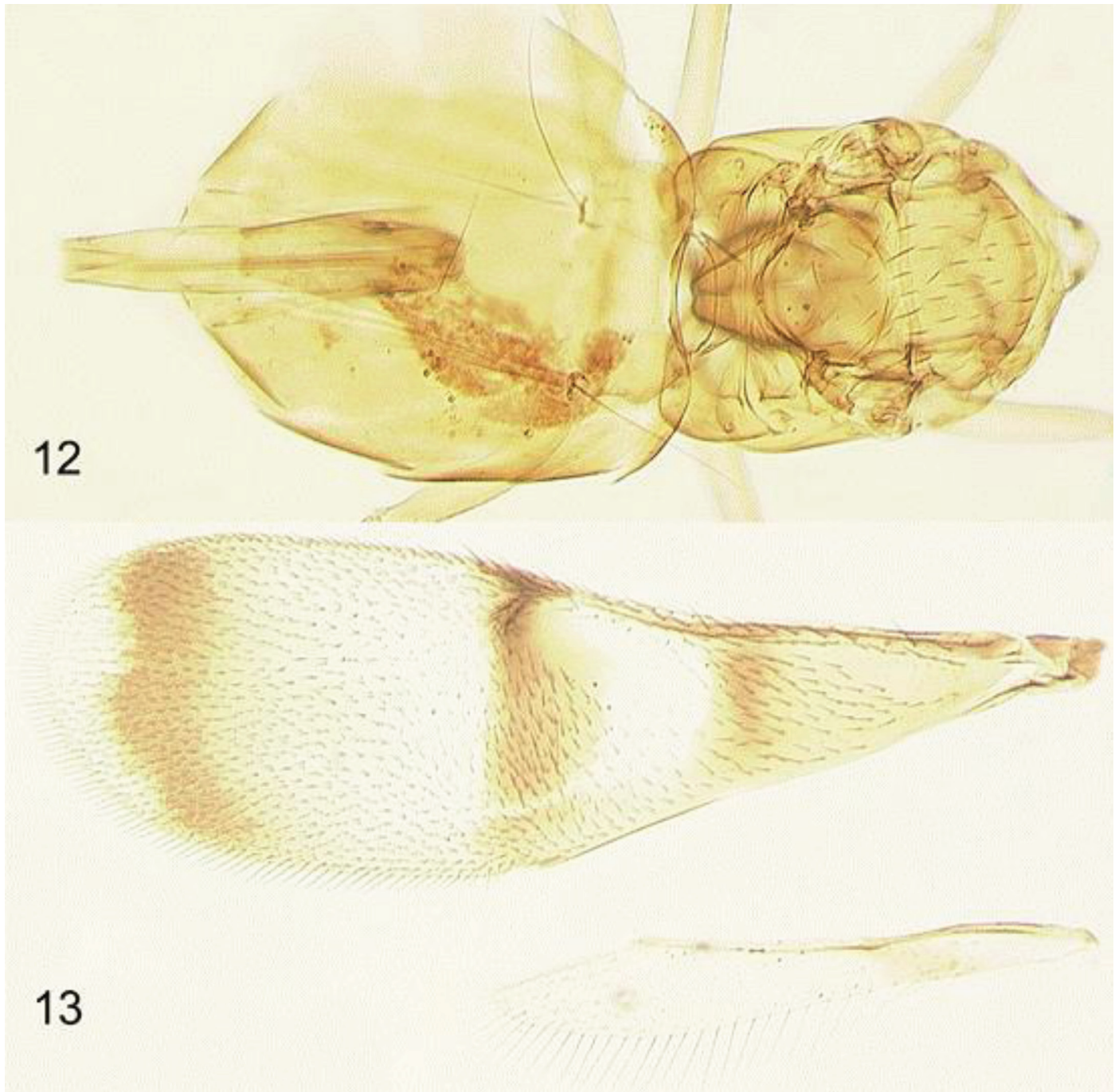
UCRC]; date unknown, E. Vélez (from *Hypogeococcus* sp. on *P. royenii*, encapsulations) [4 females on points, UCRC]. San Juan, Río Piedras, Jardín Botánico Sur, 1193 Calle Guayacan, J. C. V. Rodrigues laboratory at the University of Puerto Rico, 10 Jul 2013, Z. Rivera-Ocasio, from colony on *Hypogeococcus* sp. on *Portulaca oleracea* (the mealybug host is of Guánica dry forest origin, Guánica, 17.9561°N, 66.8722°W, Feb 2012, A. Roda, J. C. V. Rodrigues, on *P. royenii*); the parasitoid was originally from: Cabo Rojo, U. S. Fish and Wildlife Service Cabo Rojo



Figs. 9–11. *Leptomastidea hypogeococci* sp. n. female (holotype): 9, slide; 10, head (frontal view); 11, antenna.

National Wildlife Refuge, 17.9777°N, 67.1719°W, Apr 2012, E. Vélez, A. Galindo-Cardona, J. C. V. Rodrigues, on *P. royenii* [15 females, 10 males on points: BMNH (1 female, 1 male), METB (5 females, 3 males), UCRC

(4 females, 3 males), USNM (5 females, 3 males); also 1 female on slide (molecular voucher PR17-243, UCRC ENT 311808) and 1 female in 95% ethanol in a freezer, UCRC]. USA, Florida, Palm Beach Co., Seacrest

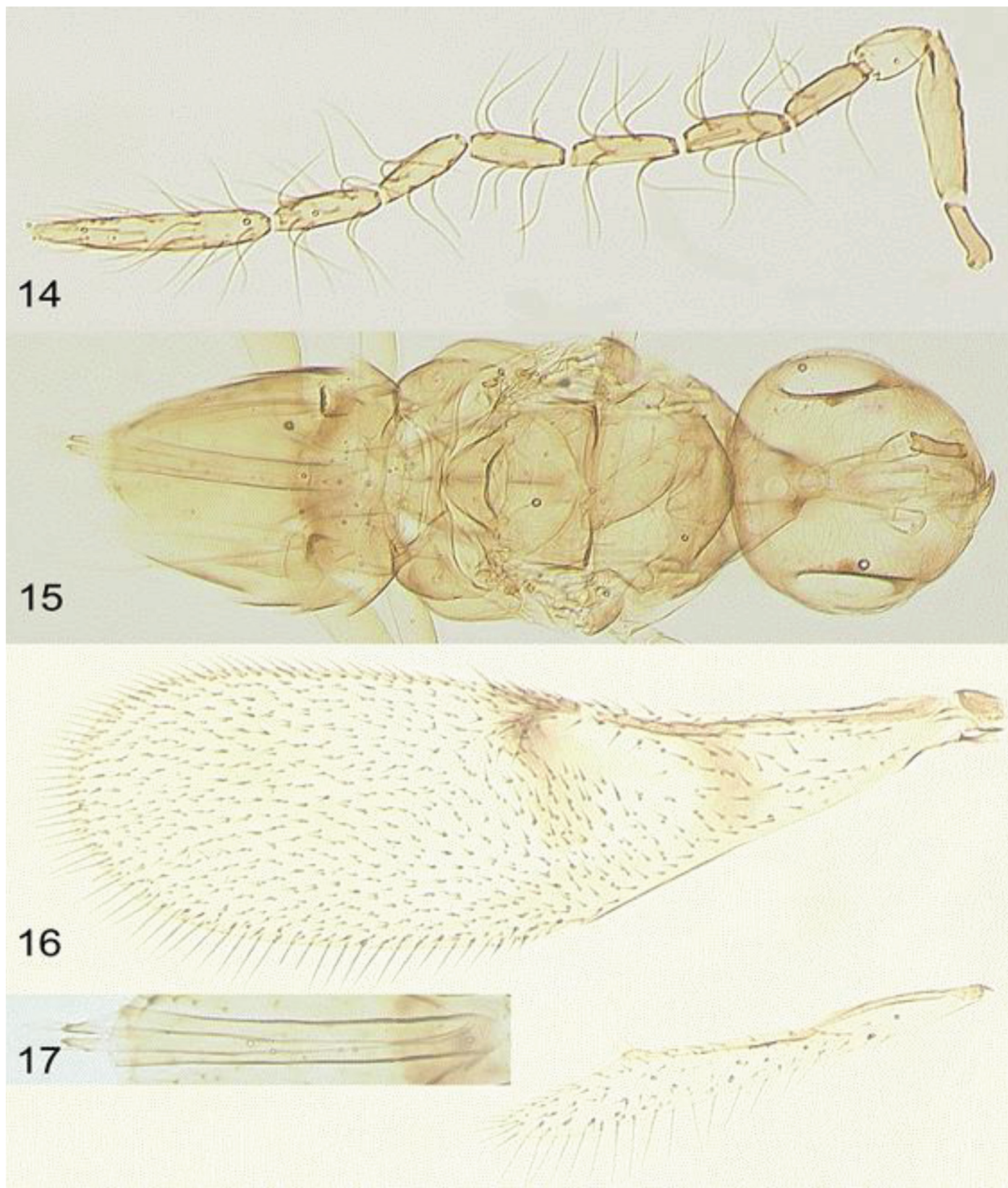


**Figs. 12–13.** *Leptomastidea hypogeococci* sp. n. female (holotype): 12, mesosoma and metasoma; 13, fore and hind wings.

Scrub Natural Area, 19 Jun 2012, S. Weihman (from *Hypogeococcus* sp. mummies on *Froelichia floridana* (Amaranthaceae)) [7 females, 7 males on points, UCRC (3 females, 3 males), USNM (4 females, 4 males); also 1 female, 1 male on slides, UCRC].

