UNIVERSITY OF CALIFORNIA
RIVERSIDE

Systematic Studies of the Parasitoid Wasp Genus *Cales* (Chalcidoidea: Aphelinidae): Combined Molecular and Morphological Approaches to Classification and Evolution

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

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December 2012

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ACKNOWLEDGEMENTS

This work would not have been possible without the boundless energy, enthusiasm and insight of my major advisor, John Heraty. He has gone above and beyond merely providing the resources necessary to perform quality research in insect systematics; he has provided a life changing and life enhancing experience. During just some of our foreign expeditions together, I have road-tripped across the South African countryside, browsed the colorful vibrancy of Nigerian street markets, hiked through Costa Rican cloud forests, and traversed the rivers of Borneo in longboats piloted by the decendants of head hunters. I am not only a well-trained scientist, but also a better citizen of the world.

Part of the text of this dissertation (Chapter 1) is a reprint of the material as it appears in *Systematic Entomology*, volume 32, issue 2, pp. 267-284, April 2011. The co-author (John Heraty) listed in that publication directed and supervised the research, which forms the basis for this dissertation. The second co-author (Emily Hartop) was an undergraduate student who helped with the scanning electron microscopy, specimen curation, and contributed to an early draft of the new species description. I am grateful John Wiley and Sons for granting the license allowing me to reproduce this work in my dissertation, and I also thank the subject editor, Lars Vilhelmsen, and three anonymous reviewers who substantially improved the manuscript with their comments and constructive criticisms. I also thank Jocelyn Berry of Biosecurity New Zealand for collecting the *Cales* specimens from New Zealand, Gary Gibson for insightful

discussions regarding chalcidoid morphology, and Lars Krogmann for graciously provided images of the internal structures of the mesosoma.

For the revision of *Cales* (Chapter 2), I am indebted to John Noyes at the Natural History Museum, London, whose extensive collecting efforts in Costa Rica provided many of the specimens used in this study. Richard Stouthamer and Paul Rugman-Jones provided helpful training and suggestions for gene amplification and sequencing. I also thank Vladimir Berezovskiy (UCR Entomology Research Museum) for slide-mounting these very challenging specimens, and John Hash and Elizabeth Murray who provided insightful comments on early drafts.

For the analyses of fore wing shape (Chapter 3) I thank Serguei Triapitsyn who provided access to the UCR-I&Q records and helped me to navigate this record-keeping system. I also thank Richard Redak and Greg Walker for helpful discussions regarding the statistical analyses.

I must thank my parents (Marta and Lewis Mottern) and sister (Lisa Barner), who have never wavered in their support and encouragement, despite my constant need to take turns down the roads less traveled. Finally, I thank Rebeccah Waterworth for her constant love and support as we shared our graduate school experiences together.

ABSTRACT OF THE DISSERTATION

Systematic Studies of the Parasitoid Wasp Genus *Cales* (Chalcidoidea: Aphelinidae): Combined Molecular and Morphological Approaches to Classification and Evolution

by

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University of California, Riverside, December 2012
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Calesinae is a small group of chalcidoid wasps that, for species with known host associations, are parasitoids of whiteflies (Hemiptera: Aleyrodidae). Prior to this study, *Cales* diversity included one species in the Neotropical region (*Cales noacki*, introduced from South America into citrus growing regions of North America, the Mediterranean and tropical Africa for biological control of woolly whitefly), and two species from Australia (*Cales spenceri* and *Cales orchamoplati*). The morphological study emphasizes the likely plesiomorphic Australian species, and includes a description of a new species from New Zealand reared from the whitefly *Asterochiton pittospori*. *Cales* shares many characteristics with Aphelinidae, though additional studies of character systems across Chalcidoidea are needed to determine the likely sister taxon.

Studies of the Neotropical Cales fauna reveal far greater diversity than previously thought. Twenty-one new species are described, and a neotype is designated for *C. noacki,* which is redescribed based on specimens molecularly determined to be conspecific with the neotype. The Neotropical Cales fauna is very morphologically

conserved, so species cannot always be determined based on morphology alone. Therefore, species boundaries are established using combined evidence from morphology, biogeography, 28S-D2-5 rDNA and a 390bp fragment of the cytochrome oxidase c subunit I (COI) gene. A molecular phylogeny and separate identification keys to male and female species are provided.

The molecular phylogenetic studies of *Cales* revealed that two molecularly distinct but morphologically cryptic species were introduced into California for control of woolly whitefly in citrus. The most common of the two was determined to be *C. noacki*, which molecularly matched specimens from Chile, and the second was newly described as *Cales rosei*, which did not molecularly match any of the specimens collected from Central or South American. We employ multivariate analyses of fore wing shape combined with biological control importation and release records to infer the likely source locality for *C. rosei*. The analyses support a Chilean origin of *C. noacki*. In addition, the wing shapes of molecularly-determined *C. rosei* specimens most closely matched biological control specimens collected near Buenos Aires, Argentina, indicating that this is the likely source locality for this species.

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INTRODUCTION

The superfamily Chalcidoidea is a hyper-diverse group of small or minute wasps, most of which are parasitoids of other insects. Currently about 22,000 species are described in 19 families, with estimates up to 500,000 species worldwide (Heraty and Gates, 2003; Noyes, 2012). The extremely small size of most members of the superfamily renders them challenging to study. From a technical perspective, specialized collecting, curation and microscopy techniques are generally required to examine specimens in sufficient detail for systematic work. However, once these skills are mastered, the true bugbears of chalcidoid systematics emerge: rampant homoplasy as a consequence of miniaturization and overwhelming species diversity. Morphological consequences of miniaturization often include reversion to plesiomorphic states, structural simplification, increased variability, and morphological novelty (Hanken and Wake, 1993). Structural simplification is especially prevalent in chalcidoid wasps. Hence, similar structures may be the result of parallel reduction rather than common ancestry. Also, similar parasitic life histories often result in similar morphological adaptations. The result is homoplasy that often obscures phylogenetic signal, at least in morphological data.

Despite these difficulties, the monophyly of some chalcidoid groups is clearly established by morphological and/or molecular synapomorphies. Other groups, including some families, are likely paraphyletic or even polyphyletic. Consequently, much systematic work remains to be done, not only at the level of alpha taxonomy to generate a better understanding of chalcidoid species diversity, but also at higher taxonomic levels within the group.

The family Aphelinidae is among the chalcidoid groups in need of systematic attention. These are minute wasps ranging from 0.5 to 2.5 mm. Most genera are parasitoids of sternorrhynchus Hemiptera (aphids, whiteflies and scale insects), and a few are known to parasitize eggs or immature Orthoptera, Coleoptera, Heteroptera, Diptera, Hymenoptera and Lepidoptera (Polaszek, 1991; Viggiani, 1984; Yasnosh, 1979). Because their hosts are often pests of agricultural or ornamental crops, many aphelinids have been used as biological control agents against plant-feeding pests.

The taxonomic history of the Aphelinidae has been unstable, and remains so to this day. Workers have not reached a consensus regarding the relationships of Aphelinidae within Chalcidoidea, nor are the relationships among subfamilies, tribes and genera well understood. Based on a shared structure the mesosternum, Gibson (1989) has suggested a monophyletic grouping of Aphelinidae+Signiphoridae. Woolley (1988) proposed that Aphelinidae was paraphyletic without the inclusion of Signiphoridae. Hayat (1985, 1994) proposed a system of 4 subfamilies and 8 tribes of Aphelinidae: Aphelininae (Aphelinini, Aphytini, Eutrichosomellini), Eriaphytinae, Coccophaginae (Coccophagini, Azotini, Pteroptricini) and Eriaporinae (Eriaporini, Euryischini). The monogeneric subfamily Calesinae (heretofore referred to as *Cales* to simplify discussion) was excluded but not placed within another chalcidoid family, leaving it *incertae sedis* within Chalcidoidea. Hayat's (1998) classification divides the family into 6 subfamilies and 8 tribes: Aphelininae (Aphelinini, Aphytini, Eretmocerini and Eutrichosomellini), Eriaphytinae, Azotinae, Coccophaginae (Coccophagini, Euxanthellini, Physcini and Pteroptricini), Eriaporinae, and Euryischiinae. Studies of aphelinid morphology (Heraty

et al., 1997) and molecular systematics (Campbell et al., 2000, Munro et al., 2011) have suggested that Aphelinidae is most likely a polyphyletic assemblage, consisting of mostly monophyletic subfamilies. However, a recent combined analysis of molecular and morphological characters for 300 chalcidoid taxa recovered a sister-group relationship between Aphelininae and Coccophaginae, with *Cales* included within the Aphelininae (Heraty et al., 2012). There status of Aphelinidae was revised to include only Aphelininae and Coccophaginae. Therefore, *Cales* is currently considered a member of the Aphelinindae *sensu stricto*.

This study includes three main objectives. First, a detailed morphological examination of *Cales* is included, with emphasis on species from Australia and New Zealand. Second, the Neotropical species of *Cales* will be revised, with species boundaries established using molecular phylogenetic techniques combined with morphology and biogeography. Finally, a study using multivariate analysis of wing shape will be used to determine the likely origins of *Cales* populations used for biological control of woolly whitefly in citrus.

CHAPTER 1 – *Cales* (Hymenoptera: Chalcidoidea): morphology of an enigmatic taxon with a review of species

ABSTRACT

Calesinae is a small group of Chalcidoidea (Hymenoptera) that are parasitoids of whiteflies (Hemiptera: Aleyrodidae). One species, *Cales noacki* Howard, has been introduced from South America into citrus growing regions of North America, the Mediterranean and Africa for biological control. The remaining species are found in Australia and New Zealand – a classic Gondwanan disjunction. The subfamily consists of a single genus, *Cales*, which is currently unplaced within Chalcidoidea. Its taxonomic position has historically been unstable, though most often *Cales* is associated with Aphelinidae. Here we present a detailed morphological study of the group with an emphasis on Australian species. Though *Cales* shares many characteristics with Aphelinidae, especially Coccophaginae and *Eretmocerus*, more studies of character systems across Chalcidoidea are needed to determine which features may be synapomorphic. Consequently, we leave *Cales incertae sedis* within Chalcidoidea. We also describe a new species from New Zealand, *Cales berryi* **n. sp.**, reared from the whitefly *Asterochiton pittospori* on lemonwood, *Pittosporum eugenioides*, and we present a key and review the four known species of *Cales*.

INTRODUCTION

Although the monophyly of some chalcidoid groups is strongly supported by morphological or molecular synapomorphies, many higher-level relationships within the superfamily remain uncertain. Establishing relationships within Chalcidoidea is hindered by a lack of comprehensive morphological studies, either across character systems or across taxa. Some detailed studies are available (*e.*g. Gibson, 1989; Heraty *et al*., 1994, 1997; Basibuyuk & Quick, 1995; Darling, 1988; Heraty & Schauff, 1998; Krogmann & Vilhelmsen, 2006), but more information is needed to determine the utility of various morphological features for phylogenetic reconstruction. Wide diversity of form and function, resulting in uncertainty regarding sister-group relationships, has left some chalcidoid groups unplaced into higher taxonomic categories, and several families are regarded as either paraphyletic or polyphyletic (Gibson *et al*., 1999; Campbell *et al*., 2000). The genus *Cales* Howard is an economically important and easily defined group that currently defies placement into any family within Chalcidoidea.

Taxonomic history. The taxonomic history of *Cales* and its higher classification is complex, and reflects the uncertainty various workers have encountered when trying to classify the group. *Cales noacki* was first described from specimens reared from *Orthezia* sp. (Hemiptera: Pseudococcidae) in Campinas, Brazil (Howard, 1907). Howard placed this species in the tribe Pteroptricini (now Aphelinidae: Coccophaginae), which was then included in the subfamily Aphelininae. Brèthes (1914) described *Diaspidophilus pallidus* from specimens reared from the white peach scale, *Pseudaulacaspis pentagona* (Targioni) (Hemiptera: Diaspididae) in Argentina, and placed it in the family Mymaridae.

Mercet (1929) synonymized *Diaspidophilus* with *Cales* and erected the new subfamily Calesinae within Aphelinidae. Dozier (1933) placed *Cales* within Trichogrammatidae while studying whitefly parasitoids in Haiti. *Paranthemus spenceri* Girault was described from Australia and placed in Mymaridae (Girault, 1915), but *Paranthemus* was later synonymized with *Cales* by Viggiani (1981). A third species was reared from an Australian whitefly, *Orchamoplatus citri* (Takahashi) (Hemiptera: Aleyrodidae), and described as *Cales orchamoplati* by Viggiani & Carver (1988). A fourth species, *Cales berryi* n. sp., was reared from the whitefly *Asterochiton pittospori* in New Zealand, and is described herein. Another species originally placed in Calesinae by Risbec (1957), *Neocales phillipiae* Risbec, was transferred to *Chartocerus* (Signiphoridae) by Polaszek (1993).

Historical reviews of *Cales* classification are sometimes in conflict, possibly because the morphological peculiarities of the group rendered many authors reluctant to draw firm conclusions regarding their taxonomic affinities. Changes in taxonomic placement of *Cales* are reviewed by Hayat (1994), Heraty and Schauff (1998) and Gibson *et al*. (1999). Briefly, *Cales* is most often included within Aphelinidae (De Santis, 1946; Ferrière, 1965; Yasnosh, 1976; Shafee & Rizvi, 1990), though several studies have suggested that *Cales* is closer to Trichogrammatidae or Eulophidae (Viggiani & Battaglia, 1984; Polaszek, 1991; Hayat, 1994; Heraty *et al*., 1997). Most recently, Hayat (1994) excluded *Cales* from Aphelinidae, and it has since remained *incertae sedis* within Chalcidoidea. Although based on limited sampling (*C. noacki* only), molecular studies have shown *Cales* to be isolated as a unique lineage, distinct from Aphelinidae,

Eulophidae or Trichogrammatidae, and potentially the sister group of Chalcidoidea, excluding Mymaridae (Campbell *et al*., 2000). A developmental study of *C. noacki* (Laudonia & Viggiani, 1986) indicates that the larval stages are distinct from other aphelinids and possibly unique within Chalcidoidea.

Biology and biological control. Most information about *Cales* biology is based upon observations of *C. noacki*. The first substantiated host species for *C. noacki* was the woolly whitefly, *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) (Dozier, 1933). It was apparently reared earlier from Pseudococcidae and Diaspididae (Howard, 1907; Bréthes, 1914), but these host records have not been substantiated through subsequent rearing. It was also reared from eggs of *Phalera bucephala* Linnaeus (Lepidoptera: Notodontidae) (Viggiani & Currado, 1978), but here may have been acting as a hyperparasitoid of another wasp (Polaszek, 1991). *Cales* are primarily endoparasitoids of the larval instars of various aleyrodids (Dozier, 1933; Debach & Rose, 1976; Rose & Woolley, 1984). The parasitoids attack second, third or fourth instar nymphs and then emerge from the mummified host remains (Miklasiewicz & Walker, 1990).

When the woolly whitefly was discovered on California citrus in the late 1960s, *C. noacki* was introduced into Baja California and Southern California from Chile and Peru for biological control (DeBach & Rose, 1976). Since then, the continued spread of the woolly whitefly has resulted in the introduction and establishment of *C. noacki* in citrus growing regions around the Mediterranean (European and Mediterranean Plant Protection Organization, 2002), including the Canary Islands, Madeira, and the Azores

(Rodríguez-Rodríguez, 1977a,b; Hernández-Suárez et al., 2003). *Cales noacki* was also introduced into Uganda and Kenya where it has successfully established and provided substantial control of woolly whitefly populations (Legg *et al*., 2003). Additional studies of *C. noacki* in North America following its introduction as a biological control agent have revealed a broader host range. *Cales noacki* is known to successfully parasitize the mulberry whitefly, *Tetraleurodes mori* (Quaintance); the acacia whitefly, *Tetraleurodes acaciae* (Quaintance); and the red-banded whitefly, *Tetraleurodes perseae* Nakahara, a minor pest of avocado in California and Mexico (Rose &Woolley, 1984; Hoddle, 2006). In their survey of whitefly parasitoids in Haiti, Evans and Serra (2002) found *C. noacki* emerging from *A. floccosus* as well as an undescribed *Aleurothrixus* species. Viggiani and Laudonia (1984) reared *C. noacki* from the viburnum whitefly, *Aleurotuba jelineki* (Frauenf.) in Campania, Italy. *Cales noacki* was found in the Azores, Canary Islands, and Madeira attacking a five different whitefly species in five different genera (Hernández-Suárez *et al*., 2003). By contrast, *C. noacki* was found to be specific to *A. floccosus* in citrus-growing regions of the Eastern Mediterranean by Vatansever and Ulusoy (2005). The apparent high degree of polyphagy and preliminary molecular data (Heraty, unpub.) suggest that the name "*C. noacki*" encompasses a cryptic species complex. However, the present study is concerned with the morphology and taxonomic status of *Cales* as a group, and does not attempt to address possible cryptic species within *C. noacki*, which will require extensive new collections of fresh sequenceable material from across South and Central America.

Thus far, *C. noacki* is the only *Cales* species that has been used as a biological control agent. However, the recent introduction of *O. citri* to New Zealand and subsequent damage caused by this pest to the New Zealand citrus industry has opened the possibility of using one or more Australian *Cales* for biological control (Pyle *et al*., 2005). Recent surveys for natural enemies of *O. citri* in New Zealand citrus have indicated that *Cales* is not yet present in the natural enemy complex (Jamieson *et al*., 2009)

Biogeography. *Cales* species exhibit a Gondwanan biogeographic pattern with one center of diversity in Australia/New Zealand, and another morphologically distinct lineage in the New World tropics. Within Chalcidoidea, this distribution is shared with Lycisini (Pteromalidae: Cleonyminae) (Gibson, 2003) and Rotoitidae, with the latter being an early branching taxon that appears to be sister to the rest of Chalcidoidea, excluding Mymaridae (Heraty, unpub.).

Purpose and scope. Here, we examine the adult morphology of *Cales* in greater detail than previous studies, with greater emphasis on Australian species, describe *Cales berryi* **n. sp.** from New Zealand, and provide a key to the world species. *Cales* is included in an ongoing comprehensive phylogenetic study of Chalcidoidea, and therefore no phylogenetic analysis is conducted herein. The many unique features of the New World *C. noacki* provide some justification for resurrecting *Paranthemus* to refer to species from Australia and New Zealand. However, there is little doubt that *Cales* is monophyletic, and the group contains relatively few species. Therefore, in the interest of simplicity and nomenclatural stability, we do not subdivide *Cales* into multiple genera.

MATERIALS AND METHODS

Curation and imaging. Specimens for point mounts or scanning electron microscopy (SEM) were dried from ethanol using hexamethyldisilizane (HMDS) (Heraty & Hawks, 1998). Slide mounts were prepared either in Hoyers medium or Canada Balsam. Scanning electron micrographs were taken with a Phillips XL30-FEG. Line drawings of wings and genitalia were produced using a camera lucida mounted on a Leica DMRB compound microscope. Pencil drawings were subsequently scanned and electronically "inked" using Adobe Illustrator CS4. Pictures of slide-mounted wings and genitalia were made using Automontage™ (Syncroscopy) with images captured by a JVC 3-CCD camera mounted on a Zeiss Axioskop2 compound microscope.

Species key. The key should work equally well for both sexes. Males are known from all described species, but females are not known for *C. spenceri*. Slide preparation of specimens will generally be required to differentiate among Australian and New Zealand species. *Cales noacki* is sufficiently distinct that identification from point and card mounted specimens is possible.

Species reviews. A synopsis of each species is provided including list of synonymy, remarks used for distinguishing among the four species, and material examined. For *C. spenceri*, *C. orchamoplati*, and the newly described species, most, if not all, of the known specimens have been examined. Known geographic distributions can be inferred from the material examined lists for these specimens, but geographic distributions for *C. spenceri* and *C. orchamoplati* are based on very few specimens and should be interpreted cautiously. Owing to its status as a biological control agent, it was

not practical to examine all known specimens of *C. noacki*. However, 104 specimens were examined, including representatives from the Caribbean, North America, North Africa, and Europe.

Species description. Quantitative data were taken from slide-mounted specimens in the type series. Measurements were only recorded if the structure was reasonably flat and mostly visible within a single focal plane. Most measurements were made of the maximum length and/or width of a structure. Measurements requiring additional explanation are as follows: fore wing length = distance from the distal end of humeral plate to apex of wing disc; fore wing width = maximum distance across wing disc perpendicular to long axis of wing; hindwing length = distance from proximal end of humeral plate to apex of wing disc; hindwing width = maximum distance from base of hamulus to the posterior margin of wing. For an explanation of how the multiporous plate sensillum length was measured, see the inset on Fig. 1.4H, and for an explanation of how the ovipositor length was measured, see Fig. 1.8E.

Morphology and terminology. Morphological terminology is not yet standardized in the chalcidoid literature. Therefore, terms are defined and explained as they are used in the text where they may be ambiguous. Terms generally follow Gibson (1997) and Kim (2003) for general morphology, Heraty *et al*. (1997) for structures of the mesofurca, and Krogmann and Vilhelmsen (2006) for some structures of the mes- and metepisternum. The present study is restricted to adult morphology. The eggs, larval instars and pupal form of *C. noacki* were described by Laudonia and Viggiani (1986).

Museums. The following institutions served as sources of material and type depositories for specimens examined for this study. **AMNZ**: Auckland Institute and Museum, Auckland, New Zealand. **ANIC**: Australian National Insect Collection, Commonwealth Scientific and Research Organization, Canberra, ACT, Australia. **BMNH**: The Natural History Museum, London, England. **CNC**: Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture Canada, Ottawa, Ontario, Canada. **DEZA**: Dipartimento di Entomologia e Zoologia Agraria dell'Università, Portici, Italy. **MACN**: Museo Argentina de Ciencias Naturales "Bernardino Rivadavia," Buenos Aires, Argentina. **NZAC**: Landcare Research, New Zealand Arthropod Collection, Auckland, New Zealand. **QM**: Queensland Museum, Brisbane, Queensland, Australia. **UCRC**: University of California, Riverside, Entomology Research Museum, Riverside, CA, USA. **USNM**: National Museum of Natural History, Washington, DC, USA.

RESULTS

Genus *Cales*

Cales Howard 1907: 82-83. Type species: *Cales noacki*, by monotypy and original designation. Deposition: USNM.

Diaspidophilus Brèthes 1914: 15-16. Type species: *Diaspidophilus pallidus*, by monotypy and original designation. Deposition: MACN. Synonymy by Gahan in Mercet 1929: 114.

Paranthemus Girault 1915: 165. Type species: *Paranthemus spenceri*, by monotypy and original designation. Deposition: QM. Synonymy by Hayat 1983.

Remarks. Like most chalcidoid groups, *Cales* is typically defined by a unique combination of characters that individually appear to be homoplastic within Chalcidoidea rather than one or more uniquely derived characters. Previous authors have referred to the sparse setation of the fore wing and linear tracks of setae, even though these are not features of the Australian species, which have an almost uniform distribution of setae. Viggiani & Battaglia (1984) illustrated the simplified male genitalia of *C. noacki*, noting the absence of a phallobase and presence of bacilliform apodemes extending into the aedeagus in addition to the aedeagal apodemes. However, the apparent lack of phallobase appears to be in error, as a characteristically expanded phallobase is present in all species examined for this study.

Morphological description

Body. Small, 0.40–0.82 mm; weakly sclerotized.

Color. Yellowish orange or pale brown. Some specimens of *C. noacki* almost white with pale yellow or brown markings on mesoscutellum and dorsal metasoma.

Head capsule. Face with straight transfacial sulcus (**tfs**) just in front of anterior ocellus (Fig. 1.1G). Scrobal depression shallow and short; scrobal sulcus (**scs**) complete and extending dorsally to delineate tfs and upper ocellar sulcus (**uos**) (Figs 1.1A, 1.6A). Malar sulcus (**msl**) present but not reaching ventral margin of eye (Figs 1.1A, 1.6A). Margins of clypeus difficult to distinguish from rest of face, but lateral limits indicated by pair of anterior tentorial pits (**atp**) and upper margin indicated by an arched epistomal sulcus (Figs 1.1A, 1.1H, 1.6A). Head posteriorly with transoccipital sulcus (**tos**) and posterior vertical occipital sulcus (**pvs**) extending from occipital foramen to tos (Fig.

1.1B); postgena inflected medially as postgenal lobe (**pgl**) below occipital foramen (Fig. 1.1B); occipital foramen separated from the mouth cavity by hypostomal bridge (**hsb**) (Figs 1.1B, 1.1D). Posterior tentorial pit (**ptp**) visible on the hypostomal bridge medial to postgenal lobe (Fig. 1.1G *inset*).

Antenna. Radicle (**rad**) approximately 2× longer than wide in Australian species (Figs 1.6B,C) and approximately 4× longer than wide in *C. noacki* (Figs 1.4F, 1.5A). Scape (**scp**) laterally flatted and subapically expanded; pedicel (**pdl**) longer than wide and tapering basally (Figs 1.4E,F,G; 1.5A,B,D,E,F; 1.6B,C). Flagellum (**f_{1-7}**) of Australian species and female *C. noacki* 4-segmented; f_1 and f_2 wider than long and fused on the medial internal surface; f_3 longer than combined lengths of f_1 and f_2; f_3 with basiconic peg sensilla (**bps**, Fig. 1.4G); clava unsegmented and tapering apically (Figs 1.4E,F,G,H; 1.5D,E,F; 1.6B,C). Flagellum of male *C. noacki* 3-segmented; f1 short and with dorsal flange (Fig. 1.5B); f_{2-3} 4× longer than wide and tapering apically; clava unsegmented and tapering apically (Figs 1.5A,B,C). Multiporous plate sensilla (**mps**) of both sexes and all species unfused along their lengths (Figs 1.4H; 1.5A,B,C,D,E,F), with male mps raised into plumose whorls along flagellum (Figs 1.4H, 1.5C, 1.5F). Clava with coeloconic sensillum (**ccs**) basally just proximal to base of mps (Fig. 1.5C). Female clava of all species with uniporous sensilla trichodea (**ust**) and styloconic sensilla (**ss**) (Fig. 1.4H).

Mouthparts. Labrum (**lbr**) projecting forward, forming a horizontal shelf, and with two short marginal setae (Fig. 1.1E). Mandible (**man**) terminating in serrate oblique tooth, ventrally with socketed tooth (**mnt**) (Figs 1.1E,F); posterolaterally with sharp mandibular process (**mnp**) overlying genal margin (Fig. 1.1F); and with 3 stout setae

arising from basal anterior surface (Figs 1.1E,F,H; 1.6A). Maxilla with three short stout setae (**mxs**, Fig. 1.1F). Maxillary palp (**mp**) 1-segmented, terminating in two stout setae (Figs 1.1D,E; 1.1H); labial palp (**lp**), reduced to single seta-like process (Fig. 1.1D); glossa (**gl**) terminating in fringe of flattened seta-like structures (Fig. 1.1E); other visible components of labiomaxillary complex include cardo (**crd**), stipes (**stp**), mentum (**mtm**), and prementum (**pmn**) (Fig. 1.1D).

Prothorax. Pronotum (**no_1**) short, membranous medially (not apparent on scanning electron micrographs), visible dorsally as thin band closely applied to mesoscutum (Figs 1.2A, 1.6D). Propleuron (**pl_1**) visible ventrally as two oblique rectangular plates divided medially (Fig. 1.2C). Prosternum (**st_1**) with tuberculate intercoxal membrane pad (**icmp**) posterior to procoxal fossae; icmp divided into anterior and posterior bands by membranous fold (Figs 1.2C,D; 1.6E).