**Additional (Non-Type) Material Examined.** ARGENTINA: Buenos Aires, Hurlingham, Gral. Simón Bolívar 1559, 34.5875°S, 58.6405°W, 14 masl, 12 Mar 2017, M. B. Aguirre (from *Hypogeococcus* sp. on *Alternanthera paronychioides* in FuEDEI lab colony) [1 female, UCRC, molecular voucher PR17-256, UCRC ENT 311809]. Salta, 25.3535°S, 64.9279°W, 762 masl, near intersection of Ruta 16 and Ruta 9, 18 Nov 2014, G. A. Logarzo, M. B. Aguirre (from *Hypogeococcus* sp. on *Alternanthera pungens*) [2 females, 1 male, UCRC, including molecu-

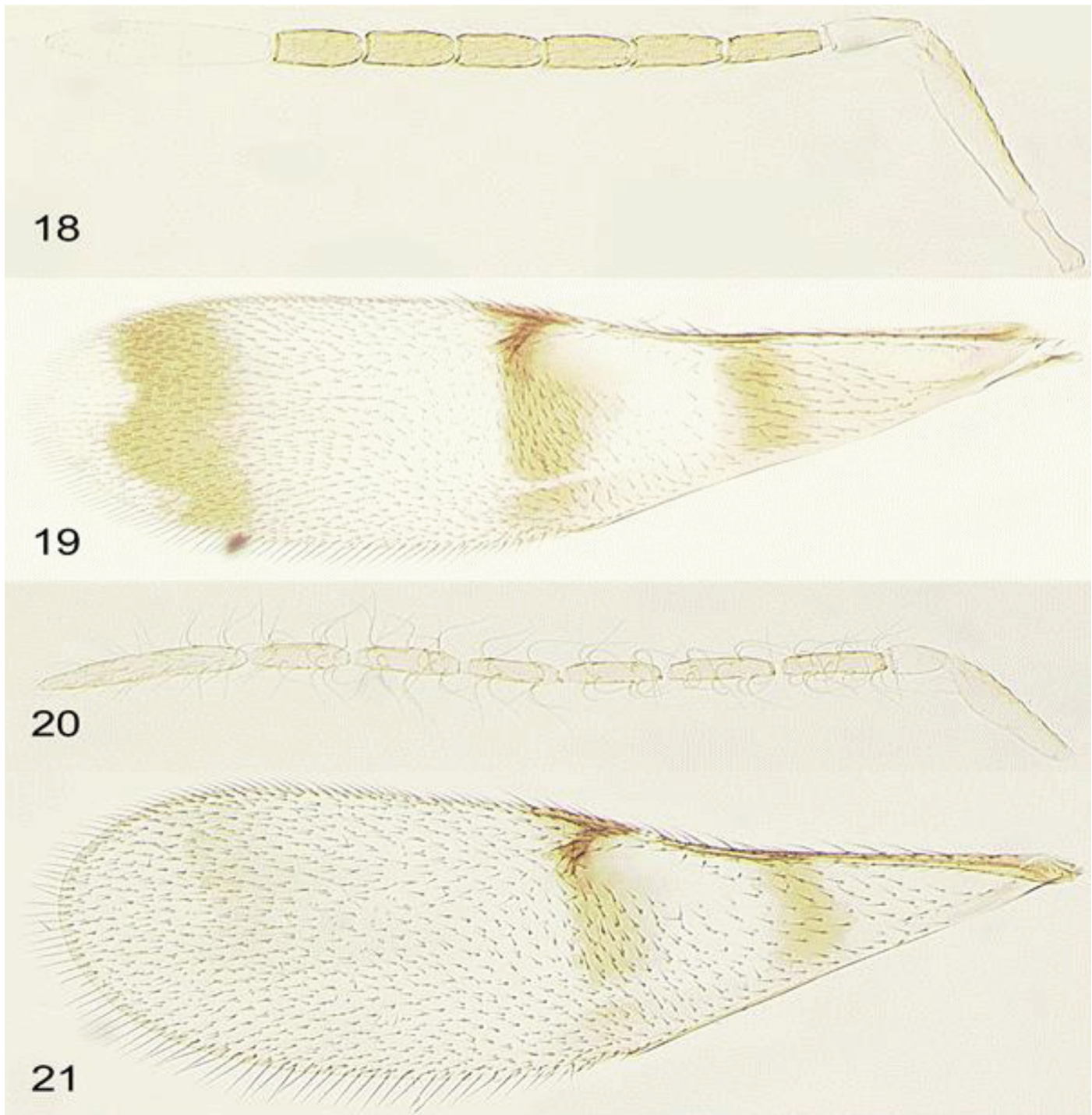
lar voucher PR17-254 (1 female, UCRC ENT 311810)]. BARBADOS: Christ Church Parish, Rockley, 8 Sep 2009, M. A. Ciomperlik (from *Hypogeococcus* sp.) [4 females, 6 males, BMNH]. [No locality indicated], 21 Apr 2009, Wendy (from *Hypogeococcus* sp.) [3 females, 3 males, BMNH]. BRAZIL, Rio de Janeiro: Quissamã, Praia Barra do Furado, 22.1063°S, 41.1502°W, 6 Dec 2016, M. B. Aguirre, S. D. Hight, M. D. Vitorino, G. A. Logarzo (from *Hypogeococcus* sp. on Amaranthaceae) [9 females, 3 males, UCRC, including molecular voucher PR17-249 (1 female, UCRC ENT 311811)]. São Pedro da Aldeia, 22.9087°S, 42.0366°W, 4 Dec 2016, G.A. Logarzo, M.D. Vitorino, S. D. Hight, M.B. Aguirre (from *Hypogeococcus* sp. on *Cereus* sp. (Cactaceae)) [1 female, UCRC]. Saquarema, 22.9567°S, 42.7522°W, 4 Dec 2016, M. B.



**Figs. 14–17.** *Leptomastidea hypogeococci* sp. n. male (paratype, Cabo Rojo, Puerto Rico, USA): 14, antenna; 15, body; 16, fore and hind wings; 17, genitalia.

Aguirre, G. A. Logarzo, M. D. Vitorino, S. D. Hight (from *Hypogeococcus* sp. on *Cereus* sp.) [3 females, 11 males, UCRC, including molecular vouchers PR17-247 (1 female, UCRC ENT 311812) and PR17-248

(1 male, UCRC ENT 311813)]. PUERTO RICO (USA): Cabo Rojo National Wildlife Refuge, 17.9787°N, 67.1708°W, 10 masl, host material collected 13 Feb 2017, parasitoids emerged 20 Feb 2017, Z. Rivera-Oc-



**Figs. 18–21.** *Leptomastidea hypogeococci* sp. n. (paratypes, Seacrest Scrub Natural Area, Palm Beach County, Florida, USA): 18, female antenna; 19, female fore wing; 20, male antenna; 21, male fore wing.

asio (from HCM, *Hypogeococcus* sp., on cactus) [6 females, 3 males, UCRC, including molecular voucher PR17-257 (1 female, UCRC ENT 311814)]. Mayagüez, 16 Jul 2009, M. A. Ciomperlik (from *Hypogeococcus* sp.) [5 females, 8 males, BMNH]. Mona Island, 12 Feb 2017, M. B. Aguirre, G. A. Logarzo (from *Hypogeococcus* sp. on Portulacaceae) [1 female, UCRC]. Vieques Island: Heliport Monte Pirata, 17 Feb 2017, G. A. Logarzo, M. B. Aguirre (from *Hypogeococcus* sp. on Portulacaceae) [1 male, UCRC]. Punta Conejos, Vieques National Wildlife Refuge, 18.1080°N, 65.3752°W, 1 masl, host material collected 30 Dec 2016,

parasitoids emerged 21 Feb 2017, Z. Rivera-Ocasio (from HCM, *Hypogeococcus* sp., on cactus [8 females, 5 males, UCRC, including molecular vouchers PR17-250 (1 female, UCRC ENT 311815) and PR17-253 (1 male, UCRC ENT 311816)]. USA, Florida, Lee Co., 30 Oct 2009, M. A. Ciomperlik (on *Portulaca* sp.) [2 females, 3 males, BMNH].

*Description.* Female (holotype and paratypes). Body length 0.726 to 1.452 mm (air-dried and critical point-dried specimens). Body mostly orange-brown to brown except pronotum white laterally; scape yellowish except brown along dorsal margin, pedicel yellow or pale except

slightly darker basally, flagellum dark brown to black, clava white; legs light brown except procoxa white.

Frontoververtex, dorsal part of pronotum, mesoscutum, axilla, scutellum, lower part of mesopleuron, and lateral parts of propodeum with minute mesh-like sculpture. Pronotum, mesoscutum, axilla, and scutellum with short, dusky setae. Propodeum laterally with inconspicuous silvery-white pubescence.

Head (Fig. 10) a little wider than high. Vertex in frontal view convex, about 0.5× head width. Occipital margin sharp. Ocelli in an obtuse triangle, posterior ocellus very far away from eye margin. Scrobes short, not meeting above; intertorular prominence weakly developed but nevertheless conspicuous. Mandible 2-dentate; labial palpus 2-segmented, maxillary palpus 3-segmented.

Antenna (Figs. 11, 18) inserted at lower eye margin level. Radicle about 0.25× total scape length, rest of scape slender, 5.2 to 5.3× as long as wide, a little wider in the middle closer to apex, with faint sculpture. Pedicel about as long as (or just slightly shorter than) F1. Funicle segments longer than wide; F1 the longest funicle segment, F2 to F6 more or less subequal in length, just slightly shorter than F1; F1 with 1 or 2, F2, F3, and F4 each with 2 or 3, F5 with 3, and F6 with 3 or 4 short mps. Clava 4.1 to 4.2× as long as wide and shorter than combined length of 3 preceding funicle segments; first claval segment with 3 or 4, second with 3 to 5 (and possibly with as many as 6), and third with 3 mps.

Mesosoma shorter than metasoma (Fig. 12). Pronotum narrow. Mesoscutum about 2× as wide as long. Scutellum a little wider than long, almost as long as mesoscutum; scutellar placoid sensilla in the middle of scutellum and close to each other.

Wings not abbreviated, fore wing extending far beyond apex of gaster. Fore wing (Figs. 13, 19) 3.0 to 3.1× as long as wide, unevenly pigmented, with 3 distinct, infusate fasciae (all as transverse, straight cross-bands), second band (behind apex of venation) narrowly interrupted, third (subapical) band entire; postmarginal vein about as long as stigmal vein; longest marginal seta 0.09 to 0.1× maximum wing width. Hind wing (Fig. 13) more or less hyaline (at most slightly infusate, more conspicuously so basally), 6.1 to 6.4× as long as wide; longest marginal seta 0.6 to 0.7× maximum wing width.

Mesobasitarsus shorter than combined length of following mesotarsal segments; mesotibial spur a little longer than or about as long as mesobasitarsus.