Mesothorax. Midlobe of the mesoscutum (**mlm**) of Australian and New Zealand species with pair of anterior mesoscutal setae (**ams**) and posterior mesoscutal setae (**pms**) (Figs 1.6D; 1.9A,B,C); Mesoscutellum (**sct**) of these species with pair of anterior scutellar setae (**ass**) and posterior scutellar setae (**pss**) (Figs 1.6D; 1.9A,B,C). Mesonotal setation of *C. noacki* variable, but typically with single pair of setae on mlm, pair of minute setae medial to mesoscutellar campaniform sensilla (**cs**) (Figs 1.2A,B), and prominent pair of pss on mesoscutellum (Figs 1.2A, 1.9D). Notaulus prominent, but not reaching transscutal articulation, and forming relatively wide separation between mlm and llm along anterior $4/5^{th}$ of their lengths (Figs 1.3A,B; 1.6D). Axilla (**ax**) advanced; with one small seta; fused with mesoscutellum posteriorly, and not distinguishable from

lateral lobe of mesoscutum (**llm**) by transscutal articulation (**tsa**) (hypothesized position of tsa represented by dotted line in Fig. 1.2A). Prospinasternal apodeme (**psa**, Fig. 1.3F) visible externally as a prospinasternal pit (**psp**) on the anterior ventral midline of the mesepisternum (**es_2**) (Figs 1.2C,D; 1.6E). Anterior thoracic spiracle (**ats**) visible within small notch in pronotal cuticle, just anterior of llm when viewed laterally (Figs 1.3B,E). Tegula (**tgl**) rounded subtriangular lobe just ventral to llm (Figs 1.3B,C,D; 1.6F). Prepectus (**pre**) mostly concealed by pronotum on intact specimens, but partially visible as small posterodorsally extending sclerite (Fig. 1.3B); visible laterally on dissected specimens as elongated subtriangular lobe (Figs 1.3D, 1.6G). Mesepisternum divided into upper mesepisternum (**ues_2**) and lower mesepisternum (**les_2**) by line of differentiated sculpture (Fig. 1.3C). Pleural sulcus (**pls**) extending from upper mesepimeron (**uep_2**), separating mesepisternum from lower mesepimeron (**lep_2**) (Figs 1.2C, 1.3C, 1.6E). Mesotrochantinal plate inflected internally (Fig. 1.2D). Lateral furcal arms (**lf**) of mesofurca anteriorly directed (Fig. 1.3F). Mesofurcal pit (**f_2p** , Figs 1.2C,D; 1.6E) present on mesepisternum anterior to mesotrochantinal plate, and separated from plate by about width of pit (Fig. 1.2D).

Metathorax. Metanotum (**no_3**) with metascutellum (**mts**) very short, visible dorsally as thin band between mesoscutellum and propodeum (**ppd**) (Figs 1.2A, 1.6D). Posterior thoracic spiracle (**pts**) visible on dorsal margin of upper mesepimeron (Figs 1.3B,E; 1.6F). Metapleuron (**pl_3**) rectangular in lateral view (Fig. 1.3A). Metepisternum extending anteriorly between mesocoxal fossae (Fig. 1.2D). Metafurcal arms (**mfa**, Fig.

1.3F) visible externally as metafurcal pits (**f_3p**) that are widely separated, each pit approximately aligned with medial margin of mesocoxal fossa (Figs 1.2C,D; 1.6E).

Propodeum. Propodeum (**ppd**) longer than metanotum and broadly joined to metasoma (Figs 1.2A, 1.6D). Propodeal spiracle (**pps**) surrounded by two or three stout setae; callus (**cal**) with single stout seta (Figs 1.3E, 1.6D).

Legs. Prominent femoral bristle (**fb**) present on posterior surface of distal ends of femora (Figs 1.2E, 1.2G). Calcar (**clc**) slightly curved and unbifurcated (Figs 1.2E,F). Single tibial spur (**tbs**) present on both mesotibia (Fig. 1.2G) and metatibia (Fig. 1.2H). Tibial combs present on foretibia (**ftc**, Fig. 1.2F) and hind tibia (**htc**, Fig. 1.2H). All tarsi 4-segmented. Basal tarsomere of foretarsus with ventral row of setae terminating in larger spatulate seta, together composing strigil (**str**, Fig. 1.2F).

Fore wing. Single prominent seta present on humeral plate (**hpl,** Figs 1.7A; 1.8A; 1.10A,B,C,D). Submarginal vein with prominent companiform sensilla (**cs**, Fig. 1.7E); single submarginal vein sensillum (**sms**, Fig. 1.7E) and single prominent seta on dorsal surface present (Figs 1.7A,C; 1.8A,B; 1.10A,B,C,D). Basal cell (**bc**) thickened, and with one or two rows of basal cell campaniform sensilla (**bcs**) posterior to submarginal vein on dorsal surface (Fig. 1.7A,E). Raised sensory hairs (**rsh**) arising from circular tubercles beneath parastigma (**pst**) (Fig. 1.7E). Marginal vein (**mv**) of *C. noacki* with 3 prominent setae (Figs 1.7A; 1.10D). Variable number of prominent setae on mv of Australian and New Zealand species, ranging from 5 to 7. Socketed sensory hairs (**sh**) present posterior to the stigma (**stg**) (Fig. 1.7F). Four stigmal vein sensilla (**svs**) on uncus (**unc**) (Fig. 1.7F). Wing disc of *C. noacki* with sparse setation; most setae arranged in three well-defined

rows (Figs 1.7A, 1.10D). Wing discs of Australian and New Zealand species more evenly setose, with some specimens showing a tendency toward setal tracks (Figs 1.7C; 1.10A,B). Marginal setae (**ms**) relatively long, 0.5–0.8× width of the fore wing (Figs 1.7A; 1.7C; 1.8A; 1.10A,B,C,D).

Hindwing. Marginal vein strongly curved posteriorly (Figs 1.7B, 1.7D, 1.8C).

Metasoma. Metasoma broadly joined to mesosoma; mesophragma extending into metasoma. First metasomal tergite (**Mt_1**) dorsal to second metasomal tergite (**Mt_2**) when viewed laterally; Mt_1 and Mt_2 clearly delineated from each other (Fig. 1.3A). Cercus not advanced and bears single seta (**cers**, Fig. 1.4A; note that both right and left setae are visible, one partially obscured by the other, giving appearance of two setae arising from single cercus). Hypopygium (**hyp**) small, extending to about midpoint of metasoma (Figs 1.4B, 1.6H).

Female genitalia. Externally visible structures of female genitalia include ovipositor sheath (**osh**), dorsal valvifer (**dv**), and ventral valvifer (**vv**) (Figs 1.4A,B,C). Ovipositor stylets (**ost**) often upturned and sabre-like when exerted (Figs 1.4A,C).

Male genitalia. Genitalia of *C. spenceri* not visible on specimens examined for this study. Genitalia of *C. noacki* simplified, consisting of aedeagus (**adg**) and reduced phallobase (**phl**) (Figs 1.4D, 1.8D). Genitalia of *C. berryi* and *C. orchamoplati* with laterally curving hook-like digitus (**dig**) on volsellus (**vls**) (Figs 1.6I; 1.8F,G). All species with expanded phallobase (subequal length and width) and paired sclerotized aedeagal rods (**adr**) in addition to aedeagal apodemes (**aap**) (Figs 1.8D; 1.8F,G). Parameres (**par**) of all species with single stout apical seta (Figs 1.8D; 1.8F,G).

Discussion

The habitus, body color, small size and life history characteristics give a "first impression" that *Cales* may be taxonomically associated with some members of the Aphelinidae, especially whitefly parasitioids in the genera *Encarsia* and *Eretmocerus*. Here we present a comparative discussion to highlight the more specific morphological evidence for and against these hypotheses, as well as the evidence suggesting affinities between *Cales* and other taxa.

Antenna. The *Cales* flagellum is at most 4-segmented, a condition shared with some Aphelininae and Trichogrammatidae, although the homology of segment fusion appears to be different from that found in either of these taxa, especially regarding fusion of the claval segments and overall number of potential flagellomeres. In all *Cales* with the exception of *C. noacki* males, f_1 and f_2 are distinct laterally (Figs 1.4F,G; 1.5E; 1.6B,C), fused medially (Figs 1.4E; 1.5F) and f_2 is distinct both laterally and medially from f3 (Figs 1.4E,F,G; 1.5D,E,F). In *C. noacki* males, f_1 is distinct both laterally and medially from the rest of the flagellum, and f_2 is fused with f_3 (Fig. 1.5B, hypothesized location of fusion indicated by dashed line). This hypothesis is based on the basal location of mps in *C. spenceri* (Fig. 1.5F) compared with the subbasal location of the mps in male *C. noacki* (Fig. 1.5B). We propose that the clava of both sexes of all species is formed from a fusion of flagellomeres 4-7. The presence of four flagellomeres in the clava is based on the presence of four distinct whorls of MPS in all male *Cales* (Figs 5A,D), and the associated constrictions of the clava in male *C. spenceri* (Fig. 1.5D). Also, the presence of a coeloconic sensillum at the base of the clava (ccs, Fig. 1.5C) appears to

be homologous to a similar sensillum on f_4 in Trichogrammatidae (e.g. *Ittys* and *Ceratogramma*) and other Chalcidoidea (J. George, pers. comm.). The segmentation of the female clava is inferred from the males as the female has fewer rows of mps and the coeloconic sensillum is absent. The mps of most Chalcidoidea are fused along their lengths (Barlin *et al*., 1981), whereas the *Cales* mps is unfused. This condition is shared with Trichogrammatidae, and *Oenrobia* (Aphelinidae: Coccophaginae).

Mouthparts. The forward-projecting labrum is similar to Rotoitidae and some Aphelinidae. The socketed ventral tooth is shared with Coccophaginae (including *Coccobius*), eriaphytine aphelinids, and some Encyrtidae (Heraty & Schauff, 1998). The three short stout setae on the maxilla are apparently unique to *Cales*.

Prothorax. The tuberculate intercoxal membranous pad is also present in Aphelininae (Rosen & Debach, 1979; Kim, 2003). The form of the icmp is variable across aphelinine taxa. In *Cales* and most Aphelininae the pad forms a continuous band posterior to the coxal fossae. A transverse membranous fold in the icmp appears to be unique to *Cales*. In *Aphytis* (Aphelinidae: Aphelininae), it is divided into left and right halves, forming a separate pad posterior to each coxal fossa (Figs 196–200 in Rosen & Debach, 1979).

Mesothorax. The pattern of dorsal setation of the *Cales* mesothorax is shared with *Eretmocerus*. Some clarification regarding the further reduction in mesonotal setation of *Cales noacki* is necessary. Typically, this species has a single pair of posterior mesoscutal setae on the midlobe of the mesoscutum. However, smaller anterior mesoscutal setae may be present, and we observed one *C. noacki* specimen with only a single anterior

mesoscutal seta on the midlobe (Fig. 1.2A). Mesoscutellar setation also appears to be variable for this species. Viggiani and Carver (1988) observed only a single pair of long setae on the mesoscutellum. However, Evans and Serra (2002) found a second pair of small anterior setae medial to the scutellar campaniform sensilla on specimens from Haiti. This pair of minute setae is also present on specimens from California and Italy examined for this study (Figs 1.2A,B). Individuals may have a complete set of anterior scutal setae on both the midlobe of the mesoscutum and mesoscutellum, or they may lack anterior setae altogether. When anterior setae are present, they are much shorter than the posterior setae.

A pleural sulcus extends from the upper mesepimeron to the lateral edge of the mesocoxal fossa. This condition is shared with Coccophaginae and Trichogrammatidae. A pit corresponding to the prospinasternal apodeme is visible on the mesepisternum of *Cales*, a condition shared by *Cirrospilus* (Eulophidae) (Krogmann & Vilhelmsen, 2006).

The *Cales* mesofurca (Fig. 1.3F) was included by Heraty *et al*. (1997) in a comparative analysis across Chalcidoidea. Structure and position of the lateral furcal arms, posterior furcal-laterophragmal muscle, furcal-pleural arm muscle, and metathoracic interfurcal muscle were considered most similar to *Eretmocerus*, with some similarities to Trichogrammatidae, Azotinae and Signiphoridae.

Metathorax. Gibson (1989) suggested a sister-group relationship between Aphelinidae *sensu lato* and Signiphoridae based on the structure of the mesocoxal articulation with the metepisternum and mesotrochantinal plate. Specifically, the mesotrochantinal plate is inflected internally with the metepisternum extending anteriorly

between the mesocoxal fossae and abutting the dorsal edge mesotrochantinal plate ("character state 3" in Gibson, 1989). In *Cales*, the mesotrochantinal plate is inflected internally, and the metepisternum extends anteriorly between the mesocoxal fossae. However, differentiating between membranous and sclerotized tissue is very difficult in small weakly-sclerotized chalcidoids such as *Cales*, either on SEM or slide mounts. Either the metepisternum is separated from the mesotrochantinal plate by membranous tissue ("character state 2" in Gibson, 1989), or the metepisternum meets the dorsal edge of the mesotrochantinal plate ("character state 3a") (G. Gibson, pers. comm.). The latter state would suggest an affinity between *Cales* and the Aphelinidae+Signiphoridae clade hypothesized by Gibson (1989), but we could not discern either state with confidence.

The posterior thoracic spiracle is visible externally in *Cales*, a character that is shared with *Chiloe* (Gibson and Huber, 2000), *Eretmocerus*, and other taxa within Chalcidoidea. However, a thorough survey of this character across Chalcidoidea is needed to determine its phylogenetic implications.

Two widely separated metafurcal pits are visible on the metepisternum of *Cales*. Krogmann and Vilhelmsen (2006) found paired metafurcal pits in some Pteromalidae, Eurytomidae, Signiphoridae, Mymaridae, and Agaonidae. Eulophidae and Aphelinidae possess a single medial metafurcal pit, and no metafurcal pits were observed on Trichogrammatidae.

Legs. All *Cales* have a prominent bristle on the posterior surface of the distal ends of the femora. This bristle is also present in all Aphelinidae *sensu lato,* most Eulophidae, some Pteromalidae and some Trichogrammatidae. Polaszek (1991) suggested that *Cales*

might be closely related to Eulophidae based on the presence of an unbifurcated straight calcar, lack of basitarsal comb (=strigil) on the foretarsus, and a reduced number of tarsal segments (from five, the presumed plesiomorphic state for Chalcidoidea, to four). All of these features are reductions that have evolved multiple times and at multiple taxonomic levels within Chalcidoidea. The structure of the calcar is similar in Trichogrammatidae, most Eulophidae, some Aphelinidae (*Euryischia* and *Eretmocerus)*, and some Eucharitidae (*Pseudochalcura*) (Basibuyuk & Quicke, 1995). Reduction from 5 to 4 tarsomeres occurs in some *Encarsia,* and all *Eretmocerus. Pteroptrix* spp. (Coccophaginae) also exhibit a reduction from 5 to 4 tarsomeres in all but a single Neotropical species, which has undergone a further reduction to 3 tarsal segments (Kim & Triapitsyn, 2003). Trichogrammatidae lack a strigil, and both Euchartidae and Eulophidae are polymorphic for this character (Basibuyuk & Quicke, 1995; Heraty 2002). However, a row of setae terminating in larger spatulate setae present on the *Cales* foretarsus may constitute a strigil (R. Burks pers. comm.).

Fore wing. The second row of campaniform sensilla on the dorsal surface of the basal cell, which are posterior to same sensilla along the submarginal vein, appear to be unique to *Cales*. Raised sensory hairs are present on the fore wings of some Trichogrammatidae, though homology with the structures on *Cales* wings is uncertain. The marginal setae are relatively long, similar to many *Encarsia* species. A striking feature of the *C. noacki* fore wing is the arrangement of discal setae into distinct rows, a character typically associated with Trichogrammatidae. However, setal lines are variably present within both Trichogrammatidae and *Cales*, suggesting that the trait is

homoplastic. *Ceratogramma*, an early branching genus of Trichogrammatidae (Owen *et al*., 2007), has relatively evenly setose wings, as do the Australian and New Zealand species of *Cales*. Consequently, setal tracks in *C. noacki* are likely an independently derived feature and not evidence for a sister group relationship with Trichogrammatidae.

Hindwing. The hindwing is similar in shape to many small Aphelinidae, except for a strongly curved marginal vein, which is similar to some Trichogrammatidae (J. George, pers. comm.).

Metasoma. Overall, the structure of the metasoma is typical of small, weakly sclerotized chalcidoids. The male genitalia of *C. noacki* are simplified, consisting of an aedeagus and reduced phallobase. This is contrary to Viggiani and Battaglia (1984) who report that the phallobase as completely absent. The aedeagal rods appear to be unique to *Cales*.

Key to the species of *Cales*

1. Fore wing disc with setae in three distinct rows (Figs 1.7A, 1.10D). Male flagellum with 3 segments; radicle long, at least 3 times as long as wide, and subequal in length to pedicel (Figs 1.4F, 1.5A). Midlobe of mesoscutum with one pair of long prominent setae and mesoscutellum with one pair of long prominent setae and second pair of minute setae (pss, ass, Figs 1.2A, 1.9D). Neotropics and introduced into North America, the Mediterranean and Africa..... *C. noacki*

– Fore wing disc evenly setose or at most tending toward rows, but setae not in 3 distinct rows (Figs 1.7C; 1.10A,B,C). Male flagellum with 4 segments; radicle short, at most 2 times as long as wide, and much shorter than pedicel (Figs 1.6B,C).

Midlobe of mesoscutum and mesoscutellum each with two pairs of long prominent setae (Figs 1.9A,B,C). Australia or New Zealand 2

2. Fore wing with longest posterior marginal seta 0.8× width of wing (Fig. 1.10B). Mesoscutum with posterior setae long, more than 1/3 length of seta extending beyond transscutal articulation when directed posteriorly (pms, Fig. 1.9B) Australia ..... *C. spenceri*

– Fore wing with longest posterior marginal seta 0.5-0.6× width of wing (Figs 1.10B,C). Mesoscutum with posterior seta short, less than 1/3 length of seta extending beyond transscutal articulation when directed posteriorly (Figs 1.9A,C. Australia or New Zealand 3

3. Fore wing with one or two rows of small campaniform sensilla on dorsal surface of basal cell, just posterior to submarginal vein (bcs, Figs 1.7E, 1.9B). Posterior margin of scutellar disc shallowly or deeply notched (Figs 1.9A,B). Fore wings hyaline. Australia ..... *C. orchamoplati*

– Fore wing basal cell without campaniform sensilla (Figs 1.8A, 1.10C), or sensilla present only as faint vestiges in proximal area posterior to submarginal vein. Posterior margin of scutellar disc rounded or shallowly notched (Figs 1.9C,D; 1.6D). Fore wing with infuscation posterior to submarginal and marginal veins (Fig. 1.10C). New Zealand..... *C. berryi* n. sp.

New species description and species reviews

***Cales berryi* Mottern and Heraty, n. sp.**

(Figs 1.9C; 1.10C; 1.5C–1.6E, 1.7C,D; 1.7F–1.8C)

Diagnosis – Cales berryi can be distinguished from other species in the genus by the following combination of characters: Radicle short, 2× longer than wide; scape 3× length of pedicel; male flagellum 4-segmented. Mesoscutellum posteriorly rounded and with two pairs of prominent setae. Fore wing with light infuscation on basal half, relatively uniform discal setation, and with campaniform sensilla absent from dorsal surface of the basal cell in most specimens. *Cales berryi* is the only species currently known from New Zealand.

Female – Body color pale brown; vertex of head and anterior half of mesoscutum orange; posterior half of mesoscutum and mesoscutellum brown; face and legs pale, almost white. Head with fine transverse colliculate sculpture, face ventral to antennae with scattered slender setae (Fig. 1.6A). Malar sulcus extending half distance to eye (msl, Fig. 1.6A). Gena broadly rounded. Maxillary palpus 1-segmented, maxilla with one stout primary seta on medial ventral edge and three secondary raised socketed setae. Antenna with radicle short, 1.5–1.9× as long as wide. Scape 4.2–5.2× as long as wide, 4.7–5.2× as long as radicle and 2.6–2.7× as long as pedicel, subapically expanded ventrally; weakly reticulate and with even scattering of semi-erect setae (Fig. 1.6B). Flagellum with 4 flagellomeres; f_1 and f_2 combined length shorter than f_3, and fused on medial surface; f_3 1.5–2.1× as long as wide, subequal in length to pedicle plus f_1 and f_2, and 0.2–0.3× as long as clava; f3 and clava with scattered mps and bps, claval setae 0.1–0.2× as long as clava;

clava unsegmented (composed of fused f_{4-7}), 4.4–5.2× as long as wide, obliquely truncate apically; mps 0.3× length of clava. Lateral lobe of mesoscutum with 2 setae (lls, Fig. 1.6D). Mid lobe of mesoscutum with two pairs of setae (ams, pms, Fig. 1.6D) and faint reticulate sculpture. Mesoscutellum with two pairs of setae (ass, pss, Fig. 1.6D). Tegula narrow in lateral view, approximately 5× longer than wide; subquadrate in dorsal view and with one seta (tgl, Figs 1.6D; 1.6F,G). Mesepisternum weakly imbricate laterally and spiculate medially (Fig. 1.6E), posteriorly raised into triangular area surrounding mesofurcal pit (f_2p, Fig. 1.6E). Metafurcal pits close to anterior margin of metepisternum (f_3p, Fig. 1.6E). Foretibial calcar 0.4–0.6× length of basitarsus. Fore wing with even infuscation posterior to the marginal and submarginal veins (sometimes very light and difficult to see on cleared specimens); 3.0–3.1× as long as broad; longest seta of posterior marginal fringe 0.4–0.5× width of wing; marginal vein with row of 6 long setae along anterior margin; discal setation relatively uniform; stigmal vein rounded, uncus distinct, usually with 4, sometimes 3, campaniform sensilla (cs) (Figs 1.8A, 1.10C; see Fig. 1.7F for similar cs on *C. noacki* fore wing). Hindwing 6.7–7.3× as long as broad, posterior marginal fringe 1.1–1.2× width of wing; discal setation uniform (Fig. 1.8C). Hypopygium deeply emarginate medially (hyp, Fig. 1.6H). Ovipositor 1.7–1.9× as long as hind basitarsus.

Male – Similar to female, except antenna with mps in transverse rows resulting from segment fusions; mps 0.5–0.6× as long as clava; clava uniformly narrowing apically, but width constricted between segment fusions (Fig. 1.6C). Phallobase broad and circular, parameres reduced to broad lobes with single apical stout seta (par, Figs

1.8F,G), digitus elongate and stout with strong laterally directed hook (dig, Figs 1.8F,G); aedeagus broadly subtriangular with 4-6 prominent sensilla.

Host – Reared from *Asterochiton pittospori* Dumbleton (Aleyrodidae) on *Pittosporum eugenioides* Cunn. (Pittosporaceae).

Etymology – Named in honour of Dr. Jocelyn A. Berry, who collected the type series.

Material examined – **Holotype: New Zealand**: ♀, slide mounted, North Island, Auckland, Mount Albert, Oakley Creek Walkway, 36°55'S 174°47'E, 12 Nov 2003, J.A. Berry, ex *Asterochiton pittospori* Dumbleton on *Pittosporum eugenoides*, deposition: NZAC [UCRC_ENT 00091228]. **Allotype:** ♂, same data as holotype, [UCRC_ENT 00091219]. **Paratypes:** 3♂♂, 2♀♀, same data as holotype, [UCRC_ENT 00091217-18, 00091220, 00091222-23]. 2♂♂, 2♀♀, pinned; AMNZ [UCRC_ENT 00091221, 00091224, 00091225, 00252060]. 2♂♂, 2♀♀, pinned; ANIC [UCRC_ENT 00091226-27 00252061-62]. 3♂♂, 1♀♀, pinned; UCRC [UCRC_ENT 00252065-67, 00252070]. 5♂♂, 5♀♀, slide mounted, UCRC [UCRC_ENT 00020119-28]. 2♂♂, 2♀♀, pinned; USNM [UCRC_ENT 00252063-64, 00252068-69]. **Other specimens: New Zealand**: ♂, slide mounted, North Island, Lake Rotoiti, 38°2'S 176°25'E, 4-9 Feb 1978, S. & J. Peck, deposition: CNC [UCRC_ENT 00050871].

***Cales noacki* Howard, 1907**

(Figs 1.1A–F; 1.2A–1.3E; 1.4A–1.5C; 1.7A,B; 1.7E,F; 1.8D; 1.9D; 1.10D)

Cales noacki Howard 1907: 82-83, by monotypy and original designation. Deposition: USNM (lost).

Diaspidophilus pallidus Brèthes 1914: 15–16, by monotypy and original designation. Deposition: MACN. Synonymy by Gahan in Mercet 1929: 114.

Cales pallidus Mercet 1929: 117, new combination; synonymy with *C. noacki* by Dozier 1933: 98.

Remarks. Cales noacki is the only member of the genus known from the New World tropics, though its range has been intentionally expanded to citrus-growing regions of North America, the Mediterranean, Africa and North Atlantic islands. It is the most distinctive species and easily recognized by the following combination of characters: wings hyaline, with discal setae arranged in three distinct rows in addition to scattered setae on the distal third of wing disc (Figs 7A, 10D). The radicle is about 4× longer than wide compared to at most 2× longer than wide in other species. The female flagellum is 4-segmented and the male flagellum 3-segmented, whereas both sexes of other species have a 4-segmented flagellum. Typically there is a single pair of stout setae on the midlobe of the mesoscutum and two pairs of setae on mesoscutellum, with the anterior pair of scutellar setae minute (ass, Figs 2A,B; 9D). Other *Cales* species have two prominent pairs of long setae on the midlobe of the mesoscutum and mesoscutellum.

Material Examined – **Haiti**: 2♀♀, slide mounted, Damien, 21 Mar 1931, H.L. Dozier, ex *Aleurothrixus haitiensis* on *Catalpa longissima*, deposition: UCRC, [UCRC_ENT 00014833]. **Italy**: 12♀♀, slide mounted head and wings; Campania, Portici (N9), 11 Nov 2003, M. Giorgini, ex *Aleurothrixus floccosus*, deposition: UCRC [UCRC_ENT 00020103-18]. **Morocco**: ♂, slide mounted, Centa Frontiere, 1 Dec 1973, M. Abbassi, ex *Aleurothrixus floccosus*, deposition: UCRC [UCRC_ENT 00251440]. ♀,

slide mounted, Centa Frontiere, 29 Nov 1973, M. Abbassi, ex *Aleurothrixus floccosus*, deposition: UCRC [UCRC_ENT 00251438]. 6♀♀, slide mounted, Tanger, 30 Nov 1973, M. Abbassi, ex *Aleurothrixus floccosus*, deposition: UCRC, [UCRC_ENT 00251439]. **Spain**: ♂, 3♀♀, slide mounted, Alora, Malaga, 4 Aug 1973, D. Rosen, citrus, deposition: UCRC [UCRC_ENT 00251443]. 3♀♀, slide mounted, Exp. Sta. "La Mayora," Malaga, Caleta De Velez, 1973, Ramon Vazquez, ex *Aleurothrixus floccosus*, deposition: UCRC [UCRC_ENT 00251442]. 5♂♂, slide mounted, Exp. Sta. "La Mayora," Malaga, Caleta De Velez, 1973, Ramon Vazquez, ex *Aleurothrixus floccosus*, deposition: UCRC [UCRC_ENT 00251441]. 2♂♂, 5♀♀, slide mounted, Malaga, 4 Aug 1973, D. Rosen, deposition: UCRC [UCRC_ENT 00251445]. ♂, 3♀♀, slide mounted, Malaga Cemetery, 4 Aug 1973, D. Rosen, ex *Aleurothrixus floccosus* on citrus, deposition: UCRC [UCRC_ENT 00251444]. 2♂♂, 14♀♀, slide mounted, Malaga Cemetery, 4 Aug 1973, D. Rosen, ex *Aleurothrixus floccosus* on citrus, deposition: UCRC [UCRC_ENT 00251437]. 2♂♂, 3♀♀, slide mounted, Malaga, Benamargosa, 4 Aug 1973, D. Rosen, ex *Aleurothrixus floccosus* on citrus, deposition: UCRC [UCRC_ENT 00251446]. 5♂♂, 6♀♀, slide mounted, Malaga, La Mayora, 4 Aug 1973, D. Rosen, ex *Aleurothrixus floccosus* on citrus, deposition: UCRC [UCRC_ENT 00251449]. ♀, slide mounted, Malaga, La Mayora, 4 Aug 1973, D. Rosen, ex *Aleurothrixus floccosus* on citrus, deposition: UCRC [UCRC_ENT 00251448]. 2♂♂, 3♀♀, slide mounted, Malaga, La Mayora, 4 Aug 1973, D. Rosen, ex *Aleurothrixus floccosus* on citrus, deposition: UCRC, [UCRC_ENT 00251447]. **USA**: ♀, slide mounted, California, Los Angeles Co., San Gabriel, 529 Dobbins Dr., 22 Apr 1982, Rose & Ferrentino, ex *Tetraleurodes mori* on

citrus and gardenia, deposition: UCRC [UCRC_ENT 00014834]. ♀, slide mounted, California, Riverside Co., Temecula, Norco Rd., 21 Jun 1997, M. Hoddle, ex *Tetraleurodes perseae*, deposition: UCRC [UCRC_ENT 00235952]. 4♂♂, 2♀♀, slide mounted, California, Riverside Co., UCR; Biological Control Grove, 350m, 33°58.308'N 117°19.125'W, 6 Jan 2006, J. Mottern, ex *Aleurothrixus floccosus* on lime, deposition: UCRC [UCRC_ENT 00237277, 00020092-96]. 6♀♀, slide mounted wings; California, Riverside Co., UCR; Biological Control Grove, 350m, 33°58.308'N 117°19.125'W, 7 Jan 2003, J. Munro, ex *Aleurothrixus floccosus* on lime, deposition: UCRC [UCRC_ENT 00020097-102]. 3♂♂, slide mounted, California, San Diego Co., So. San Diego, 2210 Leon St., deposition: UCRC [UCRC_ENT 00014910]. ♂, slide mounted, California, Ventura Co., 1.7 mi E of Santa Paula, 90m, 16 Feb 1996, M. Gates, deposition: UCRC [UCRC_ENT 00235953].