Ovipositor occupying 0.5 to 0.7× length of gaster, exerted markedly beyond gastral apex (by 0.22 to 0.3× own total length); ovipositor length:metatibia length ratio 1.2 to 1.3.

Measurements of the holotype (mm, as length or length:width). Mesosoma: 0.51; gaster: 0.645; ovipositor: 0.504. Antenna: radicle: 0.075; scape: 0.218; pedicel: 0.081; F1: 0.083; F2: 0.076; F3: 0.072; F4: 0.072; F5: 0.073; F6: 0.073; clava: 0.182. Fore wing: 0.996:0.326; longest marginal seta: 0.030; hind wing: 0.597:0.098; longest marginal seta: 0.062.

Male (paratypes). Body length 0.561 to 1.057 mm (air-dried and critical point-dried specimens). Body (Fig. 15) and appendages more or less uniformly brown except pedicel pale or light brown. Vertex in frontal view about 0.66× head width. Antenna (Figs. 14, 20) with scape minus radicle 4.2 to 4.5× as long as wide; flagellar segments with very long setae (much longer than each segment's width); funicle segments longer than wide, more or less subequal in length (F1 slightly shorter), short mps on F2 (1), F3 (0 or 1), F4 to F6 (1 or 2); clava entire, 5.8 to 6.1× as long as wide, a little wider than funicle segments, with 3 or 4 short mps. Mesosoma about as long as metasoma. Fore wing (Figs. 16, 21) 2.8 to 2.9× as long as wide, relatively less pigmented than in female, with 2 distinct infusate fasciae and sometimes also with an indistinct, irregular, rather small and faint infusate subapical spot closer to wing's anterior margin (Fig. 21);

longest marginal seta 0.15 to 0.2× maximum wing width. Hind wing (Fig. 16) almost hyaline, 6.1 to 6.6× as long as wide; longest marginal seta 0.75 to 0.9× maximum wing width. Genitalia (Fig. 17) occupying 0.7 to 0.8× length of gaster.

**Diagnosis.** This is the only species of *Leptomastidea* in the New World where the clava is contrastingly white (Fig. 8), as mentioned in the key. It differs from *L. abnormis* in having a transverse, straight (almost perpendicular to the submarginal vein, Figs. 13, 19), rather than an oblique (Fig. 3), first (proximal) infusate cross-band on the female fore wing behind the submarginal vein. *Leptomastidea hypogeococci* differs from *L. debachi* in the color patterns on the scape of the female antenna (compare Figs. 11, 18, and Fig. 6) and the configuration of infusate fasciae on the fore wing (compare Figs. 13, 19, and Fig. 7). In the review of the world species of *Leptomastidea* by Trjapitzin (2009), *L. hypogeococci* keys to Couplet 30 together with the Afrotropical species *L. jeanneli* García Mercet, from which it differs in having a white clava of the female antenna and the first (proximal) transverse infusate cross-band on the female fore wing straight, whereas in the female of *L. jeanneli* the clava is blackish-brown and the first infusate band on the fore wing (behind the submarginal vein) is strongly oblique (Prinsloo 2001).

**Distribution.** Argentina (Catamarca, Salta, Tucumán), Barbados, Florida (USA), Puerto Rico (USA) (Triapitsyn et al. 2014a), and Brazil (Rio de Janeiro).

**Hosts.** *Hypogeococcus pungens* Granara de Willink s. str. and *Hypogeococcus* sp. in Argentina, *Hypogeococcus* spp. (including *Harrisia* cactus mealybug in Puerto Rico) in Barbados, Florida (USA), and Puerto Rico (Triapitsyn et al. 2014a), and *Hypogeococcus* sp. in Brazil.

**Etymology.** The species name refers to the generic name of its mealybug hosts, *Hypogeococcus* spp.

**Comments.** Initially this species had been thought to possibly belong to either *L. antillicola* or an undescribed taxon (J. S. Noyes, personal communication). A recent comparison of our specimens with the holotype of *L. antillicola* immediately made it clear that it is not conspecific; the latter is synonymized here under *L. abnormis*. Placement of this new species in *Leptomastidea*, as defined by Noyes (2000), is correct: *L. hypogeococci* has a 2-segmented labial palpus and 3 distinct, infusate fasciae on the female fore wing; also its adults hold the wings erect over the back (Fig. 8), in the same manner as do adults of other species of this genus (Noyes 2000). There is no doubt whatsoever that *L. hypogeococci* is a native species because it parasitizes the mealybug *Hypogeococcus* sp. on the cactus *P. royenii*, both of which are native to the New World.

## Genus *Anagyrus* Howard, 1896 (Encyrtidae)

**Comments.** Members of *Anagyrus* in the Neotropical region can be recognized using the generic key in Noyes (1980). Later, Noyes (2000) provided a useful key to females of the genera in the subfamily Tetracneminae occurring in Costa Rica and a key to Costa Rican species of *Anagyrus*. More recently, Triapitsyn et al. (2014b) keyed the species in Argentina. Members of the genus are known to be mealybug parasitoids and are very diverse in the Neotropics; many species remain to be described. *Anagyrus* recognition is quite challenging, particularly because of common intraspecific variation in coloration of funicle segments of the female antenna, a character that is used extensively in taxonomic keys.

*Anagyrus cachamai* Triapitsyn, Logarzo & Aguirre, 2014

*Anagyrus cachamai* Triapitsyn, Logarzo & Aguirre in Triapitsyn et al. 2014b: 203 (key), 210–216. Type locality: El Portezuelo (28.4495°S, 65.6331°W, 653 masl), Catamarca, Argentina. Holotype female [MLPA], examined (Triapitsyn et al. 2014b).

*Anagyrus cachamai* Triapitsyn, Logarzo & Aguirre: Triapitsyn et al. 2014a: 173–174 (habitus images of both sexes).

**Type Material Examined.** Paratypes (on slides): ARGENTINA, Córdoba, Jesús María, 30.9690°S, 64.0690°W, 509 masl, 14 Feb 2014, G. A. Logarzo, M. B. Aguirre (from *Hypogeococcus pungens* on *Alternanthera pungens*) [1 female, 1 male, UCRC, molecular vouchers PR14-212 (UCRC ENT 311745) and PR14-213 (UCRC ENT 311746), respectively].

**Material Examined.** ARGENTINA: Mendoza, 32.7074°S, 68.8561°W, 816 masl, roadside of Ruta 52, collected 26 Mar 2017, M. B. Aguirre, G. A. Logarzo, S. D. Hight, S. V. Triapitsyn, emerged 28 Mar to 10 Apr 2017 (from *Hypogeococcus* sp. on *Cereus ? aethiops*) [21 females, 41 males, UCRC, including molecular voucher PR17-252 (1 female, UCRC ENT 311817)]. San Luis, San Luis, near intersection of Constitución & Lavalle, 33.2988°S, 66.3444°W, 719 masl, collected 28 Mar 2017: G. A. Logarzo, S. V. Triapitsyn, S. D. Hight, M. B. Aguirre (from *Hypogeococcus* sp. on *Alternanthera pungens*) [4 females, 4 males, UCRC]; S. D. Hight, S. V. Triapitsyn, G. A. Logarzo, M. B. Aguirre, emerged 10 Apr 2017 (from *Hypogeococcus* sp. on *Portulaca* sp.) [1 female, UCRC]. PARAGUAY: Boquerón, 22.2669°S, 60.4290°W, Fernheim Colony: 24 Sep 2016, S. D. Hight, G. A. Logarzo, M. B. Aguirre (from *Hypogeococcus* sp. on *Harrisia bonplandii*) [6 females, 5 males, UCRC, including molecular voucher PR17-246 (1 female, UCRC ENT 311818)]; 24 Sep 2016, S. D. Hight, G. A. Logarzo, M. B. Aguirre (from *Hypogeococcus* sp. on *Cleistocactus baumannii* (Cactaceae)) [14 females, 6 males, UCRC, including molecular voucher PR17-245 (1 female, UCRC ENT 311819)]. Presidente Hayes: 23.6383°S, 58.7014°W (Ruta 9, km 251): 23 Sep 2016, G. A. Logarzo, M. B. Aguirre, S. D. Hight (from *Hypogeococcus* sp. on *Harrisia tortuosa*) [3 males, UCRC]; 23 Sep 2016, G. A. Logarzo, S. D. Hight, M. B. Aguirre (from *Hypogeococcus* sp. on *Harrisia bonplandii*) [2 females, 13 males, UCRC]; 23 Sep 2016, S. D. Hight, M. B. Aguirre, G. A. Logarzo (from *Hypogeococcus* sp. on *Monvillea spegazzinii* (Cactaceae)) [2 females, 3 males, UCRC]. 23.2574°S, 59.1756°W (Ruta 9, km 318), 25 Sep 2016, S. D. Hight, M. B. Aguirre, G. A. Logarzo (from *Hypogeococcus* sp. on *Harrisia bonplandii*) [5 males, UCRC]. José Falcón, 25.2474°S, 57.7094°W, 22 Sep 2016, M. B. Aguirre, G. A. Logarzo, S. D. Hight (from *Hypogeococcus* sp. on *Portulaca* sp.) [6 females, 33 males, UCRC]. Near Colonia San Miguel, 24.3752°S, 58.1299°W, 21 Sep 2016, M. B. Aguirre, G. A. Logarzo, S. D. Hight (from *Hypogeococcus* sp. on *Portulaca* sp.) [2 females, 4 males, UCRC].

**Distribution.** Argentina (Catamarca, Córdoba, Salta, Tucumán) (Triapitsyn et al. 2014b) and Paraguay [new record].

**Hosts.** *Hypogeococcus pungens* Granara de Willink s. str. and *Hypogeococcus* spp. in Argentina (Triapitsyn et al. 2014b) and also *Hypogeococcus* spp. in Paraguay. On 29 Sep 2016 and 23 Jun 2017, this species was imported, under appropriate permits, into the quarantine laboratory at the Center for Excellence in Quarantine & Invasive Species, University of Puerto Rico, Jardín Botánico, Río Piedras, San Juan, Puerto Rico, and a colony was successfully established using the Puerto Rican *Harrisia* cactus mealybug as host.

**Comments.** See Triapitsyn et al. (2014b) for its description and illustrations, information on the distribution and host associations in Argentina, and brief notes on its biological traits. The digital images of the habitus of the dry-mounted, critical-point dried specimens for both sexes of this species were provided by Triapitsyn et al. (2014a).

*Anagyrus lapachosus* Triapitsyn, Aguirre & Logarzo, 2016

*Anagyrus lapachosus* Triapitsyn, Aguirre & Logarzo, 2016: 590–594. Type locality: Los Lapachos, 24.6019°S, 65.0812°W, Salta, Argentina. Holotype female [MLPA], examined (Triapitsyn et al. 2016).

**Material Examined.** PARAGUAY, Presidente Hayes, near Colonia San Miguel, 24.3752°S, 58.1299°W, 21 Sep 2016, M. B. Aguirre, G. A. Logarzo, S. D. Hight (from *Hypogeococcus* sp. on *Portulaca* sp.) [2 females, UCRC].

**Distribution.** Argentina (Salta) (Triapitsyn et al. 2016) and Paraguay [new record].

**Hosts.** *Hypogeococcus* sp. on *Harrisia* sp. in Argentina (Triapitsyn et al. 2016) and *Hypogeococcus* sp. on *Portulaca* sp. in Paraguay.

**Comments.** On 29 Sep 2016 and 23 Jun 2017, this species was imported, under appropriate permits, into the quarantine laboratory at the Center for Excellence in Quarantine & Invasive Species, University of Puerto Rico, Jardín Botánico, Río Piedras, San Juan, Puerto Rico, but its colony did not do well on initial trial using *Harrisia* cactus mealybug as host.

*Anagyrus quilmes* Triapitsyn, Logarzo & Aguirre, 2014

*Anagyrus quilmes* Triapitsyn, Logarzo & Aguirre in Triapitsyn et al. 2014b: 203 (key), 221, 223–228. Type locality: Vipos (near Choromoro, 26.4196°S, 65.3063°W, 788 masl), Tucumán, Argentina. Holotype female [MLPA], examined (Triapitsyn et al. 2014b).

*Anagyrus quilmes* Triapitsyn, Logarzo & Aguirre: Triapitsyn et al. 2014a: 173, 175–176 (habitus images of both sexes and color intraspecific variation of funicle segments of female antennae); Triapitsyn 2016: 28 (comparison with *A. ciomperliki* Triapitsyn).

*Anagyrus ciomperliki* Triapitsyn 2016: 27–31. Type locality: U. S. Fish and Wildlife Service Cabo Rojo National Wildlife Refuge, Cabo Rojo, Puerto Rico (USA). Holotype female [UCRC], examined (Triapitsyn 2016). **Syn. n.**

**Type Material Examined.** Paratypes of *A. quilmes* (on slides): ARGENTINA, Catamarca, El Portezuelo, 28.4701°S, 65.6354°W, 635 masl, 22 Mar 2014, G. A. Logarzo, M. Guala (from *Hypogeococcus pungens* on *Alternanthera pungens*) [1 female, 1 male, UCRC, molecular vouchers PR14-210 (UCRC ENT 311743) and PR14-211 (UCRC ENT 311744), respectively].

**Material Examined.** ARGENTINA: Catamarca, El Portezuelo, 28.4701°S, 65.6354°W, 635 masl, 15 Feb 2014, G. A. Logarzo, M. B. Aguirre (from *Hypogeococcus pungens* on *Alternanthera pungens*) [1 female, 1 male, UCRC, molecular vouchers PR14-214 (UCRC ENT 311747) and PR14-215 (UCRC ENT 311748), respectively]. Chaco, between Margarita Belén and Colonia Benitez, 20 Nov 2015, G. A. Logarzo, M. B. Aguirre (from Pseudococcidae on crass, *Crassula* sp. (Crassulaceae)) [1 female, 10 males, UCRC]. Córdoba, near Mina Clavero, Vivero Córdoba, 31.7576°S, 64.9724°W, 1015 masl, collected 31 Mar 2017: M. B. Aguirre, S. D. Hight, G. A. Logarzo, S. V. Triapitsyn, emerged 1 to 10 Apr 2017 (from *Hypogeococcus* sp. on San Pedro cactus, *Echinopsis pachanoi* (Cactaceae)) [17 females, 3 males, UCRC, including molecular voucher PR17-244 (1 female, UCRC ENT 311820)]; M. B. Aguirre, S. D. Hight, G. A. Logarzo, S. V. Triapitsyn, emerged 17 to 24 Apr 2017 (from *Hypogeococcus* sp. on *Echinopsis pachanoi*) [7 females, 4 males, UCRC]; S. D. Hight, M. B. Aguirre, S. V. Triapitsyn, G. A. Logarzo, emerged 3 to 10 Apr 2017 (from *Hypogeococcus* sp. on *Cleistocactus smaragdiflorus*) [2 females, UCRC, including molecular voucher PR17-255 (1 female, UCRC ENT 311807)]; G. A. Logarzo, S. V. Triapitsyn, S. D. Hight, M. B. Aguirre, emerged 10 Apr 2017 (from *Hypogeococcus* sp. on *Cereus* sp.) [1 male, UCRC]; S. V. Triapitsyn, G. A. Logarzo, S. D. Hight, M. B. Aguirre, emerged 10 Apr 2017 (from *Hypogeococcus* sp. on *Cereus ?forbesii*) [1 female, 3 males, UCRC]. La Rioja, Anillaco (center of town), 8 Mar 2015, N. L. Jiménez, M. E. Guala, M. B. Aguirre (from *Hypogeococcus* sp. on *Alternanthera pungens*) [1 female, UCRC]. Salta, Río Piedras, 25.3549°S, 64.9020°W, 715 masl, 16 Feb 2014, M. B. Aguirre, S. V. Triapitsyn, G. A. Logarzo (from *Hypogeococcus pungens*

on *Alternanthera paronychioides*) [1 male, UCRC, molecular voucher PR14-216 (UCRC ENT 311749)]. San Luis: San Luis, near intersection of Constitución & Lavalle, 33.2988°S, 66.3444°W, 719 masl, 28 Mar 2017, G. A. Logarzo, S. V. Triapitsyn, S. D. Hight, M. B. Aguirre, emerged 3 to 10 Apr 2017 (from *Hypogeococcus* sp. on *Alternanthera pungens*) [9 females, 4 males, UCRC, including molecular voucher PR17-251 (1 female, UCRC ENT 311806)]. Villa de Merlo, 7 Jun 2017, G. A. Logarzo (from *Hypogeococcus* sp. on *Cereus* sp.) [2 females, UCRC]. BRAZIL, Rio de Janeiro: Armação dos Búzios: APA Pau Hotel (Área de Proteção Ambiental): 5 Dec 2016, G. A. Logarzo, S. D. Hight, M. D. Vitorino, M. B. Aguirre (from *Hypogeococcus* sp. on *Cereus* sp. ("grandes")) [1 male, UCRC]; Praia das Caravelas: 22.8141°S, 41.9513°W, 5 Dec 2016, G. A. Logarzo, M. D. Vitorino, S. D. Hight, M. B. Aguirre (from *Hypogeococcus* sp. on *Cereus* sp.) [26 females, 10 males, UCRC, including molecular vouchers PR17-163 and PR17-164 (2 females, UCRC ENT 311805 and UCRC ENT 311804, respectively)]; 5 Dec 2016, G. A. Logarzo, M. D. Vitorino, S. D. Hight, M. B. Aguirre (from *Hypogeococcus* sp. on *Cereus* sp.) [3 females, UCRC]; Praia de Tucuns, 22.7999°S, 41.9303°W, 5 Dec 2016, S. D. Hight, M. D. Vitorino, G. A. Logarzo, M. B. Aguirre (from *Hypogeococcus* sp. on *Cereus* sp.) [14 females, 13 males, UCRC, including molecular vouchers PR17-161 and PR17-162 (2 females, UCRC ENT 311803 and UCRC ENT 311802, respectively)]. São Pedro da Aldeia, 22.9087°S, 42.0366°W, 4 Dec 2016, S. D. Hight, M. B. Aguirre, M. D. Vitorino, G. A. Logarzo (from *Hypogeococcus* sp. on *Cereus* sp.) [5 females, UCRC, including molecular voucher PR17-160 (1 female, UCRC ENT 311801)]. PARAGUAY, Presidente Hayes: José Falcón, 25.2474°S, 57.7094°W, 22 Sep 2016, M. B. Aguirre, G. A. Logarzo, S. D. Hight (from *Hypogeococcus* sp. on *Portulaca* sp.) [1 female, 7 males, UCRC, including molecular voucher PR17-167 (1 male, UCRC ENT 311800)]. Near Colonia San Miguel, 24.3752°S, 58.1299°W, 21 Sep 2016, M. B. Aguirre, G. A. Logarzo, S. D. Hight (from *Hypogeococcus* sp. on *Portulaca* sp.) [2 females, 1 male, UCRC]. PUERTO RICO (USA): Cabo Rojo National Wildlife Refuge, 17.9787°N, 67.1708°W, 10 masl, host material collected 13 Feb 2017, parasitoids emerged 20 Feb 2017, Z. Rivera-Ocasio (from *Harrisia cactus* mealybug, *Hypogeococcus* sp., on cactus) [5 females, 4 males, UCRC, including molecular vouchers PR17-165 and PR17-166 (2 females, UCRC ENT 311799 and UCRC ENT 311798, respectively)]. Vieques Island, Heliport Monte Pirata, 17 Feb 2017, M. B. Aguirre, G. A. Logarzo (from *Hypogeococcus* sp. on *Achyranthes aspera* (Amaranthaceae)) [1 female, 1 male, UCRC].

**Distribution.** Argentina (Catamarca, Salta, Tucumán (Triapitsyn et al. 2014b) as well as Chaco, Córdoba, and San Luis [new records]), Brazil (Rio de Janeiro) [new record], Puerto Rico (USA) (Triapitsyn 2016 [as *A. ciomperliki*]), and Paraguay (Presidente Hayes) [new record].

**Hosts.** *Hypogeococcus pungens* Granara de Willink s. str. and *Hypogeococcus* spp. in Argentina (Triapitsyn et al. 2014b), *Hypogeococcus* sp(p). in Brazil and Paraguay, and *Hypogeococcus* sp. (*Harrisia cactus* mealybug) in Puerto Rico (Triapitsyn 2016 [as *A. ciomperliki*]).