***Cales orchamoplati* Viggiani and Carver, 1988**
(Figs 9A, 10A)

Cales orchamoplati Viggiani and Carver 1988: 43–45, by original designation.

Deposition: ANIC

Remarks. Cales orchamoplati is thus far only known from Australia. It is distinguished from *C. spenceri* by its fore wing setal fringe. In *C. orchamoplati*, the longest posterior marginal seta is 0.5–0.6× the width of the fore wing, whereas in *C. spenceri* it is 0.8× the width of the wing. *Cales orchamoplati* is very similar to *C. berryi*, but the latter species usually lacks the extra rows of campaniform sensilla in the basal cell posterior to the marginal vein of the fore wing, and the wings are infuscated rather than hyaline posterior to the submarginal and marginal veins. Contrary to Viggiani and Carver

(1988), the antennal scape of *C. orchamoplati* is about 2 times as long as the pedicel, whereas the scape of *C. spenceri* and *C. berryi* is 3 times as long as the pedicel.

Material Examined – **Holotype: Australia:** ♀, slide mounted, South Australia, W.A.R.I., P. Venning's garden, 30 Nov 1976, H.M. Brookes and M. Carver, ex *Orchamoplatus citri* on lemon, deposition: ANIC [UCRC_ENT 00238174]. **Allotype: Australia:** ♂, South Australia, W.A.R.I., P. Venning's garden, 30 Nov 1976, H.M. Brookes and M. Carver, ex *Orchamoplatus citri* on lemon, deposition: ANIC [UCRC_ENT 00238175]. **Paratypes: Australia**: ♂, slide mounted, South Australia, Adelaide, 1 Dec 1976 (year erroneously written on slide label as "1986"), M. Carver, ex *Orchamoplatus citri*, deposition: DEZA [UCRC_ENT 00020090]. ♂, slide mounted, South Australia, W.A.R.I., P. Venning's garden, 30 Nov 1976, H.M. Brookes and M. Carver, ex *Orchamoplatus citri* on lemon, deposition: ANIC [UCRC_ENT 00238176]. 2♀♀, slide mounted, South Australia, W.A.R.I., P. Venning's garden, 30 Nov 1976, H.M. Brookes and M. Carver, ex *Orchamoplatus citri* on lemon, deposition: ANIC [UCRC_ENT 00238177]. ♀, slide mounted, South Australia, Adelaide, 1 Dec 1976 , M. Carver, ex *Orchamoplatus citri*, deposition: DEZA [UCRC_ENT 00020091]. **Additional specimens: Australia**: ♂, slide mounted, Queensland, Mt. Glorious, 27°19.917'S 152°45.483'E, 7-13 Feb 1998, N. Power, deposition: UCRC [UCRC_ENT 00050892].

***Cales spenceri* (Girault, 1915)**

(Figs 1G,H; 3F; 5D–F; 7C,D; 9B; 10B)

Paranthemus spenceri Girault 1915: 165, by monotypy and original designation.

Deposition: QM. Synonymy by Hayat 1983: 78.

Cales spenceri; Combination by Viggiani 1981: 47

Remarks. Cales spenceri is thus far only known from Australia, and only from males. It is distinguished from *C. orchamoplati* and *C. berryi* by the relative length of the longest posterior seta of the fore wing marginal fringe. In *C. spenceri*, the longest seta on the posterior margin of the fore wing is 0.8× the width of the wing, whereas in *C. orchamoplati* and *C. berryi* the ratio is 0.5–0.6×. The posterior mesoscutal setae of *C. spenceri* are relatively long, with more than 1/3 the length of each seta extending beyond the transscutal articulation when directed posteriorly. By contrast, the posterior mesoscutal setae of *C. orchamoplati* and *C. berryi* extend about 1/4 their lengths beyond the transscutal articulation. *Cales spenceri* is often further distinguished from *C. berryi* by the presence of additional rows of campaniform sensilla in the basal cell of the fore wing, which are often missing in *C. berryi*.

Material Examined – **Holotype: Australia**: ♂, slide mounted, Queensland, Babinda, 4 Feb 1914, A.P. Dodd, jungle, deposition: QM [UCRC_ENT 00241631]. **Additional specimen: Australia**: ♂, slide mounted, New South Wales, Border Ranges N.P., Collins Creek, 1000m, 25-26 Jan 1995, B.J. Sinclair, rainforest, deposition: CNC [UCRC_ENT 00235951].

CONCLUSIONS

Cales possesses a perplexing mix of morphological characters, evidenced by its unstable taxonomic history and its current *incertae sedis* status within Chalcidoidea. Most frequently, *Cales* are associated with the Aphelinidae. Both groups consist of small wasps that are parasitoids of sternorrhynchous Hemiptera, generally non-metallic, weakly

sclerotized, and have the meso- and metasoma broadly joined. However, Aphelinidae are most likely not a monophyletic group (Campbell *et al*., 2000; Heraty, unpub.), so the question remains with which subfamily or genus could it form a sister group relationship.

Cales shares many features with *Eretmocerus*, including a long unsegmented clava, similar setal patterns of the dorsal mesosoma, similar structure of the mesofurca, presence of the posterior thoracic spiracle reduction in tarsal segments, and simple calcar. However, unlike *Cales*, *Eretmocerus* possess a broad wing disc and linea calva. Also the male genitalia of *Cales* and *Eretmocerus* are different, with *Cales* having a broad phallobase and *Eretmocerus* having an elongated phallobase. Both groups have unique male genitalia within Chalcidoidea, but they are just as different from each other as they are from other chalcidoid groups. An affinity with coccophagine aphelinids may also be hypothesized based on presence of a ventral mandibular tooth (also found in some Encyrtidae), pleural sulcus (also found in Trichogrammatidae), and similar structure of the mesocoxal articulation, though the latter character is difficult to assess for *Cales* due to generally weak sclerotization.

Cales is an important group for biological control, and yet despite detailed morphological and biological investigations, placement of this group within Chalcidoidea is difficult. Given the apparent homoplasy of *Cales* morphology when compared with other disparate lineages of Chalcidoidea, the determination of its phylogenetic position will require morphological and molecular analyses across the entire superfamily. Therefore, *Cales* should remain unplaced for now. This study aims to contribute to this

ongoing area of chalcidoid systematics by establishing a groundplan of comparative morphology and establishing the taxonomy of one of the most enigmatic groups

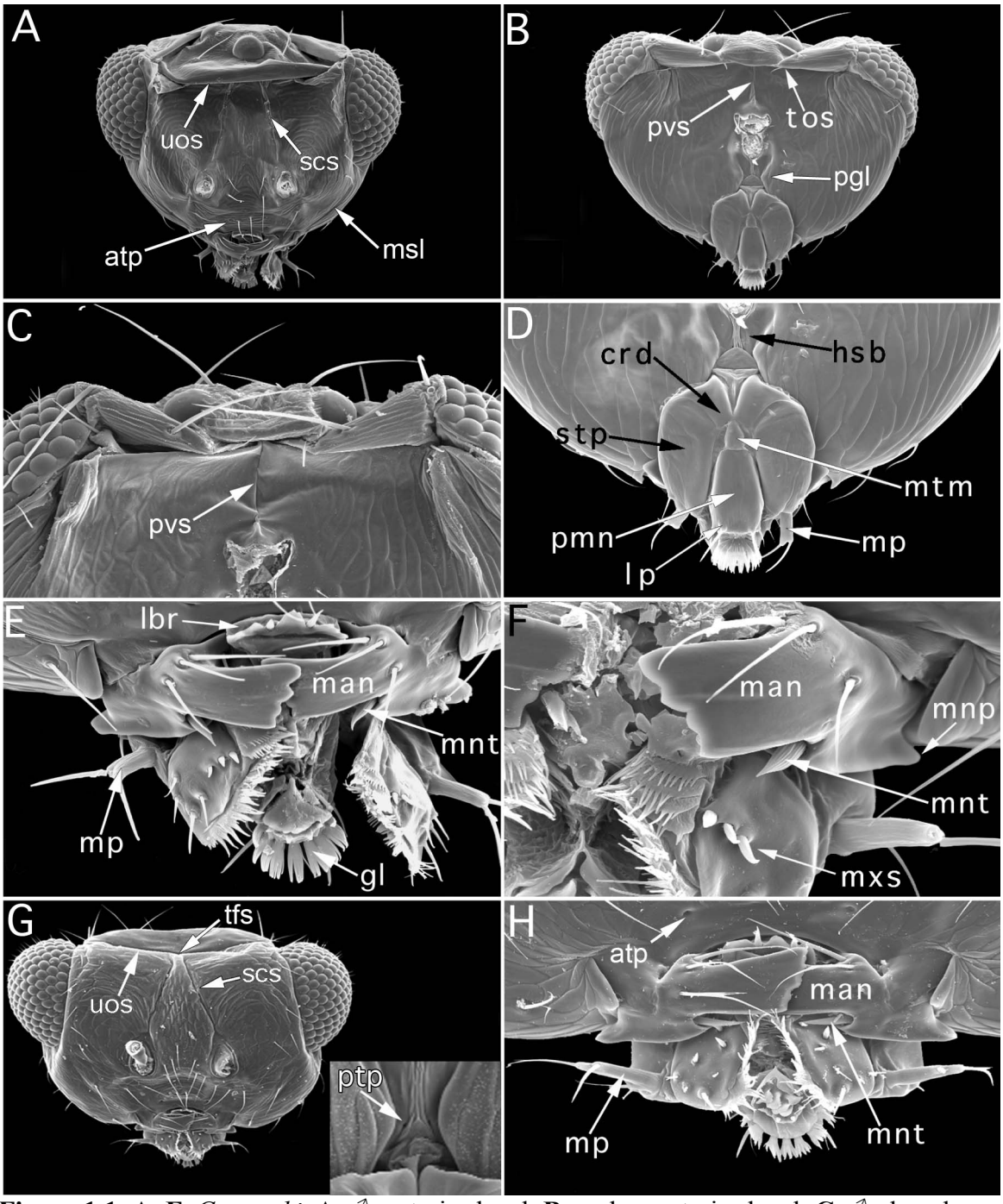


Figure 1.1. A–F, *C. noacki*. **A**, ♂, anterior head; **B**, male, posterior head; **C**, ♂, dorsal posterior head; **D**, ♂, posterior mouthparts; **E**, ♂, anterior mouthparts; **F**, ♀, mandible and maxilla detail. **G–H,** *C. spenceri,* ♂. **G**, anterior head, *inset:* posterior tentorial pit; **H**, anterior mouthparts.

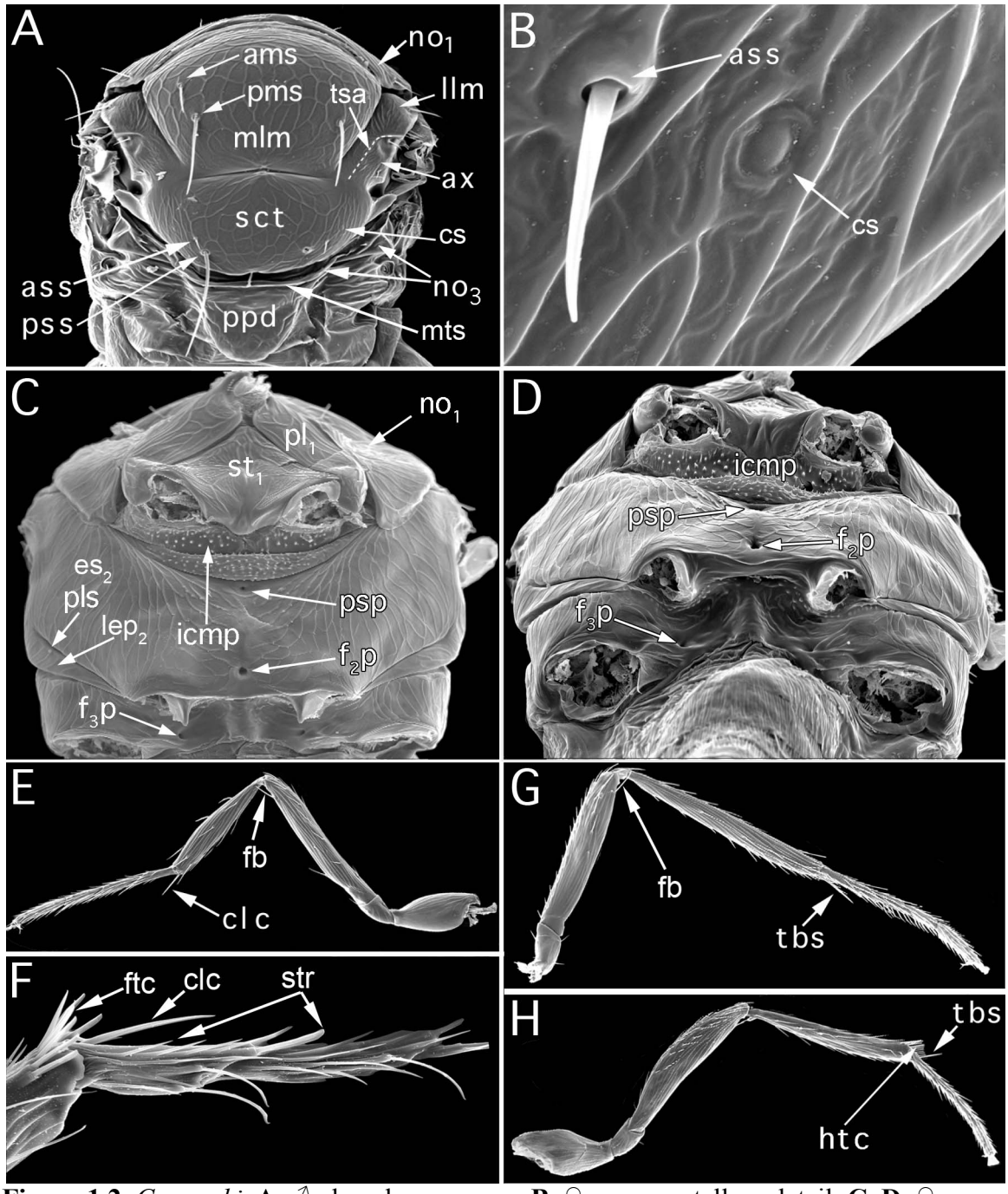


Figure 1.2. *C. noacki*. **A**, ♂, dorsal mesosoma; **B**, ♀, mesoscutellum detail; **C–D**, ♀, ventral mesosoma; **E**, ♂, foreleg; **F**, ♀, foretibia and basitarsal detail; **G**, ♀, midleg; **H**, ♀ hindleg.

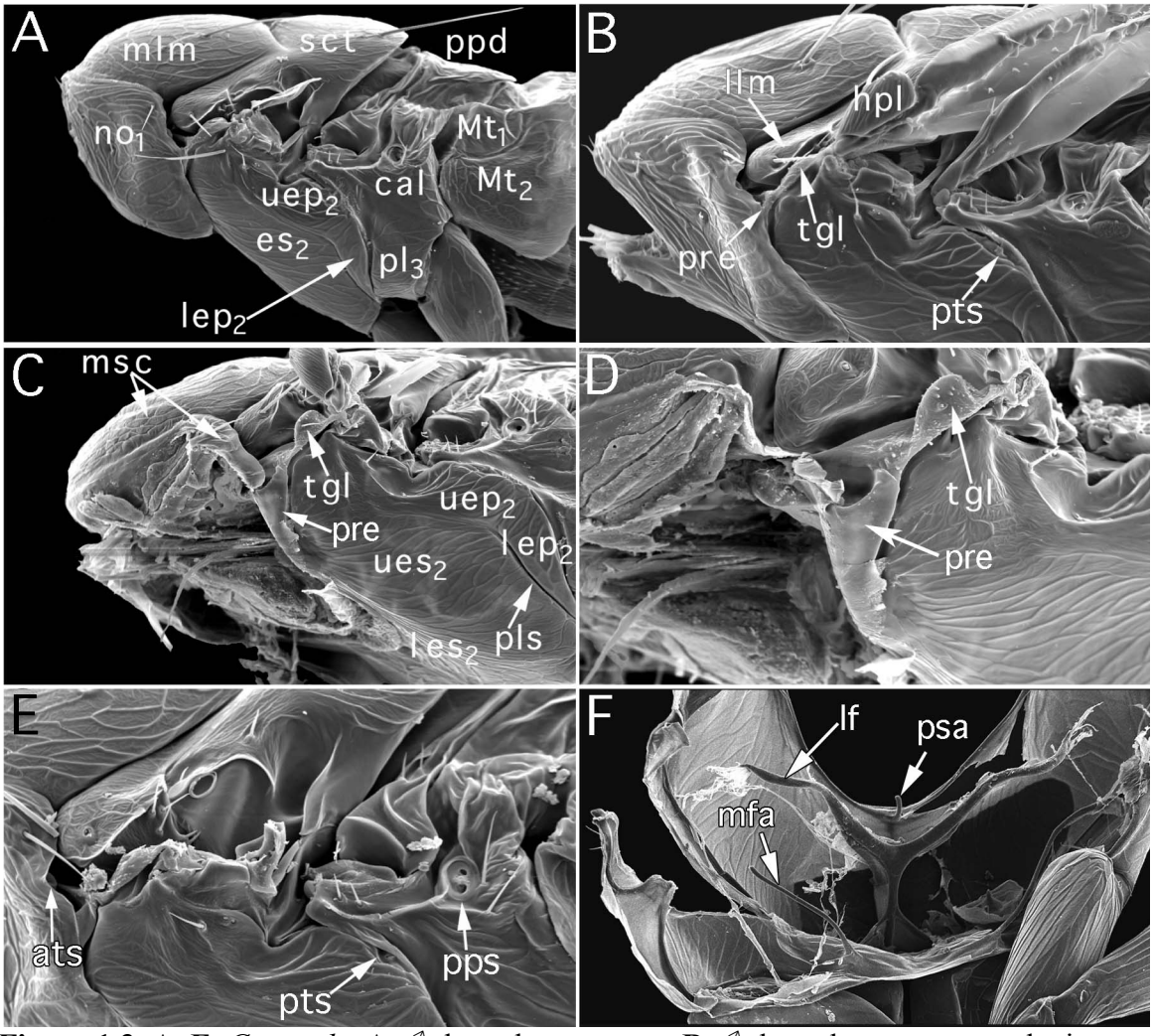


Figure 1.3. A–E, *C. noacki*. **A**, ♂, lateral mesosoma; **B**, ♂, lateral mesosoma and wing articulation; **C**, ♂, lateral mesosoma, pronotum removed; **D**, ♂, detail of lateral mesosoma, pronotum removed; **E**, ♀, lateral mesosoma, spiracles. **F**, ♀, mesofurca.

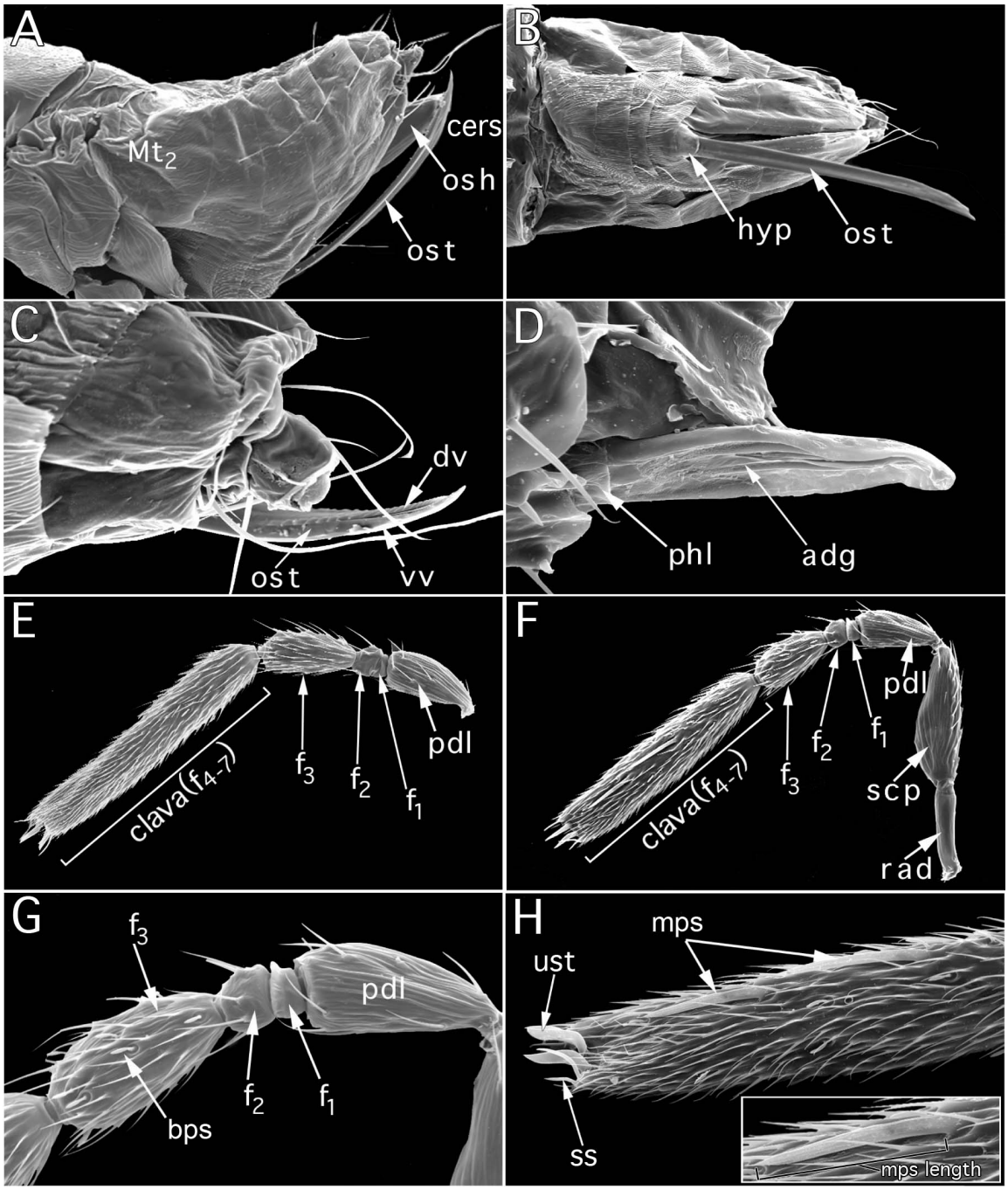


Figure 1.4. *C. noacki*. **A**, ♀, lateral metasoma; **B**, ♀, ventral metasoma; **C**, ♀, external genitalia; **D**, ♂, external genitalia; **E**, ♀, antenna, medial view; **F**, ♀, antenna, lateral view; **G**, ♀, pedicel and f_1-f_3 detail, lateral view; **H**, ♀, distal clava detail, *inset:* multiporous plate sensilla detail.

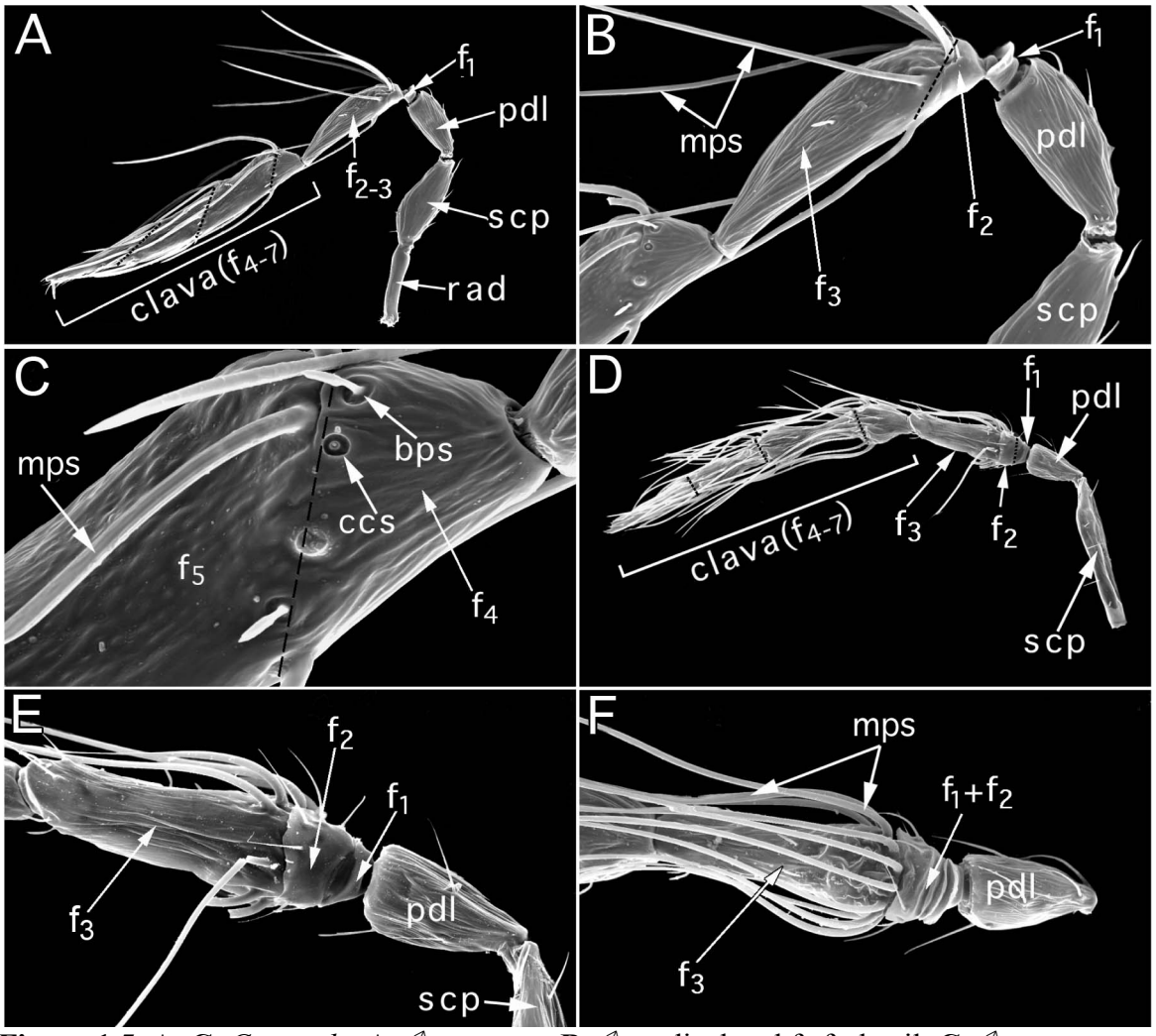


Figure 1.5. A–C, *C. noacki*. **A**, ♂, antenna; **B**, ♂, pedical and f_1-f_3 detail; **C**, ♂, proximate claval segment. **D–F,** *C. spenceri.* **D**, ♂, antenna; **E**, ♂, medial pedicel and f_1-f_3 detail; **F**, ♂, lateral pedical and f_1-f_3 detail. Hypotheses of segment fusion are indicated by dashed lines.