**Comments.** Triapitsyn (2016) described and illustrated *A. ciomperliki* based on the following differences in the proportions of female antennal segments: *A. quilmes* from Argentina having F1 shorter than pedicel but conspicuously longer than pedicel in *A. ciomperliki*. However, after that, many specimens of an *Anagyrs* sp. were reared from a *Hypogeococcus* sp. in the state of Rio de Janeiro, Brazil, that showed the intermediate state of this character, with F1 of the female antenna being either about as long as pedicel, or a little longer or shorter than pedicel. This intermediate character state cast doubt on the validity of *A. ciomperliki* as a taxon, so we decided to test its possible conspecificity with *A. quilmes* using molecular methods. The genetic analysis presented below under "Genetic Analysis" clearly indicates that they are indeed conspecific, hence the proposed synonymy of the former nominal species under the latter.

The female from Anillaco, La Rioja, Argentina, is only tentatively identified as *A. quilmes*, because the F4 is dark (see Triapitsyn 2016 for details).

### *Anagyrs* spp.

**Material Examined.** ARGENTINA, Corrientes, Mercedes, 12 Mar 2017, G. A. Logarzo, F. McKay (from *Hypogeococcus* sp. on *Alternanthera paronychioides*) [2 males, UCRC]. PUERTO RICO (USA), Vieques Island, Heliport Monte Pirata, 17 Feb 2017, M. B. Aguirre, G. A. Logarzo (from *Hypogeococcus* sp. on Portulacaceae) [1 male, UCRC].

### *Stemmatosteres* sp. (Encyrtidae)

(Figs. 22–24)

**Material Examined.** ARGENTINA, Jujuy, Cuesta del Toquero, 22.1097°S, 65.7819°W, 3750 masl, 10 Feb 2015, M. B. Aguirre, G. A. Logarzo, E. R. Hasson (from *Hypogeococcus* sp. on Cactaceae) [4 males, UCRC].

**Host.** *Hypogeococcus* sp. on Cactaceae.

**Comments.** These interesting apterous specimens (Figs. 22–24), which were collected at high altitude, are likely not conspecific with any of the known members of the genus *Stemmatosteres* Timberlake from the New World although they might be somewhat similar to *S. apterus* Timberlake that is known from California (USA) and Uruguay (Noyes 2017), but their positive identification based solely on males is impossible. Although we are not sure about habit of this undetermined species, it is very likely a primary parasitoid.

## SECONDARY PARASITOIDS (HYPERPARASITOIDS) OF *HYPOGEOCOCCUS* SPP.

*Chartocerus axillaris* De Santis, 1973 (Signiphoridae)

*Chartocerus axillaris* De Santis 1973: 152–153: Type locality: Chacras de Coria, Mendoza, Argentina. Holotype female [MLPA], examined (Triapitsyn et al. 2014a).

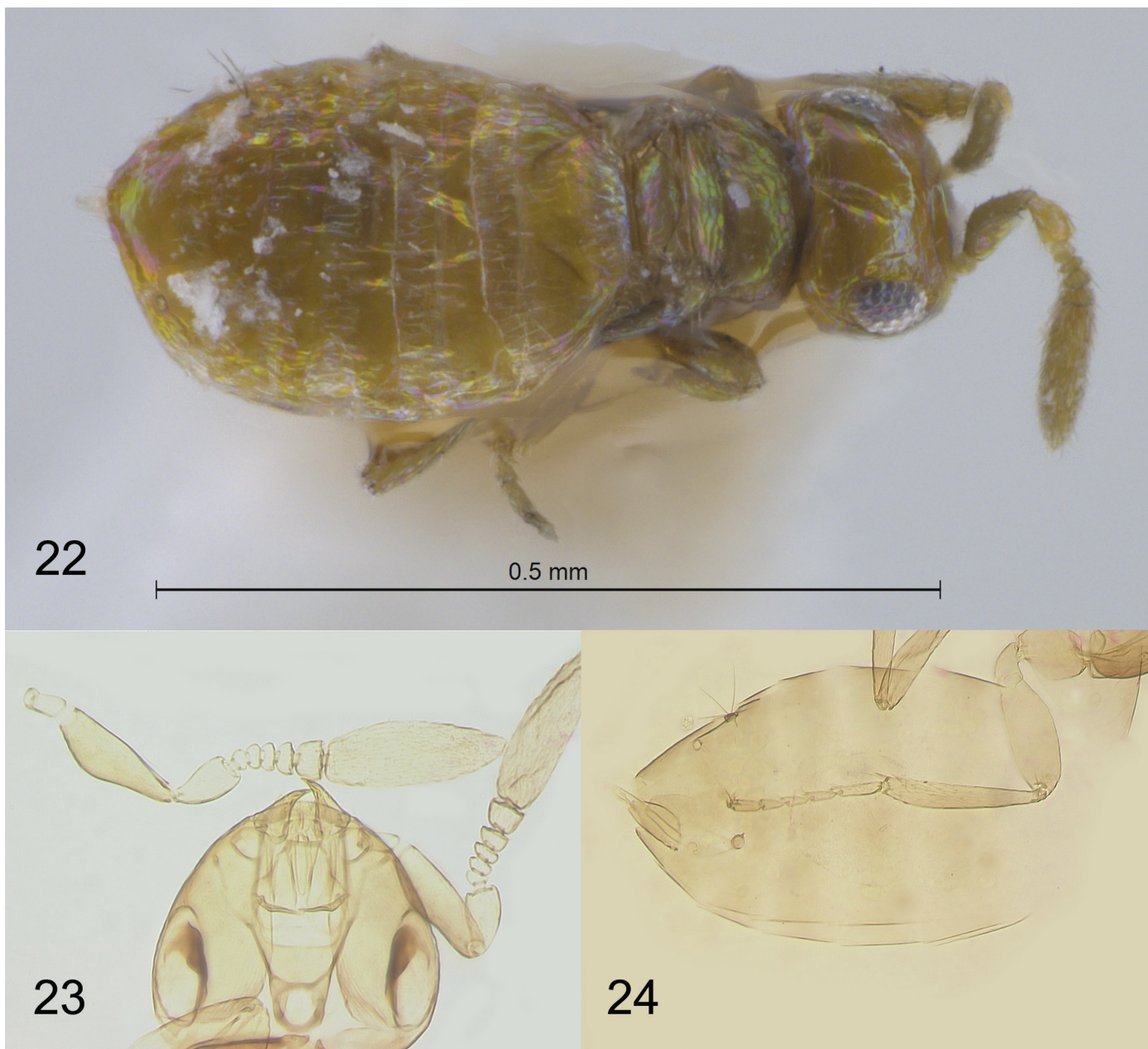
*Chartocerus axillaris* De Santis: De Santis 1979: 253 (catalog).

*Chartocerus ?axillaris* De Santis: Triapitsyn 2014a: 176–178, 180–183 (illustrations).

**Material Examined.** ARGENTINA: Córdoba, near Mina Clavero, Vivero Córdoba, 31.7576°S, 64.9724°W, 1015 masl, collected 31 Mar 2017: S. V. Triapitsyn, S. D. Hight, G. A. Logarzo, M. B. Aguirre, emerged 3 Apr 2017 (from *Hypogeococcus* sp. on a large cactus, *Cereus ?forbesii*) [1 female, UCRC]; S. D. Hight, G. A. Logarzo, S. V. Triapitsyn, M. B. Aguirre (from *Hypogeococcus* sp. on *Cleistocactus smaragdiflorus*) [2 females, UCRC]; M. B. Aguirre, S. D. Hight, G. A. Logarzo, S. V. Triapitsyn, emerged 17 to 24 Apr 2017 (from *Hypogeococcus* sp. on San Pedro cactus, *Echinopsis pachanoi*) [1 female, UCRC]. Mendoza, 32.7074°S, 68.8561°W, 816 masl, roadside of Ruta 52, collected 26 Mar 2017, M. B. Aguirre, G. A. Logarzo, S. D. Hight, S. V. Triapitsyn, emerged 28 Mar to 10 Apr 2017 (from *Hypogeococcus* sp. on *Cereus ?aethiops*) [6 females, 3 males, UCRC]. Salta: Alemania, 25.6245°S, 65.6162°W, 21 Nov 2014, G. A. Logarzo, M. B. Aguirre, M. A. Saracho Bottero (from *Hypogeococcus* sp. on *Cleistocactus* sp.) [2 females, UCRC]. Los Lapachos, 24.6019°S, 65.0812°W, 20 Nov 2014, G. A. Logarzo, M. B. Aguirre, M. A. Saracho Bottero (from *Hypogeococcus* sp. on *Harrisia* sp.) [2 females, 2 males, UCRC].

**Distribution.** Argentina (Mendoza (De Santis 1973), Catamarca, Córdoba, Salta (Triapitsyn et al. 2014a)) and Paraguay (De Santis 1979).





Figs. 22–24. *Stenmatosteres* sp. male (Cuesta del Toquero, Jujuy, Argentina): 22, habitus; 23, head and antennae; 24, gaster.

**Hosts.** Hyperparasitoid of *Hypogeococcus pungens* s. str. and *Hypogeococcus* spp. (Triapitsyn et al. 2014a), and also of a *Pseudococcus* sp. (Pseudococcidae) on *Solanum elaeagnifolium* (Solanaceae) via the encyrtid primary parasitoid *Anagyrus lopezi* (De Santis) (De Santis 1973).

**Comments.** Although initial identifications of the specimens of this species from *Hypogeococcus* spp. in Argentina were tentative (Triapitsyn et al. 2014a), chances are quite high that they were correct. Such hyperparasitoids are not generally known to be particularly host specific, and later we reared *Ch. axillaris* in Mendoza; its type locality is in the same province.

*Cheiloneurus* sp. near *banksi* (Howard) (Encyrtidae)

(Figs. 25–33)

*Cheiloneurus* n. sp. near *amplicornis* Gahan: Noyes & Triapitsyn 2018: 21 (mentioned as a hyperparasitoid from Barbados).

**Material Examined.** BARBADOS, Christ Church Parish, Rockley, 8 Sep 2009, M. A. Ciomperlik (from *Hypogeococcus* sp.) [4 females, 4 males, BMNH].

**Host.** Hyperparasitoid of the *Harrisia* cactus mealybug, *Hypogeococcus* sp., in Barbados via *Leptomastidea hypogeococci* [as *Leptomastidea* sp.] (Noyes & Triapitsyn 2018).

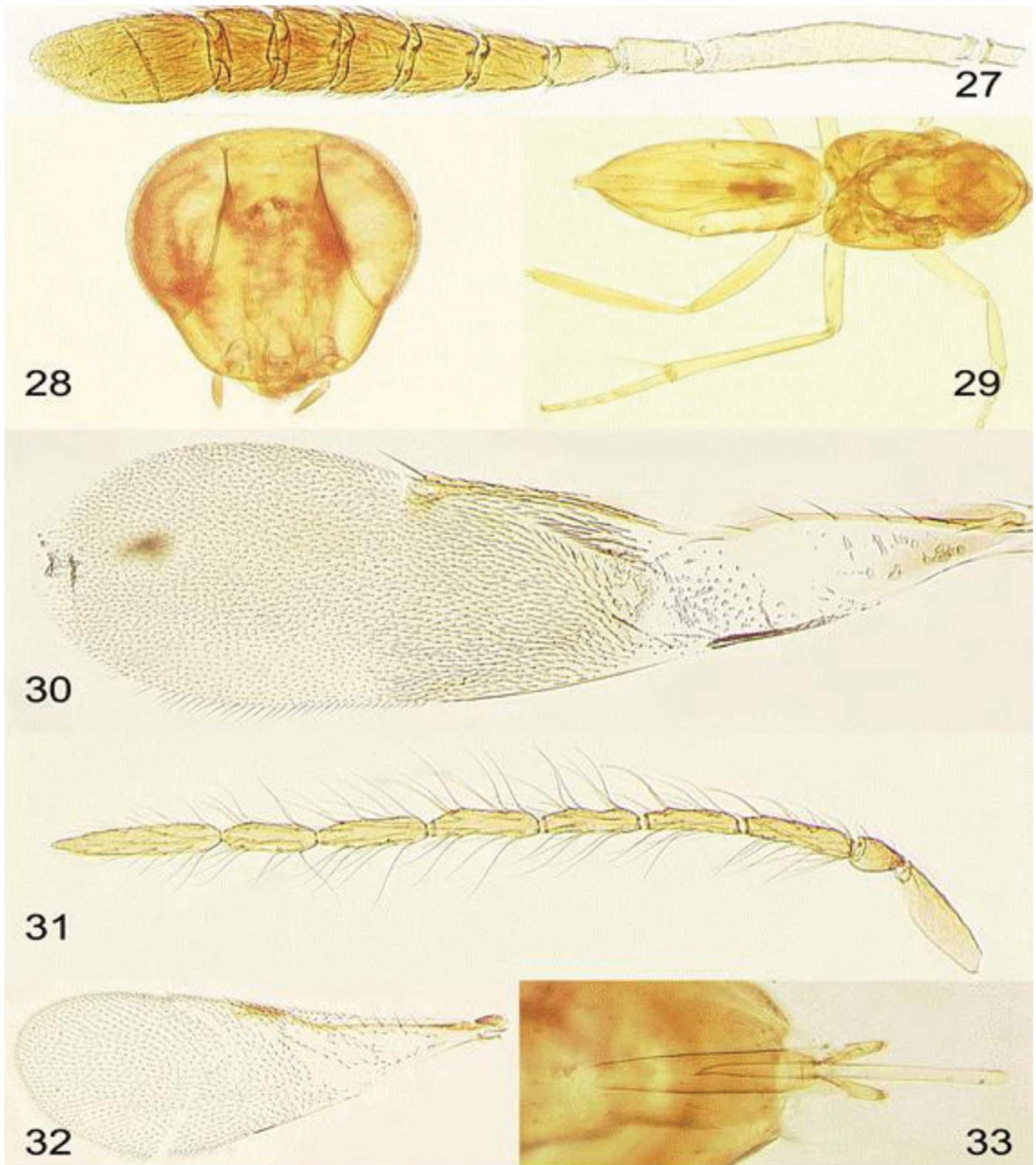
**Comments.** This species is quite likely not conspecific to any of the known species of *Cheiloneurus* Westwood from the New World although it is somewhat similar to *Ch. banksi* (Howard), of which *Ch. amplicornis* is a junior synonym (Trjapitzin 2002), per the identification labels of John S. Noyes (BMNH) and personal communication of Robert L. Zuparko (California Academy of Sciences, San Francisco, California, USA). However, we are reluctant to describe it as a new taxon because taxonomy of this genus is very difficult; its Neotropical fauna in particular is poorly known, and there is no identification key available. Its female antenna certainly does not fit any of those New World species of *Cheiloneurus* illustrated in Trjapitzin



Figs. 25–26. *Cheiloneurus* sp. near *banksi* (Rockley, Christ Church Parish, Barbados): 25, habitus of female; 26, habitus of male.

& Zuparko (2005) and Trjapitzin & Triapitsyn (2008). Illustrated here, to facilitate recognition of this interesting undescribed species, are its female

(Figs. 25, 27–30) and male (Figs. 26, 31–33). Body length of the dry-mounted females is 0.96 to 1.47 mm, and that of males is 0.83 to 0.96 mm.



Figs. 27–33. *Cheiloneurus* sp. near *banksi* (Rockley, Christ Church Parish, Barbados): 27, female antenna; 28, female head (frontal view); 29, female mesosoma and metasoma; 30, female fore wing; 31, male antenna; 32, male fore wing; 33, male genitalia.

*Prochiloneurus argentinensis* (De Santis, 1964) (Encyrtidae)

(Figs. 34–35)

*Achrysopophagus argentinensis* De Santis 1964: 357–359. Type locality: San Javier, Tucumán, Argentina. Holotype female [MLPA], photographs examined (Noyes & Triapitsyn 2018).

*Prochiloneurus* sp.: Triapitsyn et al. 2014a: 178 (female from El Portezuelo, Catamarca, Argentina), 184 (illustration).

*Prochiloneurus argentinensis* (De Santis): Löhr et al. 1990: 423 (list of hyperparasitoids); Noyes & Triapitsyn 2018: 14–15 (mentioned), 18 (illustrations of the female from Catamarca, Argentina), 19 (illustrations of the holotype), 21 (mentioned), 22–25 (resurrection as a valid taxon from the previous synonymy under *Prochiloneurus dactylopii* (Howard), taxonomic history, redescription, diagnosis, distribution, host associations), 34 (mentioned).

**Material Examined.** ARGENTINA, Córdoba, near Mina Clavero, Vivero Córdoba, 31.7576°S, 64.9724°W, 1015 masl, collected 31 Mar 2017, M. B. Aguirre, S. D. Hight, G. A. Logarzo, S. V. Triapitsyn, emerged 17 to 24 Apr 2017 (from *Hypogeococcus* sp. on San Pedro cactus, *Echinopsis pachanoi*) [2 females, UCRC].

**Other Material Examined.** ARGENTINA, Misiones, Loreto, 14 Jul 1935, A. A. Ogloblin (“s/ larva de *Pentilia egena* Musl. [sic]”) [1 female, 2 males, MLPA].

**Description.** Male (previously unknown; from Loreto, Misiones, Argentina). Illustrated are its habitus (Fig. 34) and antennae (Fig. 35). Most of mesofemur (except basally) and metatibia dark.

**Distribution.** Argentina (Catamarca, Córdoba [new record], Misiones [new record] and Tucumán), Brazil, and Guyana (De Santis 1964; Löhr et al. 1990; De Santis & Fidalgo 1994; Triapitsyn et al. 2014a [as *Prochiloneurus* sp. in Catamarca]; Noyes & Triapitsyn 2018).

**Hosts.** A hyperparasitoid of *Hypogeococcus pungens* s. str. on *Alternanthera pungens*, probably via an *Anagyrus* sp. primary parasitoid of this mealybug in Catamarca, Argentina (Triapitsyn et al. 2014a [as *Prochiloneurus* sp.]; Noyes & Triapitsyn 2018), and also of *Hypogeococcus* sp. (likely via *A. quilmes* in Córdoba), as well as of *Phenacoccus herreni* Cox & Williams (Pseudococcidae) (Löhr et al. 1990; De Santis & Fidalgo 1994). The above record of this species from a larva of *Pentilia egena* Mulsant (Coleoptera: Coccinellidae) is interesting but quite unusual, so we do not really know how trustworthy it is, even considering that A. A. Ogloblin was an experienced and very knowledgeable entomologist.

*Prochiloneurus narendrani* Noyes & Triapitsyn, 2018 (Encyrtidae) (Fig. 36)

*Prochiloneurus narendrani* Noyes & Triapitsyn 2018: 15–21. Type locality: Rockley, Christ Church, Barbados. Holotype female [BMNH], examined (Noyes & Triapitsyn 2018).

**Material Examined.** PUERTO RICO (USA): Mona Island, 12 Feb 2017, M. B. Aguirre, G. A. Logarzo (from *Hypogeococcus* sp. on Portulacaceae) [1 female, 1 male, UCRC]. Vieques Island, Heliport Monte Pirata, 17 Feb 2017, M. B. Aguirre, G. A. Logarzo (from *Hypogeococcus* sp. on *Achyranthes aspera*) [1 female, UCRC].

**Description.** Male (previously unknown; from Mona Island, Puerto Rico). Body length (of the dry-mounted, critical point dried specimen, Fig. 36) 0.76 mm. Body mostly grayish-brown except head and scutellum a little lighter and base of gaster light brown; antenna brownish, legs mostly light brown.

**Distribution.** Barbados and Puerto Rico (Mayagüez (Noyes & Triapitsyn 2018), Mona and Vieques Islands [new records]).

**Host.** Hyperparasitoid of the *Harrisia* cactus mealybug, *Hypogeococcus* sp., in Mayagüez, Puerto Rico, via *Leptomastidea hypogeococci* [as *Leptomastidea* sp.] (Noyes & Triapitsyn 2018).

## TAXONOMIC NOTES ON OTHER NEOTROPICAL SPECIES OF ANAGYRUS

*Anagyrus tanystis* De Santis, 1964

(Figs. 37–41)

*Anagyrus tanystis* De Santis 1964: 61–63. Type locality: Autonomous City of Buenos Aires, Argentina.