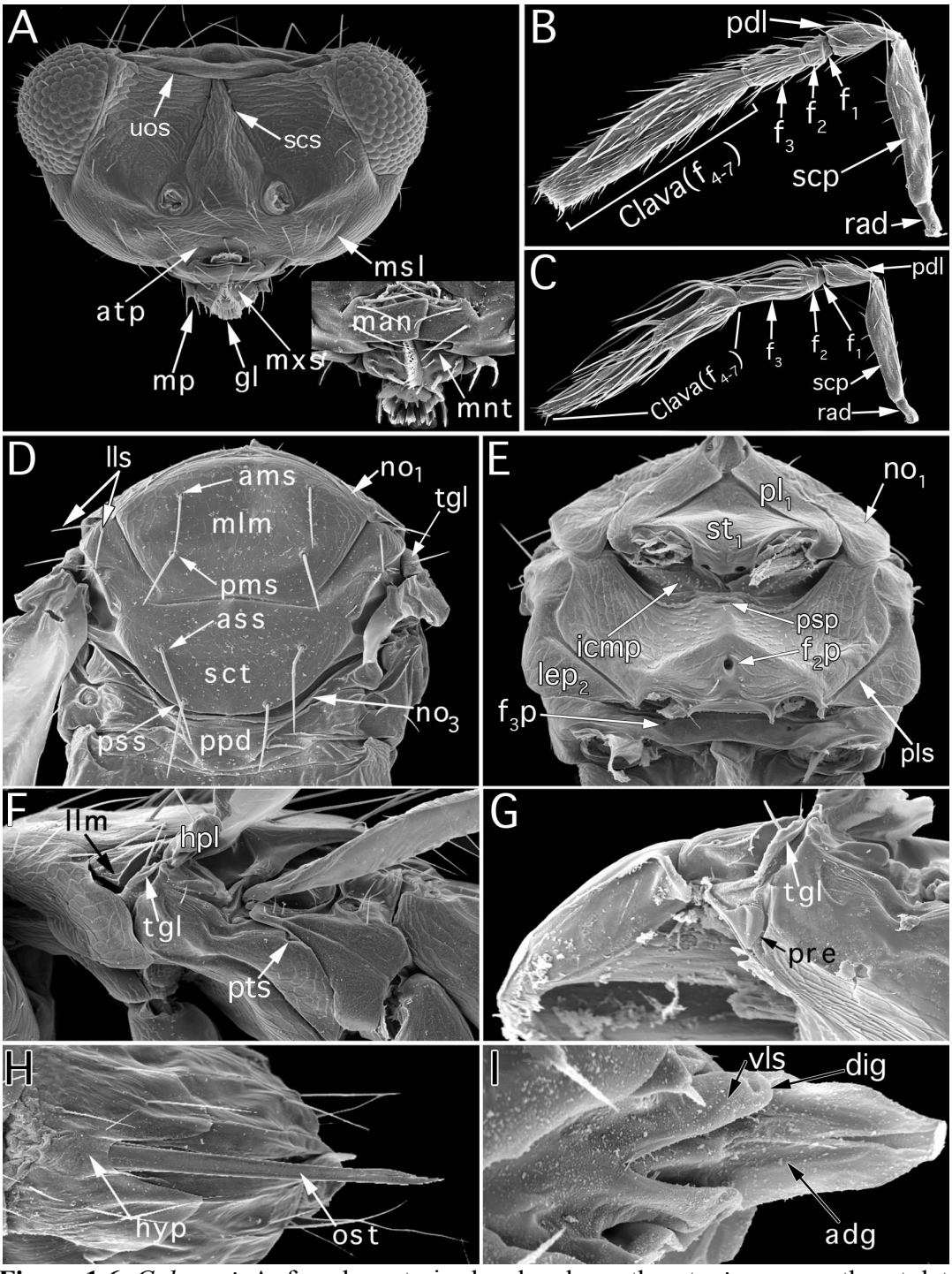


Figure 1.6. *C. berryi*. **A**, female, anterior head and mouthparts, *inset:* mouthpart detail; **B**, female, antenna, lateral view; **C**, ♂, antenna, lateral view; **D**, ♀, dorsal mesosoma; **E**, ♀, ventral mesosoma; **F**, ♀, lateral mesosoma; **G**, ♀, lateral mesosoma wing articulation; **H**, ♀, external genitalia; **I**, ♂, external genitalia.

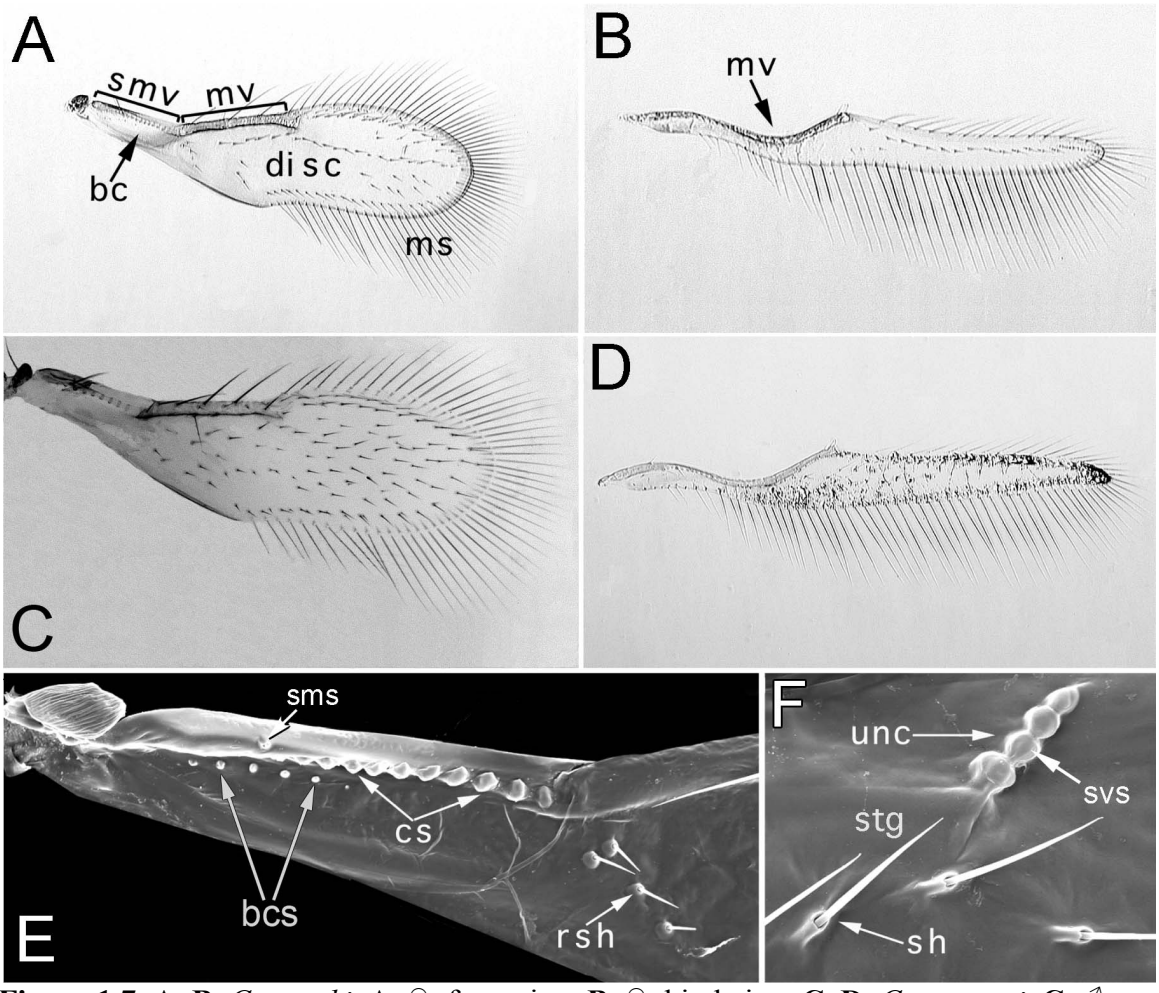


Figure 1.7. A–B, *C. noacki*. **A**, ♀, fore wing; **B**, ♀, hindwing. **C–D**, *C. spenceri*. **C**, ♂, fore wing; **D**, ♂, hindwing. **E–F**, *C. noacki*. **E**, ♀, fore wing, detail of basal cell and marginal vein; **F**, ♀, fore wing, detail of stigmal vein.

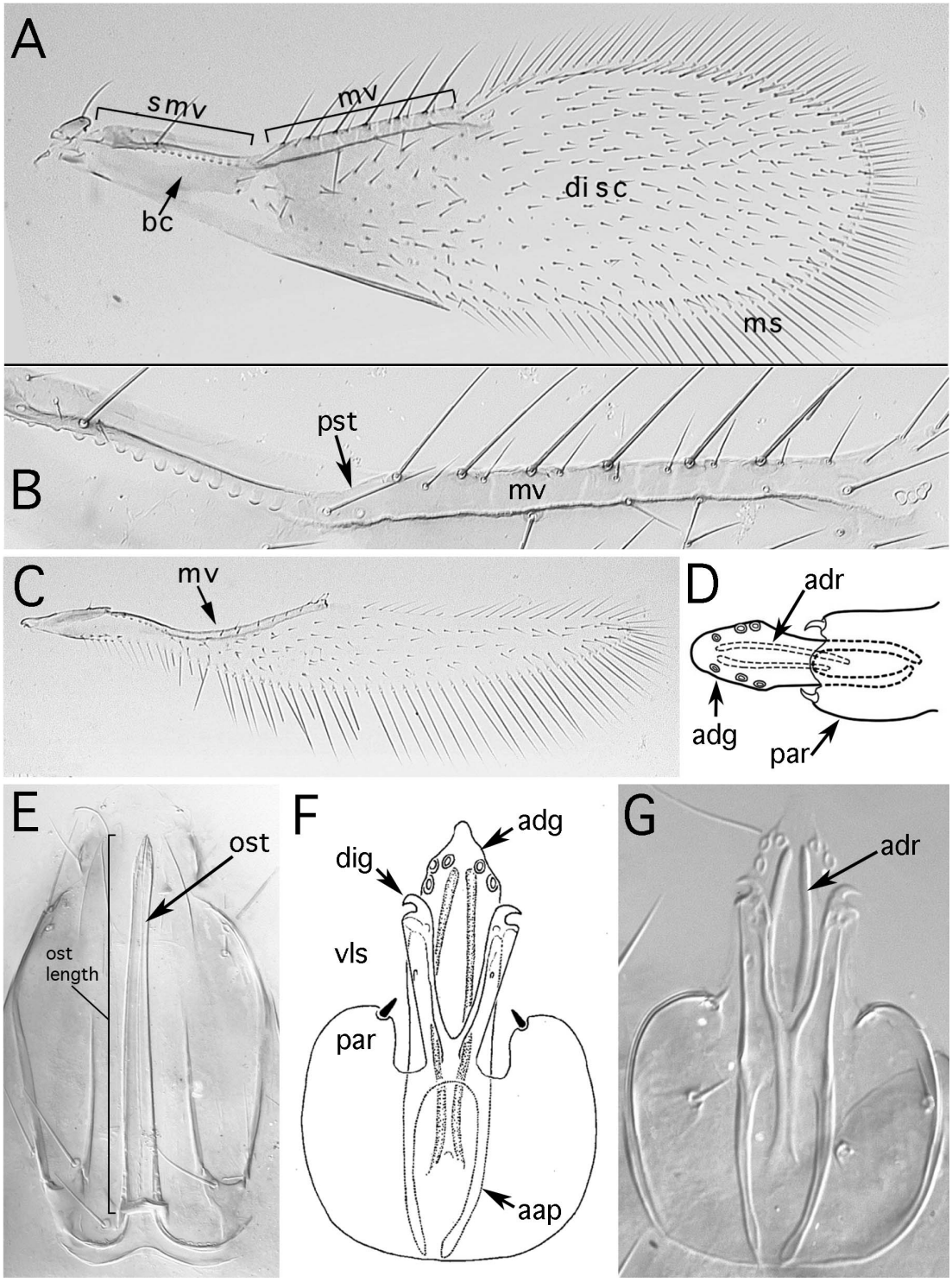


Figure 1.8. A–C, *C. berryi*. **A**, ♀, fore wing; **B**, ♀, fore wing, detail of submarginal vein and marginal vein; **C**, ♀, hindwing. **D**, *C. noacki*. ♂, genitalia. **E–G**, *C. berryi.* **E**, ♀, genitalia; **F–G**, ♂, genitalia.

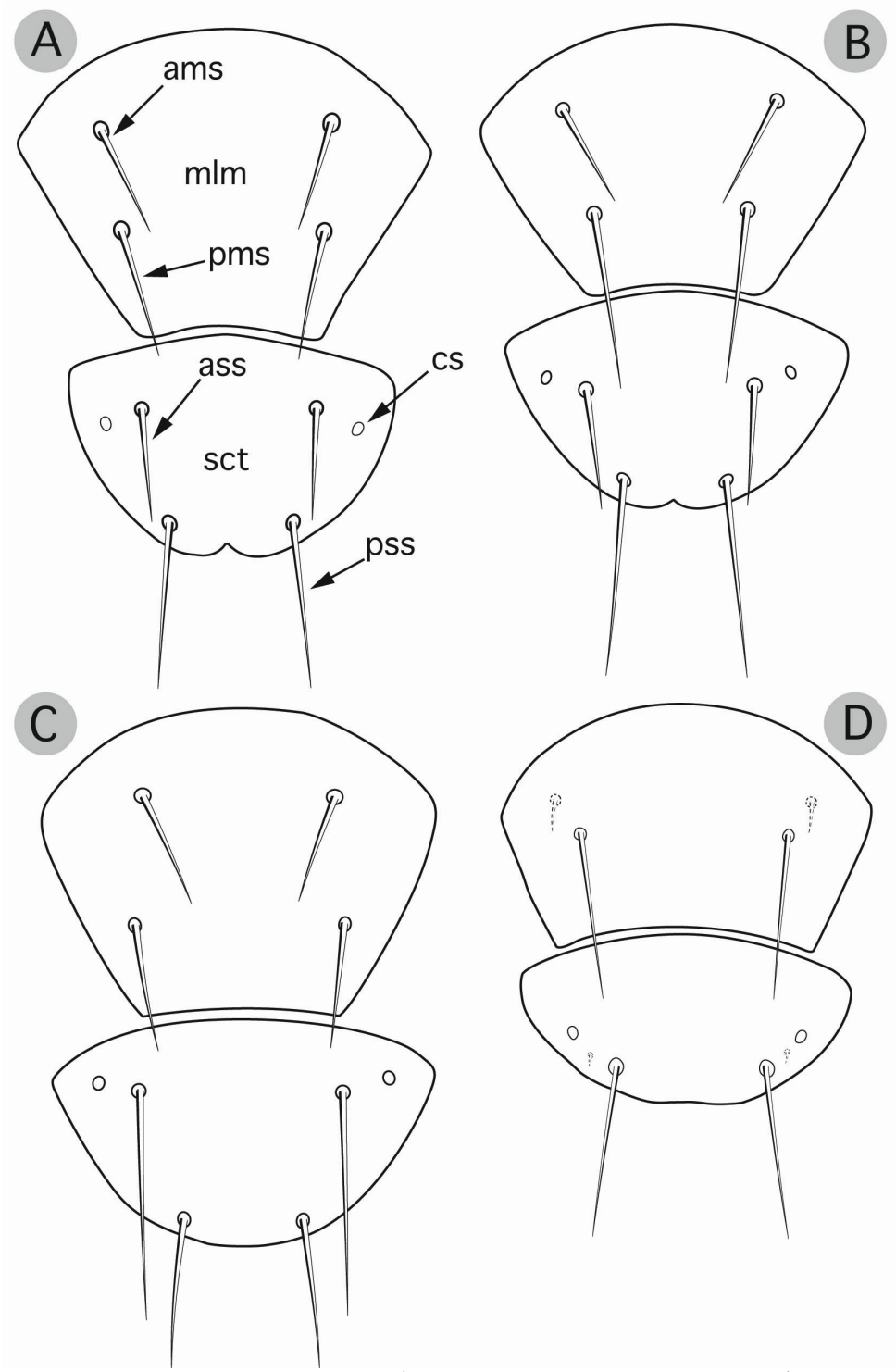


Figure 1.9. A, *C. orchamoplati*. ♂, mesoscutum. **B**, *C. spenceri*. ♂, mesoscutum. **C**, *C. berryi*. ♂, mesoscutum. **D**, *C. noacki*. ♂, mesoscutum. Minute setae on *C. noacki* mesoscutum (indicated by dashed lines) not always present. Figures not drawn to scale.

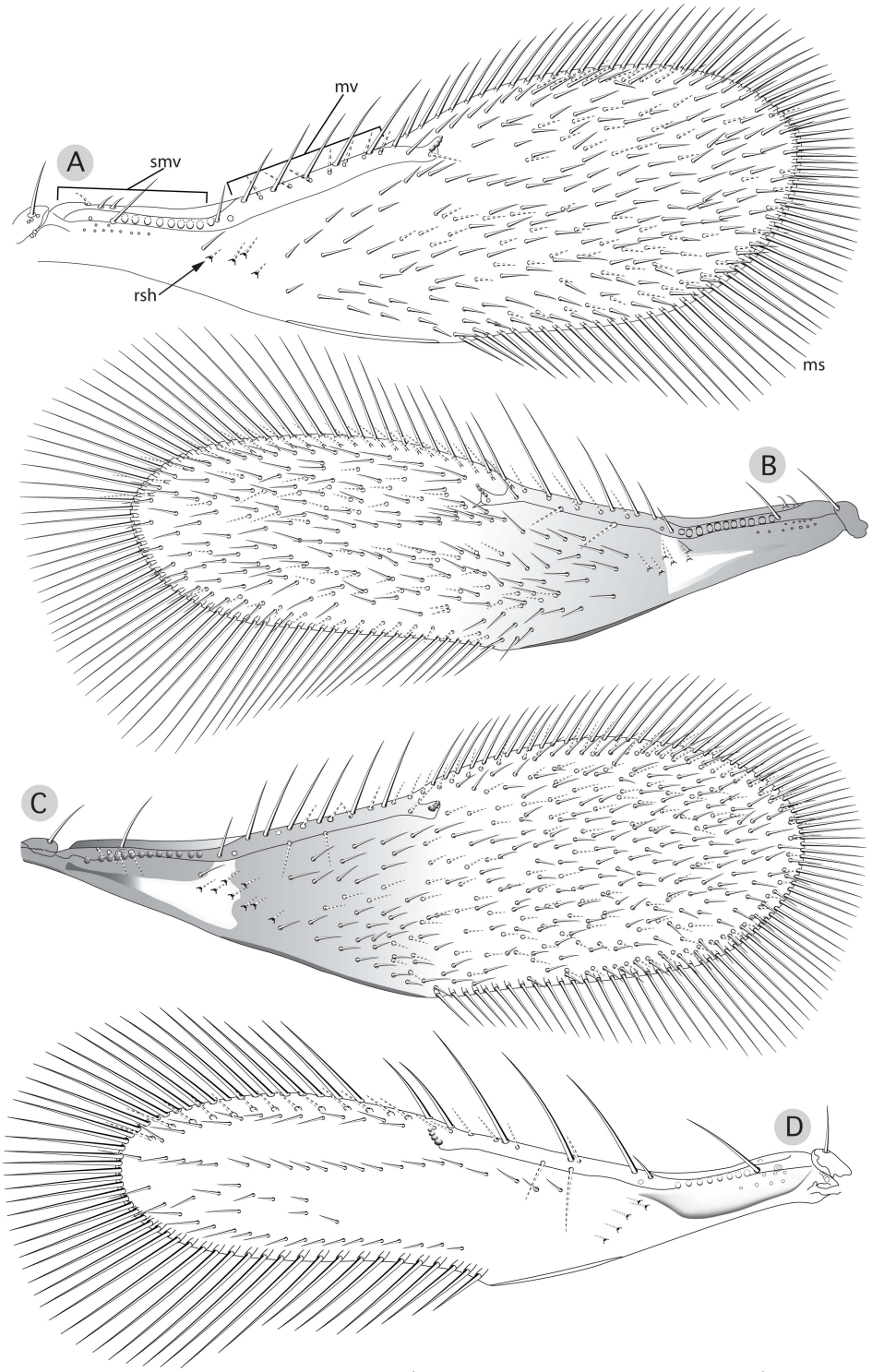


Figure 1.10. A, *C. orchamoplati*. ♂, fore wing. **B**, *C. spenceri*. ♂, fore wing. **C**, *C. berryi*. ♂, fore wing. **D**, *C. noacki*. ♂, fore wing. Dashed lines indicate setae occurring on ventral surface of wing. Figs not drawn to scale.

CHAPTER 2 – Revision of the genus *Cales* (Hymenoptera: Aphelinidae) with descriptions of new species

ABSTRACT

The genus *Cales* (Hymenoptera: Aphelinidae) includes 22 species in the Neotropical region. A neotype is designated for the single known species, *Cales noacki* Howard, and *C. noacki* is redescribed based on specimens molecularly determined to be conspecific with the neotype. Newly described species include the following: *C. bicolor* Mottern, **n. sp.**, *C. breviclava* Mottern, **n. sp.**, *C. breviscutellum* Mottern **n. sp.**, *C. brevisensillum* Mottern, **n. sp.**, *C. curvigladius* Mottern, **n. sp.**, *C. fusca* Mottern, **n. sp.**, *C. indistincta* Mottern, **n. sp.**, *C. longiseta* Mottern, **n. sp.**, *C. mogensenae* Mottern, **n. sp.**, *C. monteverdensis* Mottern, **n. sp.**, *C. multisensillum* Mottern, **n. sp.**, *C. noyesi* Mottern, **n. sp.**, *C. panamensis* Mottern, **n. sp.**, *C. parvigladius* Mottern, **n. sp.**, *C. pellonotum* Mottern, **n. sp.**, *C. peruviana* Mottern, **n. sp.**, *C. primapluvia* Mottern, **n. sp.**, *C. rosei* Mottern, **n. sp.**, *C. secundapluvia* Mottern, **n. sp.**, *C. stenoptera* Mottern, **n. sp.**, and *C. triensapluvia* Mottern, **n. sp.** Species are described based on molecular synapomorphies in 28S-D2 rDNA and COI, and supported with morphological characters whenever possible. *Cales* are highly morphologically conserved and character-poor, resulting in several cryptic species complexes. A molecular phylogeny of the known Neotropical species based on 28S-D2-5 rDNA and a 390 bp segment of COI is included, and separate identification keys to females and males are provided.

INTRODUCTION

Cales is an unusual genus of chalcidoid wasps that are parasitoids of whiteflies, at least for the few species with known host records. Most biological information about the genus is known from *Cales noacki* Howard, a biological control agent of the woolly whitefly, *Aleurothrixus floccosus* Maskell (Debach and Rose, 1976). Mottern et al. (2011) focused on a detailed taxonomic history of the group, a summary of its use in biological control, and comparative morphology among the three Australian species and the single known Neotropical species, *C. noacki*. Previous molecular analyses have consistently recovered a monophyletic *Cales,* but were unable to identify its probable sister group (Munro et. al. 2011). Citing a lack of molecular or morphological support for relationships, Mottern et al. (2011) left *Cales* as an unplaced subfamily (Calesinae) within Chalcidoidea. In subsequent analyses combining molecular and morphological data, Heraty et al. (in press) found a consistent monophyletic grouping of Aphelininae, Calesinae, Coccophaginae, Eretmocerinae, and Eriaphytinae, and hence revised the status of the family Aphelinidae to include only these subfamilies.

While collecting data for their large molecular phylogenetic analysis of Chalcidoidea, Munro et al. (2011) discovered two very distinct genetic variants within the population of *C. noacki* in the biological control orchards at the University of California, Riverside. Though these specimens were morphologically indistinguishable, their genetic divergence in 28S ribosomal DNA was consistent with very distantly related species or even different genera (Campbell et al., 1993; Babcock et al., 2001). This result led to a search for additional *Cales* specimens from throughout the Neotropics in an effort to gain

a broader understanding of the species diversity in this apparently morphologically conserved group. The relative rarity of *Cales* in entomological collections combined with the need for fresh material led us to focus primarily on recent field collections for this study. The richest source of material proved to be screen-sweep collections made by John Noyes in Costa Rica.

It has become increasingly apparent that cryptic species complexes are ubiquitous among the parasitic Hymenoptera (Smith et al., 2008; Heraty, 2009). DNA barcoding has been proposed as a method for species discovery and identification (Hajibabaei et al., 2007; Stoeckle and Hebert 2008), though this methodology has met with substantial criticism (Will & Rubinoff, 2004; Hurst & Jiggins, 2005; Meyer & Paulay, 2005; Hickerson et al., 2006; Elias et al., 2007; Wiemers & Fiedler, 2007). Furthermore, most studies focus on developing methods to distinguish among a relatively small number of biologically distinct but morphologically uniform populations (*e.g.* Stouthamer et al., 1999, 2000; Alvarez and Hoy, 2002; Kankare et al., 2005a,b; Monti et al., 2005; Bernardo et al., 2008; Desneux et al., 2009). More recent studies explore the use of integrative molecular and morphological techniques for determing species boundaries (e.g. Heetoff et al., 2011; Ceccarelli et al., 2012), though these studies do not incorporate such techniques into taxonomic revisions. Here we use the results of two gene regions, geography, and morphology to provide a revision of the Neotropical species of *Cales*, and provide both molecular and, where possible, morphological diagnostic characters to serve as a basis for future studies in the systematics and biology of *Cales*.

MATERIALS AND METHODS

Molecular phylogenetic analyses

DNA extraction, amplification and sequencing – Genomic DNA was extracted using a modified Chelex® extraction protocol (Walsh et al., 1991). Gene amplification was performed using standard PCR protocols optimized for chalcidoid DNA (Heraty et al., 2004). Amplified gene regions included 28S rDNA expansion regions D2 and D3-5, and a 390 bp segment of cytochrome oxidase subunit I (COI). In some cases, internal primers were used to amplify 28S-D2. Sequences of all primers used in this study can be found in Table 2.1. All sequencing was performed at the UCR Genomics Core Facility. Sequences were verified by comparing forward and reverse reads, and mitochondrial DNA was examined for stop codons using the software package Geneious v.5.6.6 (Biomatters, available from http://www.geneious.com/). All sequences used for this study have been uploaded to GenBank, and accession numbers are listed in Table 2.2.

Sequence Alignment – Ribosomal DNA sequences were aligned using the E-INS-i algorithm in MAFFT v.6 (Katoh et al., 2009) with default settings, except that the scoring matrix parameter was set to "1PAM /k=2" for aligning closely related sequences. Visual examination of the alignment revealed no obvious alignment errors, so no manual corrections were made to the alignment. Ribosomal and mitochondrial genes were then concatenated for a final alignment length of 1514 bp.

Analyses – Maximum likelihood (ML) analysis of the concatenated alignment (ribosomal and mitochondrial DNA) was conducted using RAxML v.7.3.2 under a GTR+Γ model (Stamatakis et al., 2008) as implemented through the CIPRES Web Portal

v.3.3 (http://www.phylo.org/). The data were analyzed with 500 rapid bootstraps using four gene partitions: 28S-D2, 28SD3-5, COI codon positions 1 and 2, and COI codon position 3. The analysis was repeated 10 times with different random seed values for the parsimony starting trees and random bootstrapping. The tree with the best final likelihood score was chosen from among these analyses. The resulting tree was visualized and drawn using FigTree v.1.3.1 (Rambaut, 2009).