*Anagyrus tanystis* De Santis: Triapitsyn et al. 2014b: 221 (key).

**Type Material Examined.** Holotype female [MACN] on slide (Fig. 37) labeled: 1. “CAPITAL FEDERAL Col: A. Zotta I-II/1913”; 2. [partially printed in red] “*Anagyrus tanystis* Det. De Santis HOLOTIPO MUSEO DE LA PLATA”; 3. [MACN type number] “A 89”.

**Distribution.** Autonomous City of Buenos Aires, Argentina (De Santis 1964).

**Hosts.** Unknown.

**Comments.** The holotype female of *A. tanystis* is dissected under 1 large square coverslip (Fig. 37); it lacks almost the entire flagellum of 1 of the antennae except for about a half of F1. Illustrated here, to facilitate its recognition, are its features (Figs. 38–41). This species is known only from a single specimen, which was not examined by Triapitsyn et al. (2014b) during preparation of the key to the described species of *Anagyrus* in Argentina.

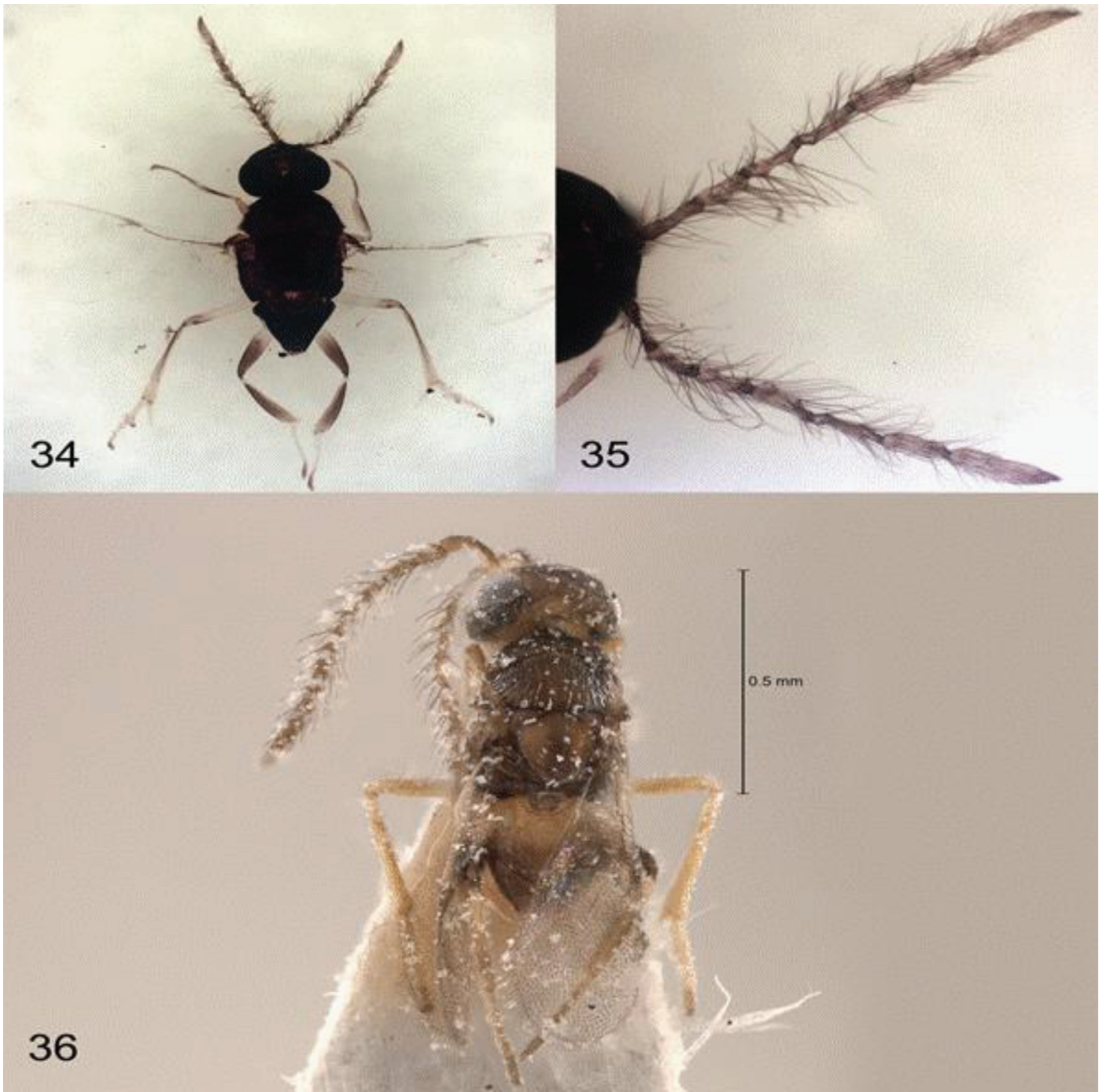
## GENETIC ANALYSIS

DNA sequences of all 3 loci (28S, ITS2 and COI) were successfully obtained for 29 of 30 specimens. The only “shortfall” was the COI of a single specimen (PR17-254) from Argentina, which failed to amplify despite multiple attempts. Direct-sequencing of the COI locus also failed for a second specimen from Argentina (PR17-256) and 3 from Brazil (PR17-247 through 249) due to co-amplification of a NUMT (GenBank accessions MG748829-832). However, a valid COI sequence was obtained for each of these specimens by amplicon cloning.

ML analyses of the concatenated 28S (MG731466-495) and COI (MG731496-524) sequences recovered 3 distinct clades (Fig. 42). These 3 clades corresponded to the species *Leptomastidea hypogeococci*, *Anagyrus cachamai*, and *Anagyrus quilmes*. Although discarded for the purpose of phylogenetic inference, sequences of ITS2 (MG731525-554) identified the same 3 clades.

*Leptomastidea hypogeococci* – DNA sequences of the rRNA loci 28S and ITS2 were identical across specimens from Puerto Rico, Argentina, and Brazil, with the slight exception that the 28S sequence of 3 specimens (PR17-253 and PR17-257 from Puerto Rico, and PR17-254 from Argentina) harbored a heterozygous peak at position 140 (G or A, rather than just a G). The Puerto Rican specimens shared an identical COI haplotype, but on average this differed from the COI of Brazilian and Argentinian specimens (obtained from cloned amplicons) by 3.7% (average pairwise p-distance = 0.037). Variation in COI among Brazilian and Argentinian specimens was 10-fold lower (average p-distance = 0.003).

*Anagyrus cachamai* – DNA sequences of 28S were more or less identical across specimens from Argentina and Paraguay, with just a separate single base insertion at position 447 and 451 in specimens PR17-246 (Paraguay) and PR17-252 (Argentina), respectively. ITS2 was more variable, with 26 single nucleotide substitutions, insertions or deletions across 5 specimens and 695 aligned bases. In addition, 2 specimens (PR14-212 and PR14-213; MG731548-548) differed from the other 3 (MG731534-536) with a 29bp deletion (positions 148-176). These 2 specimens also shared a COI haplotype (MG731518-519) that differed from that of the other 3 specimens (MG731504-506) by over



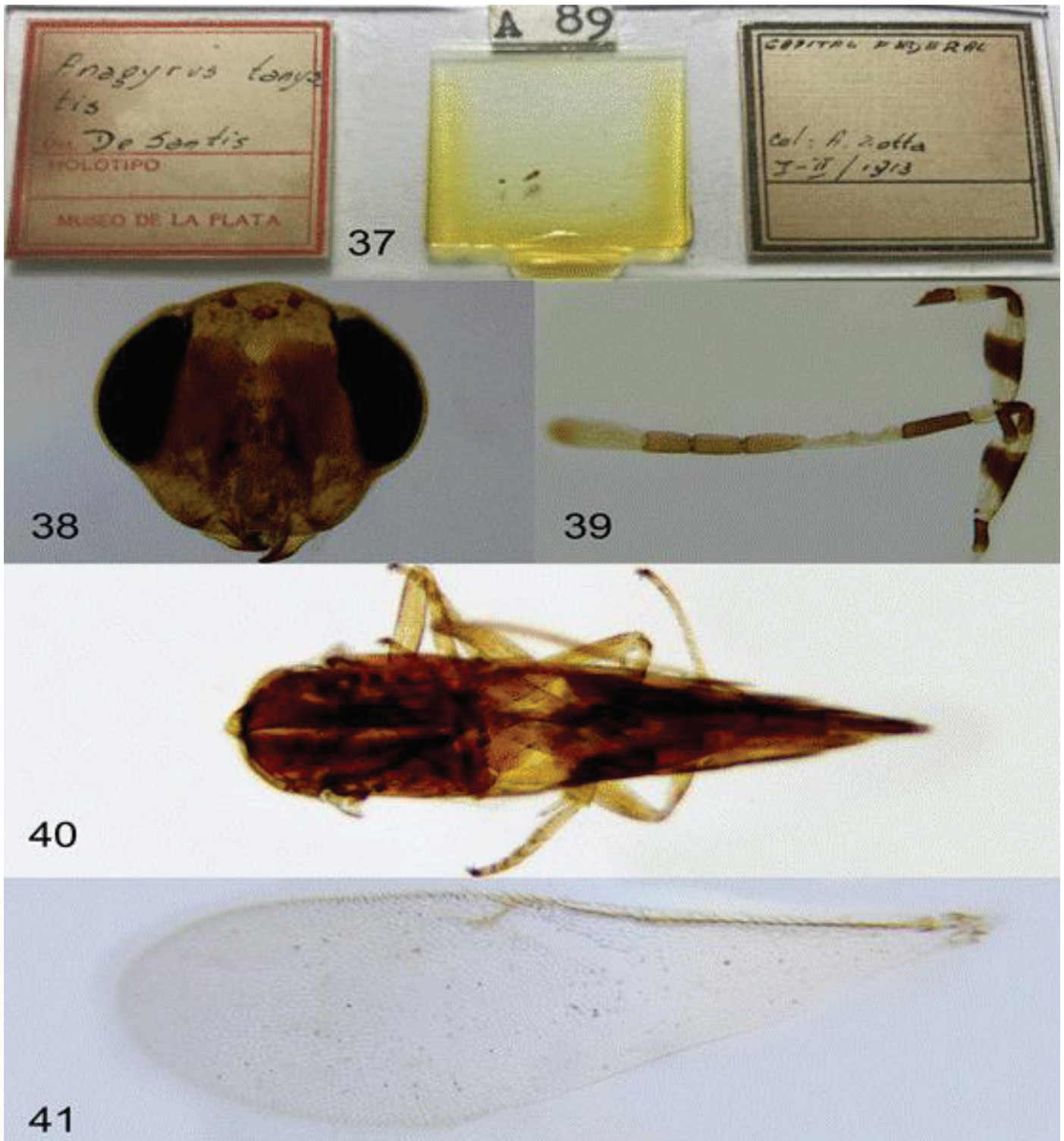
**Figs. 34–36.** *Prochiloneurus* spp. males (34–35, *P. argentinensis*, Loreto, Misiones, Argentina [photographs courtesy of D. A. Aquino]; 36, *P. narendrani*, Mona Island, Puerto Rico): 34, habitus; 35, antennae; 36, habitus.

5% (average pairwise p-distance = 0.054). Variation among the latter 3 specimens was 4-fold lower (average p-distance = 0.013).

*Anagrus quilmes* – DNA sequences of 28S and ITS2 were identical across all specimens from Puerto Rico, Argentina, Brazil, and Paraguay. Eight COI haplotypes were detected, and there was a strong phylogeographic signal in the distribution of these haplotypes (Fig. 43). Pairwise divergence between 7 of these haplotypes was typically low (average pairwise p-distance = 0.005), but the remaining haplotype, harbored by PR14-214, differed from all others by over 5% (average pairwise p-distance = 0.054).

#### BIOLOGICAL CONTROL OF THE HARRISIA CACTUS MEALYBUG IN PUERTO RICO

The biological control program for *Harrisia* cactus mealybug in Puerto Rico was advanced with 2 shipments of 2 parasitoid species from South America to the quarantine facility at the University of Puerto Rico Center for Excellence in Quarantine & Invasive Species. The species transported to the quarantine facility were *A. cachamai* and *A. lapachosus*. Both parasitoid species accepted the Puerto Rico *Harrisia* cactus mealybug, and host range tests were initiated. Quarantine host range tests are expected to

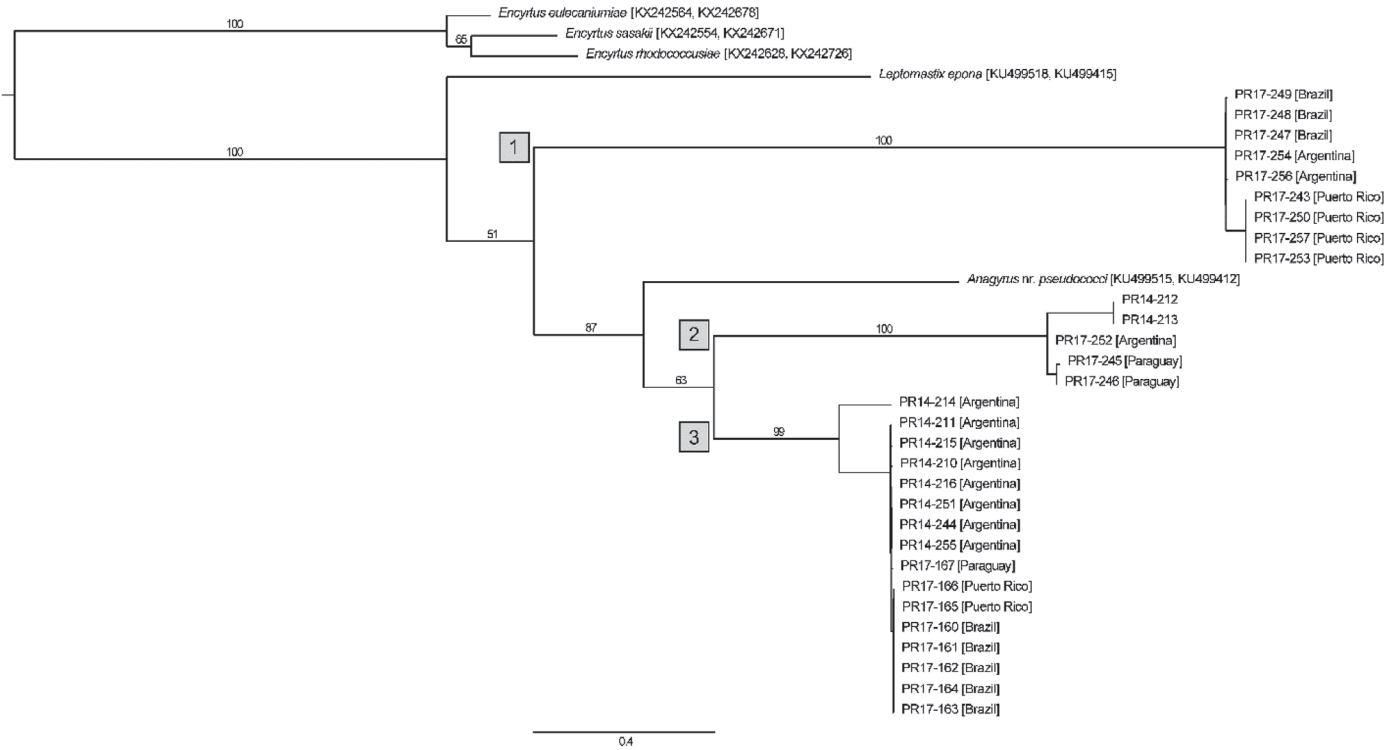


Figs. 37–41. *Anagyrus tanystis* female (holotype): 37, slide; 38, head (frontal view); 39, antennae; 40, mesosoma and metasoma; 41, fore wing.

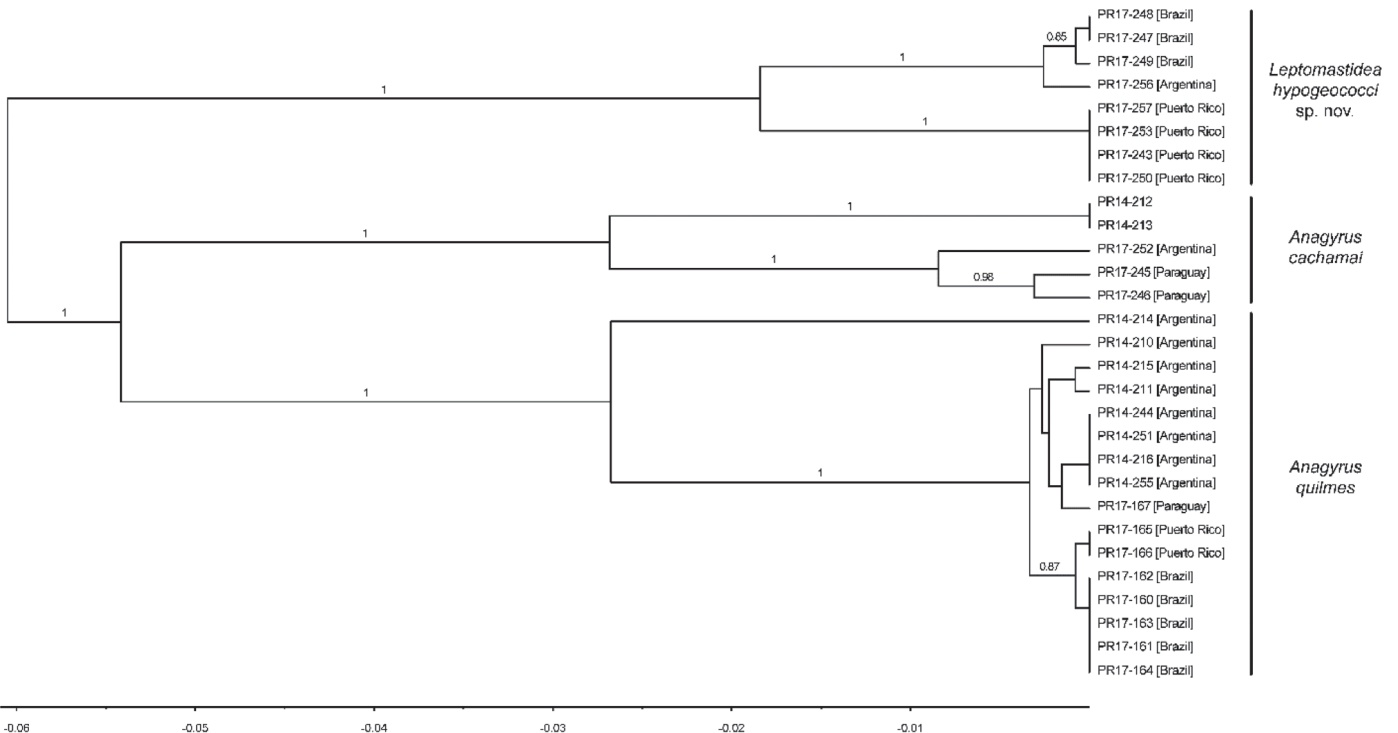
be completed by spring 2018, and a request for field release should be submitted before summer 2018. In addition, studies on natural enemy population dynamics and parasitoid biological parameters are planned for the 2 quarantined parasitoid colonies from South America, and a newly described parasitoid species already established and attacking the *Harrisia* cactus mealybug in Puerto Rico. This project is poised to successfully develop the only method that challenges the *Harrisia* cactus mealybug pest before we experience the devastation of cactus biodiversity in Puerto Rico.

## Acknowledgments

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**Fig. 42.** Phylogenetic placement of Tetracneminae specimens inferred from ML analysis of concatenated sequences of 28S and COI. The analysis was conducted in RAxML and branch support, expressed as a percentage, was assessed with 1,000 rapid bootstrap replicates. Clade 1 = *Leptomastidea hypogeococci*; clade 2 = *Anagyrus cachamai*; clade 3 = *Anagyrus quilmes*. *Encyrtus* spp. (the outgroup) belong to the subfamily Encyrtinae.



**Fig. 43.** Genetic variation in mitochondrial COI among specimens of 3 Tetracneminae (Encyrtidae) species inferred using the UPGMA method in MEGA6. The tree is drawn to scale, and branch lengths represent uncorrected p-distance. Branch support was assessed with 1,000 bootstrap replicates.

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