Bayesian analysis was conducted using MRBAYES v.3.1.2 (as implemented through the CIPRES Web Portal v.3.3 (http://www.phylo.org). The data were analyzed using four gene partitions: 28S-D2, 28SD3-5, COI codon positions 1 and 2, and COI codon position 3. The appropriate models of sequence evolution for each partition were determined with jModelTest 2 (Guindon and Gascuel, 2003; Darriba et al., 2012) using the Akaike information criterion. The analysis included two runs, each with 4 chains running for 100,000,000 generations, with trees sampled every 10,000 generations, with both runs converging (average standard deviation of split frequencies <0.01). Tracer v.1.5.0 (Rambaut and Drummond, 2007a) was used to calculate the effective sample size for each run (>200 for both), and the results of the two runs combined following the exlusion of 25% burn-in (2500 trees). Posterior probabilities were calculated and plotted onto the maximum clade credibility tree using TreeAnnotator v.1.7.2 (Rambaut and Drummond, 2007b), and the tree visualized using FigTree v.1.3.1 (Rambaut, 2009).

Maximum parsimony analyses were conducted using TNT version 1.1 (Goloboff et al., 2003; Goloboff et al., 2008b) using a New Technology Search using a sectorial search, ratchet weighting probability of 5% with 50 iterations, tree-drifting of 50 cycles,

tree-fusing of 5 rounds, and a best score hit of 10 times. Clade support values were calculated using 1000 standard bootstrap replicates.

To compare genetic distances between pairs of species, uncorrected-p pairwise distances were calculated using PAUP 4.0* (Swofford 2002).

Species diagnoses, descriptions and specimen deposition

Species diagnoses – Mottern et al. (2011) provided a detailed generic description and diagnosis for *Cales*, so these are not repeated here. Neotropical *Cales* are easily distinguishable from Australian species by the characters provided in previous generic and species descriptions (Viggiani and Carver, 1988; Mottern et al., 2011). The species diagnoses included herein focus on only those characters necessary to distinguish among New World species or species complexes. Species of *Cales* are morphologically uniform and character-poor. Also, several species are known from only a single sex. Therefore, complete diagnoses based on morphology alone are not always possible, and species must be diagnosed based on their 28S-D2 sequences (Table 2.4) or COI. Specimens that differed by three or more bases for 28S-D2 in a pairwise comparison were considered different species. This equates to greater than 0.5% difference. This cut-off was determined by examining the degree of divergence within species where multiple specimens were sampled from the same population (*C. noacki* and *C. rosei*). These two species occur sympatrically within the UCR biological control grove, and differ by 3.3% in 28S-D2. Specimens within each of these species never differed by more than two bases in 28S-D2. We use this empirically determined cut-off for the degree of sequence divergence needed to merit species status, with most species pairs exceeding 3.0%

divergence. At a minimum, species pairs determined using this method also differed by at least 4.5% in COI, which is well beyond the 2–3% divergence typically found between closely related species pairs (Hebert et al., 2003). A combination of mitochondrial and ribosomal DNA information was similarly successful in distinguishing among closely related tachinid flies in Costa Rica (Smith et al., 2007). Because of the large number of new species still possible and the few specimens sequenced for many of the included species, we do not attempt to develop diagnostic molecular markers that could be employed in RFLP or multiplex PCR methods. Instead we advocate comparison of the entire 28S or COI sequence.

Specimen imaging – Wasps were photographed in ethanol prior to DNA extraction using a Leica Z16 APOA microscope (Leica Microsystems, Wetzlar, Germany) microscope fitted with a JVC KY-F75U 3CCD digital camera (JVC Americas Corp., Wayne, New Jersey, U.S.A.). Individual images were captured using Archimed software v.5.4.1 (Microvision Instruments, Évry, France) and stacked using CombineZP (Alan Hadley, http://www.hadleyweb.pwp.blueyonder.co.uk/CZP/News.htm) using the "Do Stack" algorithm. Following extraction, specimens were slide mounted in Canada balsam, and additional photographs taken using a Zeiss Axioskop 2 mounted with a 1.4 megapixel CCD camera (model# LW1165C, Lumenera Corp., Ottawa, Ontario, Canada). Individual images were again captured using Archimed software v.5.4.1 and stacked using the "Do Weighted Average" algorithm in CombineZP.

Measurements – All measurements are diagrammed and explained in Figures 2.1 and 2.2. Measurements were taken from the stacked photographs with continual reference

to the source images to ensure specimens were mounted suitably flat for accurate measurements. Structures deemed too far out of a single plane of focus were excluded from measurement, so not all species descriptions have a compete set of measurements. All measurements were conducted using ImageJ v1.44o (National Institutes of Health, available from http://imagej.nih.gov/ij).

Morphological descriptions – Morphological terms and their abbreviations follow the terminology of Mottern et al. (2011) and are defined in Figures 2.1 and 2.2. Drawings of the mesonota are included in the photo plates to clearly show the relative positions of setae and sensilla. In some cases, the relative positions of structures in the drawing will not exactly match the relative positions of the positionally homologous structures on the left (photographic) half. These differences reflect actual asymmetry in the specimen serving as the basis for the figure and are not errors in interpretation of the photographs. Wings were excluded from measurements if they required more than about 4 images to capture the entire wing outline at 20X magnification. This occurred most frequently with wings that were not dissected from the body. For bilaterally symmetrical structures, only one side was measured per specimen, choosing the structure that is least distorted by tilting within the mounting medium, damage, or slide mounting artifacts. Keys to male and female species and species complexes require slide-mounted specimens and examination with a compound microscope. Geographic coordinates obtained by subsequent georeferencing of the specimens rather than from the original collection information are surrounded by brackets.

Specimen deposition – The following institutions served as sources of material and type depositories for specimens examined for this study. **BMNH**: The Natural History Museum, London, England. **MACN**: Museo Argentina de Ciencias Naturales "Bernardino Rivadavia," Buenos Aires, Argentina. **UCRC**: University of California, Riverside, Entomology Research Museum, Riverside, CA, USA. **USNM**: National Museum of Natural History, Washington, DC, USA.

RESULTS

Molecular phylogenetics

The ML tree, Bayesian maximum clade credibility tree, and MP strict consensus tree are mostly congruent, so support values above 60% are mapped onto the tree from the ML analysis (Fig. 2.3). All species were monophyletic with the exception of a paraphyletic *C. mogensenae* (with respect to *C. fusca*) in the ML and Bayesian analyses, though this relationship lacked support. The parsimony analysis resulted in 145 most parsimonious trees, each 746 steps long. The ML and Bayesian trees were not among the most parsimonious trees, and mapped to 765 and 758 steps, respectively. Relationships among species and major clades were mostly the same between the ML and Bayesian trees, with the exception that *C. primapluvia* is sister to *C. secundapluvia* in the Bayesian analysis and sister to *C. breviclava* in the ML analysis. Other differences between the two analyses were intraspecific variations within *C. rosei*, *C. noacki*, and *C. noyesi*.

A sister-group relationship between *C. noacki* and *C. rosei* is consistently recovered (both taxa being associated with Citrus), though it lacks either bootstrap or posterior probability support. There is some support in the ML analysis for *C. longiseta*

as the sister group to the remaining Neotropical *Cales* (Fig. 2.3, Clade 1), and this relationship is also recovered in the Bayesian tree, although the relationship lacks strong posterior probability support (53%). A moderately supported clade (Fig. 2.3, Clade 2) is also supported by two morphological apomorphies: the presence of multiporous plate sensilla on F3 of the female antenna and the separation of the posterior mesoscutellar seta from the posterior edge of the mesoscutellum by less than the diameter of the setal socket. It is unclear if the presence of mps on F3 is plesiomorphic or derived for Neotropical *Cales*, as mps are absent in *Cales orchamoplati* Viggiani and Carver (from Australia) and present in *Cales berryi* Heraty and Mottern (from New Zealand).

Pairwise uncorrected-p distances between species for both 28S-D2 and COI are given in Table 2.3. Species pairs differed by 0.5–8.6% for 28S-D2 and 4.5-12.1% for COI.

Genus *Cales* Howard, 1907

Cales Howard 1907: 82-83. Type species: *Cales noacki*, by monotypy and original designation. Deposition: USNM (lost).

Diaspidophilus Brèthes 1914: 15-16. Type species: *Diaspidophilus pallidus*, by monotypy and original designation. Deposition: MACN. Synonymy by Gahan in Mercet 1929: 114.

Paranthemus Girault 1915: 165. Type species: *Paranthemus spenceri*, by monotypy and original designation. Deposition: QM. Synonymy by Hayat 1983.

Note: In some cases, pairs of molecular species are morphologically indistinguishable (cryptic species) for males, females, or both. Therefore, the cryptic species complexes shown in the male and female keys may not match each other. If two species cannot be morphologically distinguished on the basis of males and females, they are listed as cryptic in the species diagnoses, and the reader is referred to Table 2.4, which lists the variable regions in 28S-D2 for each species.

Key to female species and cryptic species complexes

1. Ovipositor shorter than mesotibia, 0.69–0.81× length of mesotibia 2

– Ovipositor about as long or longer than mesotibia, 0.97–1.44× length of mesotibia .. 3

2. Six multiporous plate sensilla on clava; ovipositor with strong dorsal curvature (Fig. 2.9A,F), curvature remaining even after slide mounting; Gt_7, second valvifer, and ovipositor valves all hyaline; Gt_7 laterally expanded (Fig. 2.9F). Colombia ..... *C. curvigladius*

– Four multiporous plate sensilla on clava; ovipositor with at most slight dorsal curvature, usually completely flat after slide mounting; valves of ovipositor pale yellow (Fig. 2.23F). Costa Rica ..... *C. parvigladius*

3. Clava with 4 or five mps; F3 with one mps or without mps 4

– Clava with 10 mps; 3 mps on F3. French Guiana ..... *C. multisensillum*

4. Clava with 4 mps; F3 without mps 5

– Clava with 4 or 5 mps; F3 with one mps 6

5. Second valvifer hyaline, lighter than pale yellow valves (Fig. 2.4F) .... *C. bicolor*, *C mogensenae*, *C. noacki*, *C. rosei*, *C. secundapluvia*, *C. triensapluvia*

(See Table 2.4 for molecular diagnoses of these species)

– Second valvifer infuscated, about same color as pale yellow valves and lighter than hyaline Gt_7 (Fig. 2.27F). Ecuador ..... *C. primapluvia*

6. Second valvifer and Gt_7 hyaline, lighter in color than pale yellow valves (Fig. 2.11F) ..... *C. breviscutellum* and *C. indistincta*

(See Table 2.4 fore molecular diagnoses of these species)

– Second valvifer and Gt_7 infuscated, darker in color than pale yellow valves (Fig. 2.20F) ..... *C. noyesi* and *C. stenoptera*

(See Table 2.4 for molecular diagnoses of these species)

Key to male species and cryptic species complexes

1. Aedeagal rods more than half length of aedeagus (0.54–0.77×; Figs 2.13F, 2.22E) ... 2

– Aedeagal rods less then half length of aedeagus (0.44×; Fig. 2.16F). Costa Rica ..... *C. monteverdensis*

2. Antenna short and stout (Fig. 2.6F), F2-3 about 1.9× longer than wide and clava about 4.6× longer than wide ..... *C. breviclava*

– Antenna not as above, F2-3 at least 2.5× longer than wide and clava at least 5.5× longer than wide 3

3. Anterior seta of mesoscutellum long, at least 0.23× length of mesoscutellum (Fig. 2.8D) 4

– Anterior seta of mesoscutellum short, no more than 0.17× length of mesoscutellum (Fig. 2.5D) 5

4. Anterior seta of mesoscutellum about 0.4× length of mesoscutellum (Fig. 2.13D); aedeagal rods with longitudinal furrows (Fig. 2.13F). Costa Rica ..... *C. longiseta*

– Anterior seta of mesoscutellum about 0.2× length of the mesoscutellum (Fig. 2.8D); aedeagal rods smooth (Fig. 2.8F). Costa Rica ..... *C. brevisensillum*

5. Posterior mesoscutellar seta advanced anteriorly from the posterior edge of mesoscutellum by about diameter of setal socket or greater (Figs 2.4D, 2.26C). Questionable cases where socket is separated by slightly less than diameter key here 6

– Posterior mesoscutellar seta advanced anteriorly from posterior edge of mesoscutellum by much less than diameter of setal socket (Fig. 2.20D) ..... *C. indistincta*, *C. noyesi*, and *C. panamensis* (See Table 2.4 for molecular diagnoses of these species)

6. Anterior mesoscutellar seta advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum (Fig. 2.10C) ..... *C. fusca*

– Anterior mesoscutellar seta either posterior to campaniform sensillum, or, if advanced then separated anteriorly by less than diameter of sensillum (Figs 2.18D, 2.23D) ..... *C. bicolor*, *C. mogensenae*, *C. noacki*, *C. parvigladius*, *C. pellonotum*, *C. peruviana*, and *C. rosei* (See Table 2.4 for molecular diagnoses of these species)

New Species Descriptions

***Cales bicolor* Mottern, n. sp.**

(Figs 2.4, 2.5)

DIAGNOSIS: *Cales bicolor* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not anteriorly advanced from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. Females can be distinguished from *C. curvigladius C. parvigladius* and *C. primapluvia* by the following combination of characters: valves of ovipositor longer than mesotibia and without strong dorsal curvature; second valvifer hyaline, lighter in color than pale yellow valves. Males can be distinguished from *C. monteverdensis* by the relatively short aedeagal rods of the latter species, though *C. monteverdensis* is known only from a single male specimen, so it is unknown if ranges for this character overlap. *Cales bicolor* is considered morphologically cryptic with *C. brevisensillum*, *C. mogensenae*, *C. noacki*, *C. pellonotum*, *C. peruviana*, *C. rosei*, *C. secundapluvia* and *C. triensapluvia.*

DESCRIPTION: Female: (Fig. 2.4), ***color and sculpture*** (Fig. 2.4A,D) – head and body pale yellow or pale orange, with faint infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with weak reticulate sculpture. ***Antenna*** (Fig. 2.4B) – radicle 4.36–4.93× longer than wide (n=3); scape 2.30–3.48× longer than wide (n=3); pedicel 1.90–2.45×

longer than wide (n=4); F3 1.70–2.17× longer than wide (n=4); clava 4.10–5.18× longer than wide, and with 4 mps (n=3). ***Mesosoma*** (Fig. 2.4D) – midlobe of mesoscutum 1.30–1.52× wider than long (n=4); midlobe seta 0.61–0.65× length of midlobe, and setal socket advanced 0.35–0.40× length of midlobe (n=4); mesoscutellum 2.00–2.14× wider than long (n=4); anterior mesoscutellar seta short, 0.09–0.14× length of mesoscutellum and not advanced anteriorly from campaniform sensillum; posterior mesoscutellar seta 1.09–1.12× length of mesoscutellum (n=2), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket (n=4). ***Wings*** (Fig. 2.4C,E) – fore wing 4.05–4.27× longer than wide (n=3); apical seta of fore wing 0.75–0.85× width of fore wing (n=2); hind wing 9.27–9.37× longer than wide (n=2). ***Genitalia*** (Fig. 2.4F) – ovipositor 1.09–1.39× length of mesotibia (n=4); second valvifer 0.38–0.43× length of ovipositor; second valvifer and Gt_7 hyaline, valves pale yellow.

Male: (Fig. 2.5), ***color and sculpture*** (Fig. 2.5A,D) – head and body orange, darker dorsally, with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.5B) – radicle 4.52–5.30× longer than wide (n=3); scape 2.78–3.30× longer than wide (n=3); pedicel 1.79–2.29× longer than wide (n=3) F2-3 2.14–3.20× longer than wide (n=3); clava 5.56–7.61× longer than wide, longest mps of clava 0.53–0.61× length of clava (n=3). ***Mesosoma*** (Fig. 2.5D) – midlobe of mesoscutum 1.49–1.56× wider than long (n=3); setal socket of midlobe advanced 0.35-0.40× length of midlobe (n=3); mesoscutellum 1.87–2.02× wider than long (n=3); anterior mesoscutellar seta short, 0.09× length of mesoscutellum, and not advanced relative to campaniform

sensillum (n=1); socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of a setal socket (n=3). ***Wings*** (Fig. 2.5C,E) – fore wing 3.92–4.31× longer than wide (n=3); apical seta of fore wing 0.47–0.73× width of fore wing (n=3); hind wing 9.34–9.39× longer than wide (n=2). ***Genitalia*** (Fig. 2.5F) – aedeagus 0.28–0.32× length of mesotibia (n=2); aedeagal rod 0.58–0.62× length of aedeagus (n=2).

HOLOTYPE: Costa Rica: ♀, Puntarenas Prov., Res. Abs., Cabo Blanco, 30m, 9°35'00"N, 85°06'00"W, 16-17 Feb 2009, J.S. Noyes [UCRC_ENT 00313857: BMNH].

PARATYPES: Costa Rica: **1**♀, Heredia Prov., La Selva Biol. Sta., 50m, 10°25'51"N 84°01'14"W, 13 Aug 2010, R. Waterworth, rainforest trail [UCRC_ENT 00282836: UCRC]. **1**♂, Heredia Prov., Santo Domingo, INBio Parque, 9°59'00"N 84°06'00"W, 16 Feb 2008, J.S. Noyes [UCRC_ENT 00313875: BMNH]. **2**♂♂, **2**♀♀, Puntarenas Prov., Res. Abs., Cabo Blanco, 30m, 9°35'00"N 85°06'00"W, 16-17 Feb 2009, J.S. Noyes [UCRC_ENT 00313859-61, UCRC_ENT 00313892: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Latin: *bicolor* = two colors, referring to the apparent sexual dimorphism in color where males are bright orange and females are pale orange or yellow.

REMARKS: *Cales bicolor* is known from seven specimens, four females and three males. All specimens are identical for 28S-D2 with the exception of one polymorphism in one female (in a mostly conserved region not included in Table 2.3). One female specimen (UCRC ENT 313892/D3331) failed to sequence for 28S-D2, but groups with

the remaining *C. bicolor* specimens based on 28S-D3-5 and COI with 100% bootstrap support. This specimen also matches the other *C. bicolor* females morphologically, and so is placed with *C. bicolor*. COI variation ranged from 0.3-1.8% within the species.

***Cales breviclava* Mottern, n. sp.**

(Fig. 2.6)

DIAGNOSIS: *Cales breviclava* can be distinguished from all other species of *Cales* except *C. fusca* and *C. longiseta* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. *Cales breviclava* can be distinguished from *C. fusca* and *C. longiseta* by the relatively short and stout antenna. The ranges for length/width ratios for F2-3 (1.69–2.08× longer than wide) and the clava (3.86–5.41× longer than wide) do not overlap with the ranges any other known species of *Cales*.

DESCRIPTION: Male: (Fig. 2.6), ***color and sculpture*** (Fig. 2.6A,D) – head and body orange, darker dorsally with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with robust reticulate sculpture. ***Antenna*** (Fig. 2.6B) – radicle 4.30–4.93× longer than wide (n=3); scape 2.53–2.92× longer than wide (n=3); pedicel 1.61–1.78× longer than wide (n=2); F2-3 1.69–2.08× longer than wide (n=2); clava short, 3.86–5.41× longer than wide, longest mps of clava 0.57–0.59× length of clava (n=3). ***Mesosoma*** (Fig. 2.6D) – midlobe of mesoscutum 1.40–1.45× wider than long (n=4); midlobe seta 0.80–0.89× length of midlobe, setal socket advanced 0.42–0.51× length of midlobe (n=4); mesoscutellum

1.63–1.90× wider than long (n=4); anterior mesoscutellar seta short, 0.14–0.19× length of mesoscutellum, and seta advanced anteriorly of campaniform sensillum by a distance greater than diameter of campaniform sensillum (n=4); posterior mesoscutellar seta relatively long,1.09–1.56× length of mesoscutellum (n=3), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of a setal socket (n=4). ***Wings*** (Fig. 2.6C,E) – fore wing 4.05–4.33× longer than wide (n=3); apical seta of fore wing 0.88–1.19× width of fore wing (n=3); hind wing 8.06–9.63× longer than wide (n=4). ***Genitalia*** (Fig. 2.6F) – aedeagus 0.33–0.38× length of mesotibia (n=2); aedeagal rod 0.52–0.56× length of aedeagus (n=2).

Female: unknown.

HOLOTYPE: Costa Rica: ♂, Puntarenas Prov., Los Charcos de Osa, 50m, 8°40'00"N 83°30'00"W, 15-16 Feb 2010, J.S. Noyes [UCRC_ENT 00313905: BMNH].

PARATYPES: Costa Rica: **1**♂, Puntarenas Prov., Los Charcos de Osa, 50m, 8°40'00"N, 83°30'00"W, 18-19 Feb 2008, J.S. Noyes [UCRC_ENT 00313853: BMNH]. **2**♂♂, Limón Prov., Reserva Biológica Hitoy-Cerere, 100m, 9°40'00"N, 83°02'00"W, 22-23 Feb 2010, J.S. Noyes [UCRC_ENT 00313887, UCRC_ENT 00313891: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Latin noun: *breviclava* = "short club," referring to the relatively short male clava of this species.

REMARKS: All four specimens are identical for 28S-D2. The two specimens sequenced for COI differed by 1.8%.

***Cales breviscutellum* Mottern, n. sp.**

(Fig. 2.7)

DIAGNOSIS: *Cales breviscutellum* can be differentiated from all other species of *Cales* except *C. indistincta, C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by less than diameter of setal socket. Females can be differentiated from *C. multisensillum, C. noyesi* and *C. stenoptera* by the following combination of characters: one mps on F3 and five mps on clava; second valvifer and Gt_7 hyaline, lighter in color than pale yellow valves. Based on the data available (the single *C. indistincta* specimen is missing its clava), *C. breviscutellum* is considered morphologically cryptic with *C. indistincta* and *C. panamensis*.

DESCRIPTION: Female: (Fig. 2.7), ***color and sculpture*** (Fig. 2.7A,B,D) – head and body white; both midlobe and mesoscutellum with very faint reticulate sculpture. ***Antenna*** (Fig. 2.7C) – radicle 5.37–5.76× longer than wide (n=3); scape 2.72–2.93× longer than wide (n=3); pedicel 2.30–2.71× longer than wide (n=3); F3 2.42–2.88× longer than wide, and with one mps (n=3); clava 4.31–5.93× longer than wide, and with 5 mps (n=3). ***Mesosoma*** (Fig. 2.7D) – midlobe of mesoscutum 1.39–1.47× wider than long (n=3); midlobe seta 0.45× length of midlobe (n=1), and setal socket advanced 0.21× length of midlobe (n=3); mesoscutellum 2.22–2.63× wider than long (n=3); anterior mesoscutellar seta short, 0.08–0.15× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar

seta 1.16–1.21× length of mesoscutellum (n=2), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of a setal socket (n=3). ***Wings*** (Fig. 2.7E) – fore wing 4.29× longer than wide (n=1); apical seta of fore wing 0.73× width of fore wing (n=1); hind wing 10.30–10.60× longer than wide (n=2). ***Genitalia*** (Fig. 2.7F) – ovipositor 1.08–1.13× length of mesotibia (n=3); second valvifer 0.42–0.53× length of ovipositor; second valvifer and Gt_7 hyaline, valves pale yellow.

Male: unknown**.**

HOLOTYPE: Peru: ♀, Madre de Dios, Los Amigos Bio. St., trail 13, 226m, 12°34'17"S 70°05'46.2"W, 27 Dec 2010, J. Heraty [UCRC_ENT 00320305: UCRC].

PARATYPES: Ecuador: **1**♀, Orellana, Reserva Etnica Waorani, 1 km S Onkone Gare Camp, 216.3m, 0°39'25.7"S 76°27'10.8"W, 8 Oct 1995, T.L. Erwin et al., terre firme forest [UCRC_ENT 00114799: USNM]. **1**♀, Orellana, Tiputini Biodiversity Sta. nr. Yasuni National Park, 220-250m, 0°37'55"S 76°08'39"W, 9 Feb 1999, T.L. Erwin et al., terre firme forest [UCRC_ENT 00117555: USNM].

DISTRIBUTION: Neotropical, Ecuador and Peru.

ETYMOLOGY: Latin noun: *breviscutellum* = "short scutellum," referring to the relatively short and wide mesoscutellum of this species.

REMARKS: All specimens are identical for 28S-D2. Variation in COI is unknown because this gene was successfully sequenced for only one specimen (the holotype).

***Cales brevisensillum* Mottern, n. sp.**

(Fig. 2.8)

DIAGNOSIS: *Cales brevisensillum* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not anteriorly advanced from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. *Cales brevisensillum* males can be distinguished from all known males by the following combination of characters: relatively short mps on clava (longest mps 0.50× length of clava); relatively long anterior seta of mesoscutellum (0.23× length of mesoscutellum) Only *C. longiseta* has longer anterior mesoscutellar setae (0.41× length of mesoscutellum), though seta is advanced anteriorly from sensillum by at least in diameter of a sensillum in *C. longiseta*.

DESCRIPTION: Male: (Fig. 2.8), ***color and sculpture*** (Fig. 2.8A,D) – head and body orange, darker dorsally with pronounced infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.8B) – radicle 4.1× longer than wide; scape 2.83× longer than wide; pedicel 2.34× longer than wide; F2-3 2.73× longer than wide; clava 7.43× longer than wide, longest mps of clava 0.50× length of clava. ***Mesosoma*** (Fig. 2.8D) – midlobe of mesoscutum not measured due to damage during slide mounting; mesoscutellum 1.78× wider than long, with posterolateral edges relatively straight rather than curved; anterior mesoscutellar seta relatively long, 0.23×

length of mesoscutellum, and not advanced anteriorly from campaniform sensillum, or if advanced, then separated anteriorly by less than diameter of sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by about 2× diameter of a setal socket. ***Wings*** (Fig. 2.8C,E) – fore wing 4.19× longer than wide; apical seta of fore wing 0.54× width of fore wing; hind wing 8.63× longer than wide. ***Genitalia*** (Fig. 2.8F) – aedeagus 0.21× length of mesotibia; aedeagal rod 0.57× length of aedeagus (n=).

Female: unknown.

HOLOTYPE: Costa Rica: ♂, Limón Prov., Reserva Biológica Hitoy-Cerere, 100m, 9°40'00"N 83°02'00"W, 22-23 Feb 2010, J.S. Noyes [UCRC_ENT 00313886: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Latin noun: *brevisensillum* = "short sensillum," referring to the relatively short multiporous plate sensilla of the male clava.

REMARKS: Both the midlobe of the mesoscutum and the mesoscutellum were torn during slide mounting, so these structures were digitally reassembled for the photo plate (Fig. 2.8D). *Cales brevisensillum* has a strongly supported sister group relationship with *C. curvigladius* (Fig. 2.3) from Colombia.

Cales curvigladius **Mottern, n. sp.**

(Fig. 2.9)

DIAGNOSIS: *Cales curvigladius* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C. indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta

of mesoscutellum not anteriorly advanced from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. *Cales curvigladius* females can be distinguished from all known females by the following combination of characters: dorsal and ventral valves strongly curved dorsally, even on slide mounted specimens; valves, second valvifer and Gt_7 all hyaline. The relative length of the ovipositor is also shorter than in any known species (0.69× length of mesotibia), though this character should be interpreted cautiously as only one specimen (paratype, UCRC_302419) had an ovipositor mounted adequately for measurement. *Cales curvigladius* is known only from females, so it is currently considered morphologically cryptic with *C. breviscutellum*, *C. monteverdensis*, *C. pellonotum,* and *C. peruviana,* as these species are known only from males.

DESCRIPTION: Female: (Fig. 2.9), ***color and sculpture*** (Fig. 2.9A,D) – head pale orange, body pale orange to white; both midlobe and mesoscutellum with distinct hexagonal reticulate sculpture. ***Antenna*** (Fig. 2.9B) – radicle 4.47–4.66× longer than wide (n=2); scape 2.98× longer than wide (n=1); pedicel 2.18–2.36× longer than wide (n=2); F3 2.38–2.40× longer than wide (n=2); clava 4.06–4.49× longer than wide, and with 6 mps (n=2). ***Mesosoma*** (Fig. 2.9D) – midlobe of mesoscutum 1.42–1.59× wider than long (n=2); midlobe seta 0.65–0.80× length of midlobe, and setal socket advanced 0.44–0.49× length of midlobe (n=2); mesoscutellum 1.70–1.73× wider than long, with posterolateral edges relatively straight rather than curved (n=2); anterior mesoscutellar seta short, 0.15× length of mesoscutellum (n=1) and not advanced anteriorly from campaniform sensillum (n=2); posterior mesoscutellar seta 1.09× length of

mesoscutellum (n=1), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket (n=2). ***Wings*** (Fig. 2.9C,D) – fore wing 4.26× longer than wide (n=1); apical seta of fore wing 0.56× width of fore wing (n=1); hind wing 9.48× longer than wide (n=1). ***Genitalia*** (Fig. 2.9F) – ovipositor short, 0.69× length of mesotibia (n=1) and with strong dorsal curvature, even after slide-mounting (n=3); Gt_7 laterally expanded, about ¼ width of entire ovipositor apparatus at their widest point; second valvifer 0.70× length of ovipositor; second valvifer, Gt_7 and valves hyaline.

Male: unknown

HOLOTYPE: Colombia: **1**♀, Magdalena, PNN Tayrona Pueblito, 225m, 11°20'00"N 74°02'00"W, 20 Sep-26 Oct 2000, R. Henriquez [UCRC_ENT 00282840: UCRC].

PARATYPE: Colombia: **1**♀, Vichada, PNN Tuparro Cerro Tomás, 250m, 5°21'00"N 67°51'00"W, 12-22 May 2001, W. Villalba [UCRC_ENT 00251715: UCRC]. **1**♀, Cauca, PNN Gorgona Alto el Mirador, 180m, 2°58'00"N 78°11'00"W, 3-16 Aug 2000, H. Torres [UCRC_ENT 00302419: UCRC].

DISTRIBUTION: Neotropical, Colombia.

ETYMOLOGY: Latin noun: *curvigladius* = "curved sword," referring to the strong dorsal curvature of the valves of the ovipositor.

REMARKS: The two specimens that yielded molecular data differ by a single base change (0.17%) in 28S-D2, and by 1.8% in COI. A third specimen (UCRC ENT 302419) matches the others morphologically but failed to sequence. Though all three specimens

are from Colombia, they are widely geographically separated, indicating that this species has a large range, with the two most distant localities over 1100 km apart.

Cales fusca Mottern, n. sp.

(Fig. 2.10)

DIAGNOSIS: *Cales fusca* can be distinguished from all other species of *Cales* except *C. breviclava* and *C. longiseta* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. *Cales fusca* can be differentiated from *Cales longiseta* and *C. breviclava* by the following combination of characters: anterior mesoscutellar seta short, 0.17× length of mesoscutellum; clava long, 6.59× longer than wide (3.86–5.41× in *C. breviclava*).

DESCRIPTION: Male: (Fig. 2.10), ***color and sculpture*** (Fig. 2.10A,C) – head orange, body pale yellow to white; conspicuous infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum, mps of antenna, and veins and setae of wings; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.10B) – radicle 5.11× longer than wide; scape 2.73× longer than wide; pedicel 2.34× longer than wide; F2-3 2.82× longer than wide; clava 6.59× longer than wide, longest mps of clava 0.66× length of clava. ***Mesosoma*** (Fig. 2.10C) – midlobe of mesoscutum 1.47× wider than long; midlobe seta 0.74× length of midlobe, setal socket advanced 0.44× length of midlobe; mesoscutellum 2.08× wider than long; anterior mesoscutellar seta 0.17× length of mesoscutellum and advanced anteriorly from

campaniform sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket. ***Wings*** (Fig. 2.10D,E) – fore wing 4.18× longer than wide; apical seta of fore wing 0.95× width of fore wing; hind wing 9.48× longer than wide.

Female: unknown.

HOLOTYPE: Costa Rica: ♂, Limón Prov., Reserva Biológica Hitoy-Cerere, 100m, 9°40'00"N 83°02'00"W, 24-26 Feb 2008, J.S. Noyes [UCRC_ENT 00313869: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Latin adjective: fusca = "dusky," referring to the heavily infuscated portions in the fore wing, antenna and mesonotum.

REMARKS: The contrast between the orange head and the pale yellow/white body may be a useful character for this species, although one specimen of *C. curvigladius* has similar coloration. Given that only a single male specimen is known and that other species of *Cales* have marked intraspecific color variation, body and head color should be interpreted cautiously until more specimens are available for examination. The aedeagus was badly distorted during slide mounting and could not be accurately measured.

Cales indistincta **Mottern, n. sp.**

(Figs 2.11, 2.12)

DIAGNOSIS: *Cales indistincta* can be differentiated from all other species of *Cales* except *C. breviscutellum, C. multisensillum*, *C. noyesi*, *C. panamensis*, and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum;

posterior mesoscutellar setal sockets separated by less than diameter of setal socket. Females can be distinguished from *C. multisensillum*, *C. noyesi*, and *C. stenoptera* by the following combination of characters: a single mps on F3 (three mps in *C. multisensillum*); second valvifer and Gt_7 hyaline (infuscated in *C. noyesi* and *C. stenoptera*). Males can be distinguished from *C. panamensis* by the relatively long aedeagal rods of the latter species (0.77× length of aedeagus in *C. panamensis* and 0.62× in *C. indistincta*), though males are only known from a single specimen for each species. *Cales indistincta* is considered morphologically cryptic with *C. breviscutellum*.

DESCRIPTION: Female: (Fig. 2.11), ***color and sculpture*** (Fig. 2.11A,D) – head and body orange, darker dorsally with faint infuscation on lateral/posterior margin of mesoscutellum; midlobe with barely-discernable reticulate sculpture anteriorly, but nearly smooth in posterior two-thirds; mesoscutellum with some reticulate sculpture visible at lateral and posterior margins. ***Antenna*** (Fig. 2.11B) – radicle 5.56× longer than wide; scape 3.14× longer than wide; pedicel 2.22× longer than wide; F3 2.31× longer than wide, and with 1 mps. ***Mesosoma*** (Fig. 2.11D) – midlobe of mesoscutum 1.52× wider than long; socket of midlobe seta advanced 0.30× length of midlobe; mesoscutellum 2.38× wider than long; anterior mesoscutellar seta short, 0.08× length of mesoscutellum and advanced anteriorly from campaniform sensillum by at least diameter of a sensillum; posterior mesoscutellar seta 1.18× length of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket. ***Wings*** (Fig. 2.11C,E) – fore wing 4.32× longer than wide; apical seta of fore wing 0.79× width of fore wing; hind wing 10.35× longer than wide. ***Genitalia***

(Fig. 2.11F) – ovipositor 1.29× length of mesotibia; second valvifer 0.42× length of ovipositor; second valvifer and Gt_7 hyaline, and valves pale yellow.

Male: (Fig. 2.12), ***color and sculpture*** (Fig. 2.12A,D) – head and body color similar to female but with more pronounced infuscation on lateral and posterior margins of mesoscutellum; very faint reticulate sculpture on midlobe and mesoscutellum, slightly more conspicuous than sculpture in female. ***Antenna*** (Fig. 2.12B) – radicle 5.89× longer than wide; scape 3.27× longer than wide; pedicel 2.20× longer than wide; F2-3 3.62× longer than wide; clava 9.10× longer than wide, longest mps of clava 0.65× length of clava. ***Mesosoma*** (Fig. 2.12C) – midlobe of mesoscutum 1.45× wider than long (n=); midlobe setal socket advanced 0.27× length of midlobe; mesoscutellum 2.23× wider than long; anterior mesoscutellar seta short, 0.10× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by at least diameter of a sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket. ***Wings*** (Fig. 2.12D) – wings not measured because they were tilted in the mounting medium. ***Genitalia*** (Fig. 2.12E) – aedeagus 0.30× the length of the mesotibia; aedeagal rod 0.62× the length of the aedeagus.

HOLOTYPE: Costa Rica: ♂, Heredia Prov., La Selva Biol. Sta., Arborita trail, 64m, 10°25'49"N 84°00'26"W, 10 Aug 2010, J. Mottern [UCRC_ENT 00282843: UCRC].

PARATYPE: Costa Rica: **1**♀, Heredia Prov., La Selva Biol. Sta., 70m, 10°25'21.2"N 84°00'05.2"W, 10 Aug 2010, J. Heraty, secondary scrub/banana [UCRC_ENT 00282839: UCRC].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Latin adjective: *indistincta* = "not clearly indicated or obscure," referring to the faint, indistinct sculpture on the mesonotum.

REMARKS: The two known specimens of *C. indistincta* are molecularly identical for both 28S-D2 and COI. *Cales indistincta* is sister to *C. panamensis* in both Bayesian and maximum likelihood analyses, though the relationship lacks bootstrap support (Fig. 2.3).

Cales longiseta **Mottern, n. sp.**

(Fig. 2.13)

DIAGNOSIS: *Cales longiseta* can be distinguished from all other species of *Cales* except *C. breviclava* and *C. fusca* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. Males can be differentiated from all other known males by the relatively long anterior seta of mesoscutellum (0.41× length of mesoscutellum in *C. longiseta*; no more than 0.23× length of mesoscutellum in other species), and by the presence of unique longitudinal furrows in the aedeagal rods.

DESCRIPTION: Male: (Fig. 2.13), ***color and sculpture*** (Fig. 2.13A,D) – head and body orange, darker dorsally with infuscation on anterior quarter of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.13B) – radicle 4.95× longer than wide; scape 3.14× longer than wide; pedicel 2.29× longer than wide; F2-3 2.82× longer than wide; clava 7.83× longer than wide, longest mps of clava 0.69× length of

clava. ***Mesosoma*** (Fig. 2.13D) – midlobe of mesoscutum 1.62× wider than long; midlobe seta 0.80× length of midlobe, setal socket advanced 0.45× length of midlobe; mesoscutellum 2.11× wider than long; anterior mesoscutellar seta long, 0.41× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar seta 1.14× length of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by about diameter of setal socket. ***Wings*** (Fig. 2.13C,E) – fore wing relatively broad, 4.02× longer than wide; apical seta of fore wing 0.49× width of fore wing; hind wing relatively broad, 7.91× longer than wide. ***Genitalia*** (Fig. 2.13F) – aedeagus 0.24× length of mesotibia; aedeagal rod 0.54× length of aedeagus, rod with distinct longitudinal furrow (Fig. 2.13F, inset).

Female: unknown

HOLOTYPE: Costa Rica: ♂, Heredia Prov., La Selva Biol. Sta., 70m, 10°25'21.2"N 84°00'05.2"W, 10 Aug 2010, J. Heraty, secondary scrub/banana [UCRC_ENT 00282837: UCRC].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Latin noun: *longiseta* = "long seta," referring to the relatively long anterior seta of the mesoscutellum.

REMARKS: *Cales longiseta* is sister to the remaining Neotropical species of *Cales* in both Bayesian and ML analyses, though this relationship lacks strong bootstrap or posterior probability support (Fig. 2.3). Long anterior mesoscutellar setae are also present

in *Cales* from Australia and New Zealand (Mottern et al., 2011), suggesting this is a plesiomorphic condition.

Cales mogensenae **Mottern, n. sp.**

(Figs 2.14, 2.15)

DIAGNOSIS: *Cales mogensenae* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis*, and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. Females can be distinguished from all other females by the following combination of characters: valves of ovipositor longer than mesotibia; valves without strong dorsal curvature; second valvifer and Gt_7 infuscated, darker than pale yellow valves. Males can be distinguished from *C. monteverdensis* by the relatively short aedeagal rods of the latter species, though *C. monteverdensis* is known from a single male specimen, so it is unknown if ranges for this character may overlap. *Cales mogensenae* is considered morphologically cryptic with *C. brevisensillum*, *C. pellonotum*, *C. peruviana,* and *C. primapluvia*.

DESCRIPTION: Female: (Fig. 2.14), ***color and sculpture*** (Fig. 2.14A,E) – head and body pale orange, with faint infuscation on lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.14B) – radicle 5.09× longer than wide; scape 3.30× longer than wide; pedicel 2.48× longer than wide; F3 2.24× longer than wide; clava 3.99× longer than wide, and with 4 mps (n=).

Mesosoma (Fig. 2.14E) – midlobe of mesoscutum 1.47× wider than long; setal socket of midlobe seta advanced 0.37× length of midlobe; mesoscutellum 1.92× wider than long; posterolateral edges of mesoscutellum straight rather than curved; anterior mesoscutellar seta short, 0.14× length of mesoscutellum and not advanced anteriorly from campaniform sensillum; posterior mesoscutellar seta 1.18× length of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket. ***Wings*** (Fig. 2.14C,D) – fore wing 4.27× longer than wide; hind wing 10.03× longer than wide. ***Genitalia*** (Fig. 2.14F) – ovipositor 1.14× length of mesotibia; second valvifer 0.38× length of ovipositor; second valvifer and Gt_7 infuscated, darker than pale yellow valves.

Male: (Fig. 2.15), ***color and sculpture*** (Fig. 2.15A,D) – head and body orange, darker dorsally or white with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with prominent reticulate sculpture. ***Antenna*** (Fig. 2.15B) – radicle 5.03× longer than wide (n=1); scape 3.14× longer than wide (n=1); pedicel 2.43× longer than wide (n=1); F2-3 3.01–3.74× longer than wide (n=2); clava 8.25–8.51× longer than wide (n=2), longest mps of clava 0.60× length of clava (n=1). ***Mesosoma*** (Fig. 2.15D) – midlobe of mesoscutum 1.5× wider than long (n=1); setal socket of midlobe seta advanced 0.41× length of midlobe (n=1); mesoscutellum 1.88–1.89× wider than long (n=2); anterior mesoscutellar seta short, 0.11× length of mesoscutellum (n=1), and not advanced anteriorly from campaniform sensillum (n=2); socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket (n=2).

Wings (Fig. 2.15C,E) – fore wing 4.27× longer than wide (n=1); apical seta of fore wing 0.73× width of fore wing (n=1); hind wing 10.32× longer than wide (n=1). ***Genitalia*** (Fig. 2.15F) – aedeagus 0.27× length of mesotibia (n=1); aedeagal rod 0.65× length of aedeagus (n=1).

HOLOTYPE: Costa Rica: ♀, Puntarenas Prov., Res. Priv. Karen Mogensen, 305m, 9°52'00"N 85°03'00"W, 23-24 Feb 2007, J.S. Noyes [UCRC_ENT 00313910: BMNH].

PARATYPES: 2♂♂, same data as holotype, [UCRC_ENT 00313911-12: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Named in honor of Karen Mogensen, a primary benefactor of the reserve from which the holotype and paratypes were collected.

REMARKS: This species is polymorphic for color, with one male white, and the other male and the female orange. All three specimens are identical for 28S-D2. Two of the specimens were successfully sequenced for COI, and differ by 0.92%.

Cales monteverdensis **Mottern, n. sp.**

(Fig. 2.16)

DIAGNOSIS: *Cales monteverdensis* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis*, and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. *Cales monteverdensis* males can be distinguished from other known males by the very short aedeagal rods (0.44× length of aedeagus), which do not

overlap with the range of values for any other species. The anterior mesoscutellar seta is also shifted medially, nearly in line with the socket of the posterior mesoscutellar seta. *Cales parvigladius* and *C. peruviana* also have medially shifted anterior mesoscutellar setae, though not as extreme as *C. monteverdensis*. This character should be interpreted cautiously as the position of the anterior mesoscutellar seta along a transverse line can be quite variable, even within a single specimen. *Cales monteverdensis* is considered morphologically cryptic with *C. curvigladius*, *C. primapluvia*, *C. secundapluvia*, and *C. triensapluvia*, which are known only from females.

DESCRIPTION: Male: (Fig. 2.16), ***color and sculpture*** (Fig. 2.16A,D) – head and body orange dorsally, white ventrally with infuscation on lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with weak reticulate sculpture. ***Antenna*** (Fig. 2.16B) – radicle 6.52× longer than wide; scape 3.49× longer than wide; pedicel 2.37× longer than wide; F2-3 3.4× longer than wide; clava 7.03× longer than wide, longest mps of clava 0.62× length of clava. ***Mesosoma*** (Fig. 2.16D) – midlobe of mesoscutum 1.51× wider than long; socket of midlobe seta advanced 0.40× length of midlobe; mesoscutellum 1.83× wider than long; posterolateral edges of mesoscutellum straight rather than curved; anterior mesoscutellar seta short, 0.10× length of mesoscutellum, and not advanced anteriorly from campaniform sensillum by at least diameter of sensillum; anterior mesoscutellar seta shifted medially, nearly in line socket of posterior mesoscutellar seta; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by about diameter of setal socket. ***Wings*** (Fig. 2.16C,E) – fore wing 4.48× longer than wide; apical seta of fore wing 0.66× width of fore wing;

hind wing 9.74× longer than wide. ***Genitalia*** (Fig. 2.16F) – aedeagus 0.33× length of mesotibia; aedeagal rod very short relative to aedeagus, 0.44× length of aedeagus.

Female: unknown.

HOLOTYPE: Costa Rica: ♂, Puntarenas Prov., Est. Biol. Monteverde, 1500-1840m, 10°36'00"N 85°15'00"W, Feb 2007, J.S. Noyes [UCRC_ENT 00313881: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Named in honor of the Estación Biológica Monteverde where the holotype was collected.

REMARKS: *Cales monteverdensis* is sister to *C. secundapluvia* in the molecular analysis, though this relationship lacks bootstrap or posterior probability support (Fig. 2.3).

Cales multisensillum Mottern, n. sp.

(Fig. 2.17)

DIAGNOSIS: *Cales multisensillum* can be differentiated from all other species of *Cales* except *C. breviscutellum, C. indistincta*, *C. noyesi*, *C. panamensis*, and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by less than diameter of setal socket. Females can be distinguished from other known females by the presence of three mps on F3 and 10 mps on the clava. All other species have either one mps on F3, or lack mps on F3, and no more than six mps on the clava. *Cales multisensillum* is known only from

females, so it is considered morphologically cryptic with *C. panamensis*, which is known only from males.

DESCRIPTION: Female: (Fig. 2.17), ***color and sculpture*** (Fig. 2.17A,D) – head and body orange, with faint infuscation on lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with very faint reticulate sculpture. ***Antenna*** (Fig. 2.17B) – radicle 5.72× longer than wide; scape 3.92× longer than wide; pedicel 2.57× longer than wide; F3 2.95× longer than wide, and with 3 mps (all three mps are visible in Fig. 2.17A, though only two are clearly visible in Fig. 2.17B); clava 6.16× longer than wide, and with 10 mps. ***Mesosoma*** (Fig. 2.17D) – midlobe of mesoscutum 1.33× wider than long, and socket of midlobe seta advanced 0.28× length of midlobe; mesoscutellum 2.12× wider than long; anterior mesoscutellar seta short, 0.14× length of mesoscutellum and advanced anteriorly from campaniform sensillum by as least diameter of sensillum; posterior mesoscutellar seta 1.07× length of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket. ***Wings*** (Fig. 2.17C) – wing dimensions not measured as both wings are significantly tilted in mounting medium. ***Genitalia*** (Fig. 2.17E) – ovipositor 1.17× length of mesotibia; second valvifer 0.50× length of ovipositor; second valvifer and Gt_7 hyaline; valves pale yellow.

Male: unknown.

HOLOTYPE: French Guiana: ♀, Roura, Kaw Mtn., Amazone Nature Lodge, goldmine trail behind lodge, 307m, 4°33'35"N 52°12'25.8"W, 14 Sep 2010, C. Weirauch, [UCRC_ENT 00282841: UCRC].

DISTRIBUTION: Neotropical, French Guiana.

ETYMOLOGY: Latin noun: *multisensillum* = "many sensilla," referring to the large number of multiporous plate sensilla on the female antenna.

REMARKS: *Cales multisensillum* is currently the only known species of *Cales* from French Guiana. The single female specimen has an additional seta on the left side of the midlobe of the mesonotum (Fig. 2.17D). This is most likely an aberration and not a diagnostic character (similar aberrant setae occasionally occur in *C. noacki* and *C. rosei*).

***Cales noacki* Howard, 1907**

(Figs 2.18, 2.19)

Cales noacki Howard 1907: 82-83, by monotypy and original designation. Deposition: USNM, holotype lost.

Diaspidophilus pallidus Brèthes 1914: 15–16, by monotypy and original designation. Deposition: MACN. Synonymy by Gahan in Mercet 1929: 114.

Cales pallidus Mercet 1929: 117, new combination; synonymy with *C. noacki* by Dozier 1933: 98.

DIAGNOSIS: *Cales noacki* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C. indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis*, and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not separated anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal socket separated from posterior edge of mesoscutellum by at least diameter of setal socket. Females can be distinguished from *C. curvigladius*, *C. mogensenae*, *C. parvigladius* and *C. primapluvia* by the following

combination of characters: valves of ovipositor about as long or longer than mesotibia; valves without strong dorsal curvature; second valvifer and Gt_7 hyaline, lighter in color than pale yellow valves. Males can be differentiated from *C. monteverdensis* and *C. brevisensillum* by the following combination of characters: aedeagal rods relatively long, greater than 0.44× length of aedeagus; anterior seta of mesoscutellum short, less than 0.23× length of mesoscutellum. *Cales noacki* is considered morphologically cryptic with *C. bicolor*, *C. pellonotum*, *C. peruviana*, *C. rosei*, *C. secundapluvia*, and *C. triensapluvia*.

DESCRIPTION: The *C. noacki* holotype has been lost (M. Gates, pers. comm.). The species is redescribed here based upon the female neotype (designated herein) and additional specimens that have been identified as the same species based on the sequence of 28S-D2, COI, and 18S. **Female:** (Fig. 2.18), ***color and sculpture*** (Fig. 2.18A,D) – head and body white to orange, sometimes multicolored, with faint infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.18B) – radicle 4.05–5.73× longer than wide (n=11); scape 2.76–3.30× longer than wide (n=11); pedicel 2.28–2.9× longer than wide (n=11); F3 1.19–2.54× longer than wide (n=11); clava 4.16–4.84× longer than wide, and with 4 mps (n=10). ***Mesosoma*** (Fig. 2.18D) – midlobe of mesoscutum 1.42–1.68× wider than long (n=7); midlobe seta 0.60–0.79× length of midlobe (n=4), and setal socket advanced 0.31–0.45× length of midlobe (n=7); mesoscutellum 1.84–2.16× wider than long (n=8); anterior mesoscutellar seta short, 0.09–0.12× length of mesoscutellum and not advanced anteriorly from campaniform sensillum (n=7); posterior mesoscutellar seta 1.05–1.34× length of mesoscutellum (n=7), socket of

posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket (n=7). ***Wings*** (Fig. 2.18C,E) – fore wing 3.92–4.28× longer than wide (n=19); apical seta of fore wing 0.66–0.98× width of fore wing (n=18); hind wing 9.02–9.96× longer than wide (n=7). ***Genitalia*** (Fig. 2.18F) – ovipositor 0.97–1.23× length of mesotibia (n=4); second valvifer 0.32–0.43× length of ovipositor; second valvifer and Gt_7 hyaline; valves pale yellow.

Male: (Fig. 2.19), ***color and sculpture*** (Fig. 2.19A,D) – head and body white to orange, sometimes multicolored, with faint infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.19B) – radicle 3.57–5.22× longer than wide (n=7); scape 2.68–3.41× longer than wide (n=7); pedicel 2.05–2.78× longer than wide (n=7); F2-3 3.32–4.14× longer than wide (n=7); clava 6.94–8.51× longer than wide, longest mps of clava 0.55–0.67× length of clava (n=7). ***Mesosoma*** (Fig. 2.19D) – midlobe of mesoscutum 1.35–1.68× wider than long (n=9); midlobe seta 0.64–0.77× length of midlobe, setal socket advanced 0.30–0.45× length of midlobe (n=9); mesoscutellum 1.73–1.95× wider than long (n=9); anterior mesoscutellar seta short, 0.07–0.11× length of mesoscutellum, and not advanced anteriorly from campaniform sensillum (n=9); posterior mesoscutellar seta 0.94–1.13× length of mesoscutellum (n=4), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket (n=4). ***Wings*** (Fig. 2.19C,E) – fore wing 3.87–4.23× longer than wide (n=9); apical seta of fore wing 0.71–0.87× width of fore wing (n=8); hind wing

9.00–9.66× longer than wide (n=7). ***Genitalia*** (Fig. 2.19F) – aedeagus 0.23–0.30× length of mesotibia (n=5); aedeagal rod 0.51–0.61× length of aedeagus (n=5).

NEOTYPE: Chile: ♀, Región de Tarapacá, Oasis de Pica, 20°28'54.4"S 69°19'14.7"W, 13 May 2010, Osman Peralta Collao, *ex Aleurothrixus floccosus* on *Citrus limon* [UCRC_ENT 00282456: UCRC].

ADDITIONAL MATERIAL:

The following specimens have been determined to be conspecific with the neotype based upon 18S, 28S-D2 and COI. **Chile**: **2**♂, **1**♀, same data as neotype, [UCRC_ENT 00282454-55, UCRC_ENT 00282457: UCRC]. **Italy**: **2**♂♂, **14**♀♀, Campania, Portici (NA), [40°48'50"N 14°20'20"E], 11 Nov 2003, M. Giorgini, ex *Aleurothrixus floccosus* [UCRC_ENT 00020103-18: UCRC]. **USA**: **1**♂, California, Riverside Co., UCR, Biological Control Grove, 350m, 33°58'18"N 117°19'08"W, 20 Dec 2002-4 Jan 2003, J. Heraty & J. Munro, ex *Aleurothrixus floccosus* [UCRC_ENT 00020139: UCRC]. **4**♀♀, same locality, 7 Jan 2003, J. Munro, ex *Aleurothrixus floccosus* on lime [UCRC_ENT 00020098, UCRC_ENT 00020100-01, UCRC_ENT 00020102: UCRC]. **4**♂♂, **3**♀♀, same locality, 4 Jan 2006, J. Mottern, ex *Aleurothrixus floccosus* on orange [UCRC_ENT 00282354-55, UCRC_ENT 00282358-59, UCRC_ENT 00282361, UCRC_ENT 00282364, UCRC_ENT 00282366: UCRC]. **3**♂♂, **3**♀♀, same locality, 6 Jan 2006, J. Mottern, ex *Aleurothrixus floccosus* on lime [UCRC_ENT 00020092-95, UCRC_ENT 237277, UCRC ENT 282828: UCRC]. **1**♀, California, Riverside Co., Temecula, Norco Rd., 21 Jun 1997, M. Hoddle, ex *Tetraleurodes perseae* [UCRC_ENT 00235952: UCRC]. The following specimens have been determined to be conspecific with the

neotype based on analyses of wing shape (Mottern and Heraty, in prep., a) and not on molecular analyses. These specimens are from likely *C. noacki* source populations imported into the United States. All 38 specimens are mounted on a single slide. **Chile**: **20**♂♂, **18**♀♀, Region V, de Quillota Prov., La Cruz, [32°49'34"S 71°13'38"W], 16 Apr 1970, Sergio Rojas, ex *Aleurothrixus floccosus* on citrus, [UCRC_ENT 00320495 (38): UCRC]. The following specimens have been determined to be either conspecific with the neotype or very closely related based on morphometrics of wing shape. Each museum number represents a single slide, and numbers in parentheses indicate the number of specimens on the slide if greater than one. **Brazil**: **33**♂♂, **31**♀♀, São Paulo, [23°32'56"S 46°38'19"W],10-13 May 1976, M. Rose, ex *Aleurothrixus floccosus* on citrus, [UCRC_ENT 00320470 (5), UCRC_ENT 00320471 (2), UCRC_ENT 00320472 (2), UCRC_ENT 00320473 (2), UCRC_ENT 00320499 (16), UCRC_ENT 00320559 (2), UCRC_ENT 00320560 (14), UCRC_ENT 00320561 (14), UCRC_ENT 00320562 (5), UCRC_ENT 00320563 (2): UCRC]. **Argentina**: **2**♀♀, Tucumán Prov., San Miguel de Tucumán, [26°49'59"S, 65°13'00"W], May 1976, M. Rose, ex *Aleurothrixus floccosus* on citrus, [UCRC_ENT 00320493 (2)]. **8**♂♂, **10**♀, same locality, 2 May 1976, M. Rose, ex *Aleurothrixus floccosus* on ant-tended citrus, [UCRC_ENT 00320544 (3), UCRC_ENT 00320546 (6), UCRC_ENT 00320553 (2), UCRC_ENT 00320545 (7): UCRC]. **5**♂♂, **12**♀♀, same locality, 3 May 1976, M. Rose, ex *Aleurothrixus floccosus* on citrus, [UCRC_ENT 00320554 (8), UCRC_ENT 00320555, UCRC_ENT 00320556 (6), UCRC_ENT 00320557, UCRC_ENT 00320515: UCRC]. **13**♂, **22**♀, same locality, 4 May 1976, M. Rose, ex *Aleurothrixus floccosus* on citrus, [UCRC_ENT 00320490 (2),

UCRC_ENT 00320491, UCRC_ENT 00320492 (2), UCRC_ENT 00320510 (2), UCRC_ENT 00320512 (4), UCRC_ENT 00320513 (2), UCRC_ENT 00320514 (9), UCRC_ENT 00320516 (2), UCRC_ENT 00320522 (2), UCRC_ENT 00320536 (9): UCRC]. **1**♂, **4**♀, same locality 7 May 1976, M. Rose, ex *Aleurothrixus floccosus* on sour orange, [UCRC_ENT 00320475 (2), UCRC_ENT 00320476 (2), UCRC_ENT 00320511: UCRC].

HOST: *Aleurothrixus floccosus* Maskell and *Tetraleurodes perseae* Nakahara.

DISTRIBUTION: Specimens molecularly determined to be conspecific with the neotype are known from Chile, Italy, and U.S.A. (California).

REMARKS: *Cales noacki* is an important biological control agent of the woolly whitefly, *A. floccosus* in California and the Mediterranean region (Onillon & Onillon, 1972; Onillon 1974; DeBach and Rose, 1976; Rose and Woolley, 1984; Miklasiewicz and Walker, 1990). Its native and biological control ranges are unclear because multiple species (at least *C. noacki* and *C. rosei*) were imported into California and Europe during biological control efforts against the woolly whitefly in the 1960s and 1970s. These importations were all identified as "*C. noacki*" at the time, and collection sources included localities in Brazil, Argentina, and Chile. Both *C. noacki* and *C. rosei* are known to occur in the field in California, though only *C. noacki* has been confirmed to exist in Europe. *Cales noacki* and *C. rosei* are currently the only two species known to be associated with citrus.

Cales noacki specimens differed from each other by 0–0.17% for 28S-D2 and 0–4.1% for COI, and by a single base change in 18S (data not shown). *Cales noacki* is sister

to *Cales rosei* in all analyses, though this relationship lacks bootstrap or posterior probability support (Fig. 2.3). Despite their sister group relationship and cryptic morphology, there is significant molecular divergence between these two species (3.3–3.7% for 28S-D2 and 8.2–10.5% for COI).

Cales noyesi **Mottern, n. sp.**

(Figs 2.20, 2.21)

DIAGNOSIS: *Cales noyesi* can be differentiated from all other species of *Cales* except *C. breviscutellum, C. indistincta*, *C. multisensillum*, *C. panamensis*, and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by less than diameter of setal socket. Females can be differentiated from *C. breviscutellum*, *C. indistincta*, and *C. multisensillum* females by the following combination of characters: second valvifer and Gt_7 infuscated, darker than pale yellow valves; F3 with one mps; clava with five mps. *Cales noyesi* is considered cryptic with *C. panamensis* and *C. stenoptera*.

DESCRIPTION: Female: (Fig. 2.20), ***color and sculpture*** (Fig. 2.20A,D) – head and body pale yellow to orange, darker dorsally, with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.20B) – radicle 4.41–4.85× longer than wide (n=5); scape 2.60–3.22× longer than wide (n=6); pedicel 2.30–2.87× longer than wide (n=4); F3 2.46–3.29× longer than wide, and with 1 mps (n=5); clava 4.02–5.57× longer than wide, and with 5 mps (n=5). ***Mesosoma*** (Fig. 2.20D) – midlobe

of mesoscutum 1.27–1.66× wider than long (n=8); midlobe seta 0.59–0.68× length of midlobe (n=3), and setal socket advanced 0.29–0.37× length of midlobe (n=7); mesoscutellum 1.91–2.13× wider than long (n=8); anterior mesoscutellar seta short, 0.04–0.08× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by at least diameter of sensillum (n=8); posterior mesoscutellar seta 1.06–1.13× length of mesoscutellum (n=3), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket (n=8). ***Wings*** (Fig. 2.20C,E) – fore wing 4.29–4.39× longer than wide (n=7); apical seta of fore wing 0.75–0.91× width of fore wing (n=7); hind wing 10.21–10.69× longer than wide (n=6). ***Genitalia*** (Fig. 2.20F) – ovipositor long, 1.27–1.44× length of mesotibia (n=8); second valvifer 0.38–0.64× length of ovipositor; second valvifer and Gt_7 infuscated, darker in color than pale yellow valves.

Male: (Fig. 2.21), ***color and sculpture*** (Fig. 2.21A,D) – head and body white to orange, sometimes multicolored, with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.21B) – radicle 5.29–6.47× longer than wide (n=7); scape 2.52–3.76× longer than wide (n=7); pedicel 1.92–2.27× longer than wide (n=6); F2-3 3.34–4.67× longer than wide (n=6); clava 7.29–10.20× longer than wide, longest mps of clava 0.70–0.86× length of clava (n=7). ***Mesosoma*** (Fig. 2.21D) – midlobe of mesoscutum 1.37–1.63× wider than long (n=8); midlobe seta 0.53× length of midlobe (n=2), setal socket advanced 0.24–0.40× length of midlobe (n=8); mesoscutellum 1.90–2.17× wider than long (n=8); anterior mesoscutellar seta short, 0.07–0.11× length of

mesoscutellum, and advanced anteriorly from campaniform sensillum by at least diameter of sensillum (n=8); posterior mesoscutellar seta 1.04–1.29× length of mesoscutellum (n=2), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket (n=8). ***Wings*** (Fig. 2.21E) – fore wing 4.28–4.55× longer than wide (n=6); apical seta of fore wing 0.90–1.15× width of fore wing (n=6); hind wing 9.74–11.47× longer than wide (n=6). ***Genitalia*** (Fig. 2.21C) – aedeagus 0.27–0.34× length of mesotibia (n=6); aedeagal rod 0.61–0.72× length of aedeagus (n=6).

HOLOTYPE: Costa Rica: ♀, Puntarenas Prov., Res. Abs., Cabo Blanco, 30m, 9°35'00"N 85°06'00"W, 16-17 Feb 2009, J.S. Noyes [UCRC_ENT 00313899: BMNH].

PARATYPES: Costa Rica: **7**♂♂, **6**♀♀, same data as holotype, [UCRC_ENT 00313863-68, UCRC_ENT 00313870, UCRC_ENT 00313893-98: BMNH]. **1**♂, **1**♀, Puntarenas Prov., Los Charcos de Osa, 50m, 8°40'00"N 83°30'00"W, 18-19 Feb 2008, J.S. Noyes [UCRC_ENT 00313851-52: BMNH]. **1**♂, Heredia Prov., Braulio Carrillo N.P., 429m, 10°09'44.6"N 83°56'19.4"W, 12 Aug 2010, J. Mottern [UCRC_ENT 00282835: UCRC].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Named in honor of Dr. John Noyes, who collected most of the type series.

REMARKS: *Cales noyesi* is sister to *C. stenoptera* in our molecular analysis, and is morphologically cryptic with this species. Within *C. noyesi,* specimens diverged by 0–0.5% for 28S-D2 and 0–10.8% for COI. However, including only the specimens collected

at the type locality, all are identical for 28S-D2 and diverge by 0–2.3% for COI. The three specimens not collected at the type locality (Fig. 2.3, D2960, D3329, and a male specimen that is molecularly identical to D3329) lacked sufficient divergence in 28S-D2 to be considered separate species, although they are from molecularly and geographically distinct populations.

Cales panamensis **Mottern, n. sp.**

(Fig. 2.22)

DIAGNOSIS: *Cales panamensis* is considered morphologically cryptic with *C. breviscutellum, C. indistincta*, *C. multisensillum*, *C. noyesi*, and *C. stenoptera*. It can be differentiated from all other species of *Cales* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by less than diameter of setal socket.

DESCRIPTION: Male: (Fig. 2.22), ***color and sculpture*** (Fig. 2.22A,D) – head and body white, with infuscation on anterior third of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with very faint reticulate sculpture. ***Antenna*** (Fig. 2.22B) –pedicel 2.31× longer than wide; F2-3 3.35× longer than wide; clava 9.65× longer than wide, longest mps of clava 0.73× length of clava. ***Mesosoma*** (Fig. 2.22D) – midlobe of mesoscutum 1.43× wider than long; midlobe seta 0.59× length of midlobe, setal socket advanced 0.28× length of midlobe; mesoscutellum 2.19× wider than long; anterior mesoscutellar seta short, 0.11× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by at least

diameter of a sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket. ***Wings*** (Fig. 2.22C) – fore wing 4.57× longer than wide; apical seta of fore wing long, 1.01× width of fore wing. ***Genitalia*** (Fig. 2.22E) – aedeagus 0.28× length of mesotibia; aedeagal rod 0.77× length of aedeagus.

Female: unknown

HOLOTYPE: Panama: ♂, Chiriquí, Qbda. Arena, 7 km NNE Lago Fortuna Dam, 1085m, 8°46'38"N 82°12'32"W, 6 Jan 2001, M. Yoder & J.B. Woolley, [UCRC_ENT 00282842: UCRC].

DISTRIBUTION: Neotropical, Panama.

ETYMOLOGY: Named for the Republic of Panama where the holotype was collected.

***Cales parvigladius* Mottern, n. sp.**

(Figs 2.23, 2.24)

DIAGNOSIS: *Cales parvigladius* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis*, and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. Females can be distinguished from all other known females by the following combination of characters: valves of ovipositor very short, 0.81× length of mesotibia, and without strong dorsal curvature; second valvifer and Gt_7 hyaline; valves pale yellow. Males can be distinguished from *C. brevisensillum* by the relatively short

anterior seta of the mesoscutellum (0.08× length of mesoscutellum in *C. parvigladius* and 0.23× in *C. brevisensillum*). *Cales parvigladius* is considered morphologically indistinguishable from *C. pellonotum* and *C. peruviana*.

DESCRIPTION: Female: (Fig. 2.23), ***color and sculpture*** (Fig. 2.23A,D) – head and body orange and white, with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.23B) – radicle 4.21× longer than wide; scape 3.05× longer than wide; pedicel 2.34× longer than wide; F3 2.15× longer than wide, and with 1 mps; clava 5.28× longer than wide, and with 4 mps. ***Mesosoma*** (Fig. 2.23D) – midlobe of mesoscutum 1.81× wider than long; socket of midlobe seta advanced 0.44× length of midlobe; mesoscutellum 1.88× wider than long; anterior mesoscutellar seta short, 0.08× length of mesoscutellum and advanced anteriorly from campaniform sensillum by less than diameter of sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket. ***Wings*** (Fig. 2.23C,E) – fore wing 4.09× longer than wide; apical seta of fore wing 0.07× width of fore wing; hind wing 10.5× longer than wide. ***Genitalia*** (Fig. 2.23F) – ovipositor 0.81× length of mesotibia; second valvifer 0.49× length of ovipositor; second valvifer and Gt_7 hyaline, and valves pale yellow.

Male: (Fig. 2.24), ***color and sculpture*** (Fig. 2.24A,D) – head and mesosoma orange dorsally and white ventrally; legs and most of metasoma white; with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.24B) –

radicle 5.24× longer than wide; scape 2.74× longer than wide; pedicel 2.04× longer than wide; F2-3 3.46× longer than wide; clava 8.02× longer than wide. ***Mesosoma*** (Fig. 2.24D) – midlobe of mesoscutum 1.55× wider than long; socket of midlobe seta advanced 0.47× length of midlobe; mesoscutellum 1.84× wider than long; posterolateral edges of mesoscutellum straight rather than curved; anterior mesoscutellar seta short, 0.08× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by less than diameter of sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket. ***Wings*** (Fig. 2.24C,E) – fore wing 4.0× longer than wide; apical seta of fore wing 0.93× width of fore wing; hind wing 9.94× longer than wide. ***Genitalia*** (Fig. 2.24F) – aedeagus 0.24× length of mesotibia; aedeagal rod 0.62× length of aedeagus.

HOLOTYPE: Costa Rica: ♀, Limón Prov., Reserva Biológica Hitoy-Cerere, 100m, 9°40'00"N 83°02'00"W, 22-23 Feb 2010, J.S. Noyes [UCRC_ENT 00313885: BMNH].

PARATYPE: Costa Rica: **1**♂, Limón Prov., Reserva Biológica Hitoy-Cerere, 100m, 9°40'00"N 83°02'00"W, 22-23 Feb 2010, J.S. Noyes [UCRC_ENT 00313890: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Latin noun: *parvigladius* = "small sword," referring to the relatively short ovipositor of this species

REMARKS: The two *C. parvigladius* specimens differ from each other by 0.17% (a single base change) in 28S-D2 and 0.87% in COI.

***Cales pellonotum* Mottern, n. sp.**

(Fig. 2.25)

DIAGNOSIS: *Cales pellonotum* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C. indistincta*, *C. longiseta*, *C. monteverdensis*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket; aedeagal rods greater than ½ the length of the aedeagus. *Cales pellonotum* is considered morphologically cryptic with *C. bicolor*, *C. mogensenae*, *C. noacki*, *C. parvigladius*, *C. peruviana*, *C. primapluvia*, *C. rosei, C. secundapluvia* and *C. triensapluvia*.

DESCRIPTION: Male: (Fig. 2.25), ***color and sculpture*** (Fig. 2.25A,C) – head and body orange, darker dorsally with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.25D) – radicle 4.98× longer than wide; scape 3.24× longer than wide; pedicel 1.90× longer than wide; F2-3 3.53× longer than wide; clava 9.79× longer than wide, longest mps of clava 0.55× length of clava, mps with smokey infuscation. ***Mesosoma*** (Fig. 2.25C) – midlobe of mesoscutum 1.61× wider than long; socket of midlobe seta advanced 0.4× length of midlobe; mesoscutellum 1.87× wider than long; anterior mesoscutellar seta short, 0.08× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by less than diameter of sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by about

diameter of setal socket. ***Wings*** (Fig. 2.25B) – fore wing 4.28× longer than wide; apical seta of fore wing 0.77× width of fore wing; hind wing 9.04× longer than wide. ***Genitalia*** (Fig. 2.25E) – aedeagus 0.25× length of mesotibia; aedeagal rods very thin, tapering anteriorly, and 0.69× length of aedeagus.

Female: unknown

HOLOTYPE: Costa Rica: ♂, Limón Prov., Reserva Biológica Hitoy-Cerere, 100m, 9°40'00"N 83°02'00"W, 22-23 Feb 2010, J.S. Noyes [UCRC_ENT 00313889: BMNH].

DISTRIBUTION: Neotropical, Costa Rica.

ETYMOLOGY: Greek noun: *pellonotum* = "dusky back," referring to the extensive infuscation of the mesonotum.

REMARKS: *Cales pellonotum* is one of the least morphologically diagnosable *Cales* species. It has the most common arrangement of setae and sensilla on the mesoscutellum, and is known only from males. The single specimen has very thin aedeagal rods, a character that may prove useful once surveyed across a larger samples size.

Cales peruviana **Mottern, n. sp.**

(Fig. 2.26)

DIAGNOSIS: *Cales peruviana* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C. indistincta*, *C. longiseta*, *C. monteverdensis*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket; aedeagal rods greater than 0.5× the

length of the aedeagus. *Cales peruviana* is considered morphologically cryptic with *C. bicolor*, *C. mogensenae*, *C. noacki*, *C. parvigladius*, *C. pellonotum*, *C. primapluvia*, *C. rosei, C. secundapluvia* and *C. triensapluvia*.

DESCRIPTION: Male: (Fig. 2.26), ***color and sculpture*** (Fig. 2.26A,C) – head and body orange, darker dorsally with infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with very faint reticulate sculpture. ***Antenna*** (Fig. 2.26B) – radicle 5.52× longer than wide; scape 2.73× longer than wide; pedicel 2.30× longer than wide; F2-3 3.24× longer than wide; clava 8.65× longer than wide, longest mps of clava 0.66× length of clava. ***Mesosoma*** (Fig. 2.26C) – midlobe of mesoscutum badly distorted during slide mounting; mesoscutellum 1.87× wider than long; posterolateral edges of mesoscutellum straight rather than curved; anterior mesoscutellar seta short, 0.09× length of mesoscutellum, and not advanced anteriorly from campaniform sensillum; socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket. ***Wings*** (Fig. 2.26D,E) – fore wing 4.37× longer than wide; apical seta of fore wing 0.86× width of fore wing. ***Genitalia*** – aedeagus 0.30× length of mesotibia; aedeagal rod 0.65× length of aedeagus.

Female: unknown.

HOLOTYPE: Peru: ♂, Madre de Dios, Los Amigos Bio. St., trail 23 nr 10, 247m, 12°33'30.2"S 70°05'37.7"W, 20-24 Dec 2010, J. Heraty [UCRC_ENT 00302407: UCRC].

DISTRIBUTION: Neotropical, Peru.

ETYMOLOGY: This species is named for the Republic of Peru where the holotype was collected.

REMARKS: The midlobe of the mesoscutum on the single male specimen has been stretched or otherwise distorted during slide mounting, so neither the photograph nor the drawing (Fig. 2.26D) are likely good representations of the true shape. Because of the distortion, no measurements are reported from the midlobe of the mesoscutum. Like *Cales pellonotum*, *C. peruviana* is known only from males, and has the most common mesoscutellar condition.

Cales primapluvia **Mottern, n. sp.**

(Fig. 2.27)

DIAGNOSIS: *Cales primapluvia* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C. indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. Females can be distinguished from *C. bicolor*, *C. curvigladius*, *C. mogensenae*, *C. noacki*, *C. parvigladius, C. rosei*, *C. secundapluvia* and *C. triensapluvia* by the following combination of characters: valves of ovipositor about as long or longer than mesotibia; valves without strong dorsal curvature; second valvifer infuscated, darker in color than pale yellow valves; Gt_7 hyaline, lighter in color than pale yellow valves. *Cales primapluvia* is considered morphologically cryptic with *C. brevisensillum*, *C. monteverdensis*, *C. pellonotum* and *C. peruviana*.

DESCRIPTION: Female: (Fig. 2.27), ***color and sculpture*** (Fig. 2.27A,D) – head and body white, with narrow band of infuscation on posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.27B) – radicle 4.65× longer than wide; pedicel 2.57× longer than wide; F3 2.21× longer than wide; clava 5.2× longer than wide, and with 4 mps. ***Mesosoma*** (Fig. 2.27E) – midlobe of mesoscutum 1.43× wider than long; midlobe seta 0.65× length of midlobe, and setal socket advanced 0.26× length of midlobe; mesoscutellum 2.1× wider than long; anterior mesoscutellar seta short, 0.10× length of mesoscutellum and not advanced anteriorly from campaniform sensillum by greater than or equal to diameter of sensillum; posterior mesoscutellar seta 1.2× length of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by about diameter of setal socket. ***Wings*** (Fig. 2.27C,D) – fore wing 4.01× longer than wide; apical seta of fore wing 0.58× width of fore wing; hind wing 9.58× longer than wide. ***Genitalia*** (Fig. 2.27F) – ovipositor 1.26× length of mesotibia; second valvifer 0.44× length of ovipositor; second valvifer infuscated; Gt_7 either hyaline or with very light infuscation, lighter than second valvifer; valves pale yellow, about same color as second valvifer but darker than out plates of ovipositor.

HOLOTYPE: Ecuador: ♀, Orellana, Reserva Etnica Waorani, 1 km. S. Onkone Gare Camp, 216.3m, 0°39'25.7"S 76°27'10.8"W, 2 Jul 1995, T.L. Erwin et al., terre firme forest [UCRC_ENT 00114359: USNM].

DISTRIBUTION: Neotropical, Ecuador.

ETYMOLOGY: Latin noun: *primapluvia* = "first rain." This specimen was collected via insecticidal fogging of the Ecuadorian forest canopy. During this collection event, multiple morphologically indistinguishable species of *Cales* "rained" down upon the collection funnels. This is the first of those species to be described.

REMARKS: Though morphologically very similar to *C. secundapluvia* and *C. triensapluvia*, *C. primapluvia* is very molecularly divergent from all other *Cales*, differing by a 2.7–8.4% in 28S-D2 from other Neotropical species.

***Cales rosei* Mottern, n. sp.**

(Figs 2.28, 2.29)

DIAGNOSIS: *Cales rosei* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C. indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not separated anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal socket separated from posterior edge of mesoscutellum by at least diameter of setal socket. Females can be distinguished from *C. curvigladius*, *C. mogensenae*, *C. parvigladius* and *C. primapluvia* by the following combination of characters: valves of ovipositor about as long or longer than mesotibia; valves without strong dorsal curvature; second valvifer and Gt_7 hyaline, lighter in color than pale yellow valves. Males can be differentiated from *C. monteverdensis* and *C. brevisensillum* by the following combination of characters: aedeagal rods relatively long, greater than 0.44× length of aedeagus; anterior seta of mesoscutellum short, less than 0.23× length of mesoscutellum. *Cales noacki* is considered morphologically cryptic with

C. bicolor, *C. pellonotum*, *C. peruviana*, *C. noacki*, *C. secundapluvia* and *C. triensapluvia*.

DESCRIPTION: Female: (Fig. 2.28), ***color and sculpture*** (Fig. 2.28A,D) – head and body white to orange, sometimes multicolored, with faint infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.28B) – radicle 5.87–6.69× longer than wide (n=3); scape 2.99–3.36× longer than wide (n=3); pedicel 2.05–2.61× longer than wide (n=3); F3 1.97–2.47× longer than wide (n=3); clava 3.97–5.45× longer than wide, and with 4 mps (n=3). ***Mesosoma*** (Fig. 2.28D) – midlobe of mesoscutum 1.48–1.71× wider than long (n=3); midlobe seta 0.64–0.75× length of midlobe, and setal socket advanced 0.38–0.52× length of midlobe (n=3); mesoscutellum 1.83–1.88× wider than long (n=3); anterior mesoscutellar seta short, 0.12–0.13× length of mesoscutellum and not advanced anteriorly from campaniform sensillum (n=3); posterior mesoscutellar seta 1.04–1.09× length of mesoscutellum (n=3), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket (n=3). ***Wings*** (Fig. 2.28C,E) – fore wing 3.98–4.15× longer than wide (n=5); apical seta of fore wing 0.52–0.88× width of fore wing (n=6); hind wing 8.79–9.59× longer than wide (n=5). ***Genitalia*** (Fig. 2.28F) – ovipositor 1.21–1.34× length of mesotibia (n=4); second valvifer 0.38–0.52× length of ovipositor (n=2); second valvifer and Gt_7 hyaline; valves pale yellow.

Male: (Fig. 2.29), ***color and sculpture*** (Fig. 2.29A,D) – head and body white to orange, sometimes multicolored, with faint infuscation on anterior half of midlobe of

mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.29B) – radicle 3.97–5.51× longer than wide (n=4); scape 2.66–3.19× longer than wide (n=4); pedicel 2.01–2.42× longer than wide (n=5); F2-3 2.48–3.76× longer than wide (n=5); clava 5.94–8.92× longer than wide, longest mps of clava 0.63–0.75× length of clava (n=5). ***Mesosoma*** (Fig. 2.29D) – midlobe of mesoscutum 1.39–1.66× wider than long (n=4); midlobe seta 0.56–0.73× length of midlobe (n=2), setal socket advanced 0.37–0.44× length of midlobe (n=4); mesoscutellum 1.87–1.88× wider than long (n=3); anterior mesoscutellar seta short, 0.11× length of mesoscutellum, and not advanced anteriorly from campaniform sensillum (n=3); posterior mesoscutellar seta 1.05–1.13× length of mesoscutellum (n=3), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket (n=3). ***Wings*** (Fig. 2.29C,E) – fore wing 3.74–4.02× longer than wide (n=6); apical seta of fore wing 0.52–0.90× width of fore wing (n=7); hind wing 8.12–9.65× longer than wide (n=5). ***Genitalia*** (Fig. 2.29F) – aedeagus 0.25–0.34× length of mesotibia (n=3); aedeagal rod 0.52–0.60× length of aedeagus (n=3).

HOLOTYPE: USA: ♀, California, Riverside Co., UCR; Biological Control Orchard, 350m, 33°58'18"N 117°19'08"W, 4 Jan 2006, J. Mottern, ex *Aleurothrixus floccosus* on orange [UCRC_ENT 00282363: UCRC].

PARATYPES: USA: **4**♂♂, California, Riverside Co., UCR; Biological Control Grove, 350m, 33°58'18"N 117°19'08"W, 20 Dec 2002-4 Jan 2003, J. Heraty & J. Munro, ex *Aleurothrixus floccosus*, [UCRC_ENT 00020135-38: UCRC]. **2**♀♀, same locality, 7 Jan 2003, J. Munro, ex *Aleurothrixus floccosus* on lime [UCRC_ENT 00020097,

UCRC_ENT 00020099: UCRC]. **3**♂♂, **3**♀♀, same locality, 4 Jan 2006, J. Mottern, ex *Aleurothrixus floccosus* on orange [UCRC_ENT 00282356-57, UCRC_ENT 00282360, UCRC_ENT 00282362, UCRC_ENT 00282365, UCRC_ENT 00282367: UCRC]. **1**♂, same locality, 6 Jan 2006, J. Mottern, ex *Aleurothrixus floccosus* on lime [UCRC_ENT 00020096: UCRC].

ADDITIONAL MATERIAL: The following specimens are determined as conspecific with *C. rosei* based on an analysis of wing shape (Mottern and Heraty, in prep., a) and not on molecular analyses. These specimens are from the likely source populations imported into the United States for biological control of woolly whitefly. Each museum number represents a single slide, and numbers in parentheses indicate the number of specimens on the slide if greater than one. **Argentina**: **10**♂, **10**♀♀, Buenos Aires Prov., Saenz-Peña, 34°35'58"S 58°31'58"W, 20 Apr 1976, M. Rose, ex *Aleurothrixus floccosus* [UCRC_ENT 00326576-95: UCRC]; **50**♂♂, **37**♀♀, Tigre, 34°25'29"S 58°34'47"W, 21 Apr 1976, M. Rose, ex *Aleurothrixus floccosus* on citrus [UCRC_ENT 00320482 (14), UCRC_ENT 00320484 (4), UCRC_ENT 00320494 (20), UCRC_ENT 00320507(20), UCRC_ENT 00320508 (2), UCRC_ENT 00320535 (2), UCRC_ENT 00320537 (13), UCRC_ENT 00320538 (2), UCRC_ENT 00320539 (4), UCRC_ENT 00320540 (20): UCRC]; **20**♂♂, **18**♀♀, José C. Paz, 34°30'54"N 58°45'58"W, 28-29 Apr 1976, M. Rose, ex *Aleurothrixus floccosus* on citrus [UCRC_ENT 00320483 (12), UCRC_ENT 00320487 (13), UCRC_ENT 00320509 (13): UCRC].

HOST: *Aleurothrixus floccosus*.

DISTRIBUTION: Nearctic, California. Introduced from Southern South America.

ETYMOLOGY: Named in honor of Mike Rose.

REMARKS: *Cales rosei* was imported into California in the 1970s as part of a biological control program against the woolly whitefly (DeBach and Rose, 1976). At the time, all Neotropical *Cales* were known as *C. noacki*, so it is not certain which of the South American collection sites yielded *C. rosei*. Based upon importation records maintained at the University of California Insectary and Quarantine Facility and an analysis of wing shape (Mottern and Heraty, in prep., a), *C. rosei* is most likely native to Argentina, and specimens from the likely source localities are included here as additional material examined.

***Cales secundapluvia* Mottern, n. sp.**

(Fig. 2.30)

DIAGNOSIS: *Cales secundapluvia* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. Females can be distinguished from *C. curvigladius*, *C. mogensenae*, *C. parvigladius* and *C. primapluvia* by the following combination of characters: valves of ovipositor about as long or longer than mesotibia; valves without strong dorsal curvature; second valvifer and Gt_7 hyaline, lighter in color than pale yellow valves. *Cales primapluvia* is considered morphologically cryptic with *C. bicolor*, *C.*

brevisensillum, *C. monteverdensis*, *C. noacki*, *C. pellonotum*, *C. peruviana, C. rosei, C. secundapluvia* and *C. triensapluvia*.

DESCRIPTION: Female: (Fig. 2.30), ***color and sculpture*** (Fig. 2.30A,E) – head and body white, with faint infuscation on lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.30B) – radicle 4.4× longer than wide; scape 2.77× longer than wide; pedicel 2.41× longer than wide; F3 1.98× longer than wide; clava 5.29× longer than wide, and with 4 mps. ***Mesosoma*** (Fig. 2.30E) – midlobe of mesoscutum 1.32× wider than long; socket of midlobe seta advanced 0.35× length of midlobe; mesoscutellum 2.09× wider than long; anterior mesoscutellar seta short, 0.10× length of mesoscutellum and not advanced anteriorly from campaniform sensillum; posterior mesoscutellar seta 1.16× length of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket. ***Wings*** (Fig. 2.30C,D) – fore wing 3.89× longer than wide; apical seta of fore wing 0.49× width of fore wing; hind wing 9.06× longer than wide. ***Genitalia*** (Fig. 2.30F) – ovipositor 1.15× length of mesotibia; second valvifer and Gt_7 hyaline, and valves pale yellow.

Male: unknown.

HOLOTYPE: Ecuador: ♀, Orellana, Tiputini Biodiversity Station; Trans. 7 Sta. 1, 220-250m, 0°37'55"S 76°08'39"W, 4 Jul 1998, T.L. Erwin, et al., terre firme forest [UCRC_ENT 00116631: USNM].

DISTRIBUTION: Neotropical, Ecuador.

ETYMOLOGY: Latin noun: *secundapluvia* = "second rain." This is the second species described from a cryptic species complex that "rained" down from the Ecuadorian forest canopy (see etymology for *C. primapluvia*).

***Cales stenoptera* Mottern, n. sp.**

(Figs 2.31, 2.32)

DIAGNOSIS: *Cales stenoptera* can be differentiated from all other species of *Cales* except *C. breviscutellum, C. indistincta*, *C. multisensillum*, *C. noyesi* and *C. panamensis* by the following combination of characters: anterior seta of mesoscutellum advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by less than diameter of setal socket. Females can be differentiated from *C. breviscutellum*, *C. indistincta* and *C. multisensillum* by the following combination of characters: second valvifer and Gt_7 infuscated, darker than pale yellow valves; F3 with one mps; clava with five mps. *Cales stenoptera* is considered cryptic with *C. noyesi* and *C. panamensis*.

DESCRIPTION: Female: (Fig. 2.31), ***color and sculpture*** (Fig. 2.31A,D) – head and mesosoma pale yellow, metasoma mostly white; faint infuscation on anterior third of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with faint reticulate sculpture. ***Antenna*** (Fig. 2.31B) – radicle 4.99–6.12× longer than wide (n=3); scape 2.90–3.04× longer than wide (n=3); pedicel 2.35–2.64× longer than wide (n=3); F3 2.69–2.93× longer than wide, and with 1 mps (n=3); clava 4.43–5.23× longer than wide, and with 4 mps (n=3). ***Mesosoma*** (Fig. 2.31D) – midlobe of mesoscutum 1.47–1.61× wider than long (n=3); midlobe seta 0.54× length of midlobe

(n=1), and setal socket advanced 0.32–0.36× length of midlobe (n=3); mesoscutellum 1.96–2.18× wider than long (n=3); anterior mesoscutellar seta short, 0.07–0.13× length of mesoscutellum and advanced anteriorly from campaniform sensillum by greater than diameter of sensillum (n=3); posterior mesoscutellar seta 0.98–1.21× length of mesoscutellum (n=2), socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket, but separated from campaniform sensillum by about 5× diameter of sensillum (n=3). ***Wings*** (Fig. 2.31C,E) – fore wing narrow, 4.46–4.60× longer than wide (n=2); apical seta of fore wing long, 0.97–1.01× width of fore wing (n=2); hind narrow, 11.01–12.04× longer than wide (n=2). ***Genitalia*** (Fig. 2.31F) – ovipositor 1.06–1.12× length of mesotibia (n=3); second valvifer 0.37–0.40× length of ovipositor (n=3); second valvifer and Gt_7 infuscated, darker than pale yellow valves.

Male: (Fig. 2.32), ***color and sculpture*** (Fig. 2.32A,D) – head and body white; infuscation on anterior half of midlobe of mesoscutum and lateral/posterior margin of mesoscutellum; both midlobe and mesoscutellum with faint reticulate sculpture. ***Antenna*** (Fig. 2.32B) – radicle 6.14× longer than wide; scape 3.11× longer than wide; pedicel 2.3× longer than wide; F2-3 3.32× longer than wide; clava 10.36× longer than wide, longest mps of clava 0.65× length of clava. ***Mesosoma*** (Fig. 2.32D) – midlobe of mesoscutum 1.56× wider than long; midlobe seta 0.53× length of midlobe, setal socket advanced 0.29× length of midlobe; mesoscutellum 2.02× wider than long; anterior mesoscutellar seta short, 0.09× length of mesoscutellum, and advanced anteriorly from campaniform sensillum by greater than diameter of sensillum; posterior mesoscutellar seta 0.95× length

of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by less than diameter of setal socket, but separated from campaniform sensillum by about 5× diameter of sensillum. ***Wings*** (Fig. 2.32C) – fore wing appears narrower than in most species, but too tilted within mounting medium to measure accurately; hind wing 10.23× longer than wide. ***Genitalia*** (Fig. 2.32E) – aedeagus 0.23× length of mesotibia; aedeagal rod 0.71× length of aedeagus.

HOLOTYPE: Ecuador: ♀, Orellana, Rio Piraña Bridge, Reserva Etnica Waorani, Onkone Gare Camp, 216.3m, 0°39'25.7"S 76°27'10.8"W, 7 Jul 2006, T.L. Erwin, M.C. Pimienta et al, terre firme forest [UCRC_ENT 00248658: USNM].

PARATYPES: Ecuador: **1**♂, same data as holotype [UCRC_ENT 00245028: USNM]. **Peru**: **1**♀, Madre de Dios, Los Amigos Biol. Station, trail 14, 231m, 12°34'36.3"S 70°05'06.7"W, 20 Dec 2010, J. Heraty [UCRC_ENT 00320304: UCRC]. **1**♀, Manu Prov., Villa Carmen, Pillcopata, 583m, 12°53'42"S 71°24'30"W, 28 Nov 2011, J. Heraty, secondary forest [UCRC_ENT 00320306: UCRC].

DISTRIBUTION: Neotropical, Ecuador and Peru.

ETYMOLOGY: Greek noun: stenoptera = "narrow wing," referring to the relatively narrow fore wing of this species

REMARKS: *Cales stenoptera* is morphologically and molecularly very close to *C. noyesi*, differing from the latter species by 0.7–1.0% for 28S-D2 and 6.9–11.0% for COI. Intraspecific molecular divergence ranged from 0.0–0.3% for 28S-D2 and 1.5–6.9% for COI. This species is polymorphic for color, with the two Ecuadorian specimens pale yellow to white and the Peruvian specimens orange.

***Cales triensapluvia* Mottern, n. sp.**

(Fig. 2.33)

DIAGNOSIS: *Cales triensapluvia* can be distinguished from *C. breviclava*, *C. breviscutellum*, *C. fusca*, *C indistincta*, *C. longiseta*, *C. multisensillum*, *C. noyesi*, *C. panamensis* and *C. stenoptera* by the following combination of characters: anterior seta of mesoscutellum not advanced anteriorly from mesoscutellar campaniform sensillum by at least diameter of sensillum; posterior mesoscutellar setal sockets separated by at least diameter of setal socket. Females can be distinguished from *C. curvigladius*, *C. mogensenae*, *C. parvigladiu,* and *C. primapluvia* by the following combination of characters: valves of ovipositor about as long or longer than mesotibia; valves without strong dorsal curvature; second valvifer and Gt_7 hyaline, lighter in color than pale yellow valves. *Cales triensapluvia* is considered morphologically cryptic with *C. bicolor*, *C. brevisensillum*, *C. monteverdensis*, *C. noacki*, *C. pellonotum*, *C. peruviana, C. rosei* and *C. secundapluvia*.

DESCRIPTION: Female: (Fig. 2.33), ***color and sculpture*** (Fig. 2.33A,E) – head and body white (orange inclusion is visible in head and antenna of habitus photo); both midlobe of mesoscutum and mesoscutellum with reticulate sculpture. ***Antenna*** (Fig. 2.33B) – radicle 3.91× longer than wide; scape 3.44× longer than wide; pedicel 2.54× longer than wide; F3 1.98× longer than wide; clava 6.28× longer than wide, and with 4 mps. ***Mesosoma*** (Fig. 2.33E) – midlobe of mesoscutum 1.36× wider than long; socket of midlobe seta advanced 0.33× length of midlobe; mesoscutellum 1.92× wider than long; anterior mesoscutellar seta short, 0.11× length of mesoscutellum and not advanced

anteriorly from campaniform sensillum; posterior mesoscutellar seta long, 1.34× length of mesoscutellum, socket of posterior mesoscutellar seta separated from posterior edge of mesoscutellum by at least diameter of setal socket. ***Wings*** (Fig. 2.33C,D) – fore wing 4.31× longer than wide; apical seta of fore wing 0.68× width of fore wing; hind wing 9.9× longer than wide. ***Genitalia*** (Fig. 2.33F) – ovipositor is mounted laterally, so measurements should be interpreted cautiously; ovipositor 1.25× length of mesotibia; second valvifer 0.47× length of ovipositor; second valvifer and Gt_7 hyaline; valves pale yellow.

Male: unknown.

HOLOTYPE: Ecuador: ♀, Orellana, Tiputini Biodiversity Sta. nr. Yasuni National Park, 220-250m, 0°37'55"S 76°08'39"W, 24 Oct 1998, T.L. Erwin et al., terre firme forest [UCRC_ENT 00117247: USNM].

DISTRIBUTION: Neotropical, Ecuador.

ETYMOLOGY: Latin noun: *triensapluvia* = "third rain." This is the third species described from a cryptic species complex that "rained" down from the Ecuadorian forest canopy (see etymology for *C. primapluvia*).

CONCLUSIONS

Cales are rarely collected, morphologically uniform, and character poor, a combination that makes them a difficult subject for traditional taxonomy. We have taken a combined molecular/morphological taxonomic approach in the revision with the goal of identifying those species that can be diagnosed based on morphology and providing a

robust molecular framework for distinguishing among all species including those that are morphologically cryptic.

Prior to this study, all Neotropical *Cales* (as well as populations exported to the Nearctic and Palearctic regions for biological control of woolly whitefly) were considered a single species, *C. noacki*. Molecular phylogenetic analysis of ribosomal and mitochondrial DNA has revealed a much richer fauna. Ribosomal DNA, particularly the 28S-D2 region proved to be the most useful for distinguishing among species. This gene is easy to amplify, even for specimens preserved under less than ideal conditions, and has historically proven valuable for distinguishing among closely related species of Chalcidoidea (Babcock and Heraty, 2000; Babcock et al., 2001; Manzari et al., 2002; Triapitsyn et al., 2006).

Perhaps most remarkable is the ability of morphologically cryptic species to remain cryptic despite substantial molecular divergence in both 28S and COI. *Cales noacki* and *C. rosei*, for example, differ from each other by 3.3–3.7% in 28S-D2, and 8.2-10.5% in COI. Nevetheless, both males and females are morphologically cryptic for these species. Measurements suggest some statistical differences in fore wing proportions and ovipositor lengths, but ranges for both these structures overlap for the two species. *Cales noacki* and *C. rosei* are also capable of attacking the same host in both spatial and temporal sympatry; both can be collected from the same tree at the same time in the biological control groves at the University of California, Riverside. The mechanisms allowing resource sharing for such biologically similar parasitoids are a fertile field for future study. The cryptic species phenomen has potentially important implications for

biological control. Parasitoid species thought to be generalists may prove to be cryptic complexes of specialists (e.g. Smith et al., 2007), broadening the list of potential biological control agent candidates. Very few parasitoid cryptic species complexes have been studied within an applied ecological context, so it remains unclear whether species groups attacking a single host species are likely to interfere with each other or provide additive control.

This study likely represents a starting point rather than a summation of our understanding of *Cales* diversity. Because of their cryptic morphology, molecular analyses will be crucial for future studies of *Cales*, taxonomy, life history and use in biological control.

Table 2.1. Primers used for sequencing. Modifications indicated by "*". Numbers following Ribosomal primers refer to complimentary 5' start position in *Drosophila melanogaster* (Tautz et. al 1988). Numbers following cytochrome oxidase subunit I (COI) primers refer to complimentary 5' start position in *Drosophila yakuba* (Folmer et. al 1994).

Primer	Sequence (5' – 3')	Reference
28S D2-3551 F	CGG GTT GCT TGA GAG TGC AGC	Modified from Campbell et al. 2000*
28S D2i-3686 F	GAA ACC GTT CAG GGG TAA ACC	
28S D2-4039 R	CTC CTT GGT CCG TGT TTC	
28S D3-4046 F	TTG AAA CAC GGA CCA AGG AG	Modified from Nunn et al. 1996*
28S D3-4413 R	TCG GAG GGA ACC AGC TAC TA	Modified from Nunn et al. 1996*
28S D5-4625 R	CGC CAG TTC TGC TTA CCA	Modified from Schulmeister 2003*
COI NJ-2197 F	TAT ATT TTA ATT YTW CCW GGA TTT GG	Modified from Simon et al. 1994
COI MD-2614 R	ATT GCA AAT ACT GCA CCT AT	Dowton and Austin, 1997

Table 2.2. GenBank accession numbers for sequences used in this study. In some cases, sequences exist for specimens that were destructively sampled for sequencing. These specimens will have a D-number, but will not have a UCRC_ENT museum number, and are not listed in the material examined. Holotypes/Neotypes are indicated by "*".

Specimen identifiers			GenBank accession no.		
Species	**Museum no.**	**D no.**	**28S-D2**	**28S-D3-5**	**COI**
C. bicolor	282836	2961	TBD	TBD	TBD
*C. bicolor**	313857	3348	TBD	TBD	TBD
C. bicolor	313859	3453	TBD	TBD	TBD
C. bicolor	313860	3454	TBD	TBD	TBD
C. bicolor	313861	3455	TBD	TBD	TBD
C. bicolor	313875	3347	TBD	TBD	TBD
C. bicolor	313892	3331	TBD	TBD	TBD
C. breviclava	313853	3334	TBD	TBD	TBD
C. breviclava	313887	3346	TBD	TBD	TBD
C. breviclava	313891	3446	TBD	TBD	TBD
*C. breviclava**	313905	3336	TBD	TBD	TBD
C. breviscutellum	114799	3462	TBD	TBD	TBD
C. breviscutellum	117555	3466	TBD	TBD	TBD
C. breviscutellum*	320305	3408	TBD	TBD	TBD
C. brevisensillum*	313886	3343	TBD	TBD	TBD
C. curvigladius	251715	2829	TBD	TBD	TBD
C. curvigladius*	282840	2956	TBD	TBD	TBD
C. fusca*	313869	3335	TBD	TBD	TBD
C. indistincta	282839	2965	TBD	TBD	TBD
C. indistincta*	282843	2959	TBD	TBD	TBD
C. longiseta	282837	2962	TBD	TBD	TBD
C. mogensenae*	313910	3345	TBD	TBD	TBD
C. mogensenae	313911	3337	TBD	TBD	TBD
C. mogensenae	313912	3447	TBD	TBD	TBD
C. monteverdensis*	313881	3338	TBD	TBD	TBD
C. multisensillum*	282841	2957	TBD	TBD	TBD
C. noacki	20092	2461	TBD	TBD	TBD
C. noacki	20093	2449	TBD	TBD	TBD
C. noacki	20094	2459	TBD	TBD	TBD
C. noacki	20095	2464	TBD	TBD	TBD
C. noacki	20098	1287	TBD	TBD	TBD
C. noacki	20100	1289	TBD	TBD	TBD
C. noacki	20101	1290	TBD	TBD	TBD
C. noacki	20102	1291	TBD	TBD	TBD
C. noacki	20105	1426-9	TBD	TBD	TBD
C. noacki	20106	1426-8	TBD	TBD	TBD
C. noacki	20107	1426-7	TBD	TBD	TBD
C. noacki	20110	1426-4	TBD	TBD	TBD
C. noacki	20112	1426-2	TBD	TBD	TBD
C. noacki	20113	1426-1	TBD	TBD	TBD

Table 2.2. Cont.

Specimen identifiers			GenBank accession no.		
Species	**Museum no.**	**D no.**	**28S-D2**	**28S-D3-5**	**COI**
C. noacki	20139	1443-1	TBD	TBD	TBD
C. noacki	235952	1519	TBD	TBD	TBD
C. noacki	237277	2447	TBD	TBD	TBD
C. noacki	282354	2890	TBD	TBD	TBD
C. noacki	282355	2891	TBD	TBD	TBD
C. noacki	282358	2894	TBD	TBD	TBD
C. noacki	282359	2895	TBD	TBD	TBD
C. noacki	282361	2897	TBD	TBD	TBD
C. noacki	282364	2900	TBD	TBD	TBD
C. noacki	282366	2902	TBD	TBD	TBD
C. noacki	282454	2908	TBD	TBD	TBD
C. noacki	282455	2909	TBD	TBD	TBD
*C. noacki**	282456	2910	TBD	TBD	TBD
C. noacki	282457	2911	TBD	TBD	TBD
C. noacki	282828	2445	TBD	TBD	TBD
C. noacki	–	1518	TBD	TBD	TBD
C. noacki	–	2446	TBD	TBD	TBD
C. noacki	–	2454	TBD	TBD	TBD
C. noyesi	282835	2960	TBD	TBD	TBD
C. noyesi	313851	3329	TBD	TBD	TBD
C. noyesi	313852	3344	TBD	TBD	TBD
C. noyesi	313863	3341	TBD	TBD	TBD
C. noyesi	313864	3333	TBD	TBD	TBD
C. noyesi	313865	3339	TBD	TBD	TBD
C. noyesi	313866	3456	TBD	TBD	TBD
C. noyesi	313867	3457	TBD	TBD	TBD
C. noyesi	313868	3458	TBD	TBD	TBD
C. noyesi	313870	3459	TBD	TBD	TBD
C. noyesi	313893	3342	TBD	TBD	TBD
C. noyesi	313894	3330	TBD	TBD	TBD
C. noyesi	313895	3448	TBD	TBD	TBD
C. noyesi	313896	3449	TBD	TBD	TBD
C. noyesi	313897	3450	TBD	TBD	TBD
C. noyesi	313898	3451	TBD	TBD	TBD
*C. noyesi**	313899	3452	TBD	TBD	TBD
*C. panamensis**	282842	2958	TBD	TBD	TBD
*C. parvigladius**	313885	3340	TBD	TBD	TBD
C. parvigladius	313890	3445	TBD	TBD	TBD
*C. pellonotum**	313889	3444	TBD	TBD	TBD
*C. peruviana**	302407	3377	TBD	TBD	TBD
*C. primapluvia**	114359	3465	TBD	TBD	TBD

Table 2.2. Cont.

Specimen identifiers			GenBank accession no.		
Species	**Museum no.**	**D no.**	**28S-D2**	**28S-D3-5**	**COI**
C. rosei	20096	2463	TBD	TBD	TBD
C. rosei	20097	1286	TBD	TBD	TBD
C. rosei	20099	1288	TBD	TBD	TBD
C. rosei	20135	1443-3	TBD	TBD	TBD
C. rosei	20136	1443-4	TBD	TBD	TBD
C. rosei	20137	1443-5	TBD	TBD	TBD
C. rosei	20138	1443-6	TBD	TBD	TBD
C. rosei	282356	2892	TBD	TBD	TBD
C. rosei	282357	2893	TBD	TBD	TBD
C. rosei	282360	2896	TBD	TBD	TBD
C. rosei	282362	2898	TBD	TBD	TBD
*C. rosei**	282363	2899	TBD	TBD	TBD
C. rosei	282365	2901	TBD	TBD	TBD
C. rosei	282367	2903	TBD	TBD	TBD
*C. secundapluvia**	116631	3470	TBD	TBD	TBD
C. stenoptera	245028	2954	TBD	TBD	TBD
*C. stenoptera**	248658	2828	TBD	TBD	TBD
C. stenoptera	320304	3407	TBD	TBD	TBD
C. stenoptera	320306	3409	TBD	TBD	TBD
*C. triensapluvia**	117247	3468	TBD	TBD	TBD

Table 2.3. Pairwise percent differences (uncorrected p) in 28S-D2 rDNA (below diagonal) and cytochrome oxidase subunit I (COI, above diagonal). Values are the minimum distance for comparisons with a range of variation. Cells marked with "–" are missing data for COI.

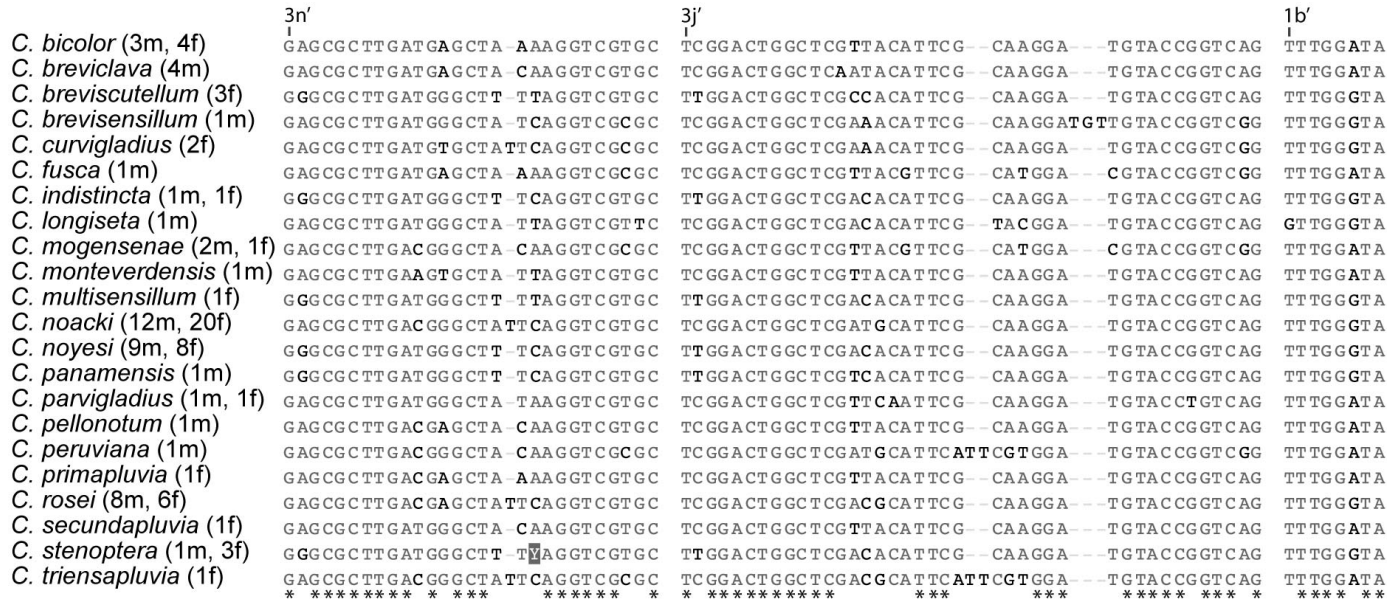
	C. bicolor	*C. breviclava*	*C. breviscutellum*	*C. brevisensillum*	*C. curvigladius*	*C. fusca*	*C. indistincta*	*C. longiseta*	*C. mogensenae*	*C. monteverdensis*	*C. multisensillum*	*C. noacki*	*C. noyesi*	*C. panamensis*	*C. parvigladius*	*C. pellonotum*	*C. peruviana*	*C. primapluvia*	*C. rosei*	*C. secundapluvia*	*C. stenoptera*	*C. triensapluvia*
C. bicolor		8.2	9.2	9.5	6.9	9.2	9.7	8.8	4.5	–	9.7	8.2	9.0	–	7.8	8.1	7.7	–	9.2	8.2	7.2	–
C. breviclava	1.5		9.0	9.2	7.9	10.5	12.5	8.8	5.0	–	10.0	8.5	10.5	–	9.7	10.9	8.5	–	8.8	9.2	9.5	–
C. breviscutellum	4.5	4.2		10.0	9.2	11.8	10.9	7.1	6.3	–	8.2	8.2	9.7	–	9.0	10.4	6.4	–	9.5	7.4	8.7	–
C. brevisensillum	4.5	4.2	3.9		6.7	11.8	10.9	9.9	10.4	–	10.0	8.5	11.0	–	8.3	10.4	9.2	–	8.8	9.2	9.0	–
C. curvigladius	4.7	4.5	4.4	0.5		11.1	10.3	9.9	8.6	–	10.0	10.0	8.7	–	9.6	9.5	8.5	–	9.2	7.4	7.2	–
C. fusca	2.5	3.5	6.7	5.9	6.0		12.1	11.6	8.6	–	10.2	9.8	11.5	–	9.6	10.9	10.5	–	8.5	10.8	10.2	–
C. indistincta	4.4	3.7	2.3	3.2	3.7	6.4		11.6	10.0	–	8.4	10.0	9.1	–	9.6	8.6	8.4	–	10.0	10.3	6.6	–
C. longiseta	6.5	6.0	4.3	6.0	6.2	8.2	5.4		7.7	–	9.2	9.2	10.8	–	8.7	10.4	5.4	–	9.5	9.2	8.5	–
C. mogensenae	2.8	2.8	5.9	5.0	5.4	1.3	5.5	7.7		–	8.6	6.8	9.5	–	6.3	8.1	7.2	–	6.5	8.1	6.8	–
C. monteverdensis	2.0	1.8	4.0	4.0	4.2	4.2	3.7	5.7	3.3		–	–	–	–	–	–	–	–	–	–	–	–
C. multisensillum	4.9	4.4	0.5	3.9	4.4	7.0	2.5	4.5	6.2	4.4		9.0	9.2	–	9.6	10.0	8.5	–	9.7	9.7	8.7	–
C. noacki	5.0	4.7	3.7	4.2	4.7	7.0	4.0	5.5	5.9	4.2	3.9		9.0	–	9.6	7.7	7.7	–	8.2	9.2	9.7	–
C. noyesi	4.9	4.4	1.2	3.7	4.2	6.9	2.2	5.0	6.0	4.4	1.3	3.4		–	8.7	11.8	7.7	–	9.0	9.0	6.9	–
C. panamensis	6.1	5.7	3.2	5.0	5.7	8.6	2.9	7.6	7.6	5.0	3.6	5.9	2.5		–	–	–	–	–	–	–	–
C. parvigladius	2.2	2.8	5.4	4.7	5.2	4.0	4.9	7.1	3.9	2.5	5.7	5.5	5.5	6.8		8.1	8.5	–	5.9	9.1	7.0	–
C. pellonotum	1.7	2.0	4.5	4.5	4.7	3.5	4.5	6.4	2.5	1.7	4.9	4.3	4.7	6.3	2.7		9.5	–	7.6	11.8	8.1	–
C. peruviana	3.2	2.5	5.4	4.7	5.2	3.3	4.9	7.2	2.3	2.8	5.5	4.8	5.4	6.7	4.2	3.2		–	7.9	8.5	6.7	–
C. primapluvia	3.8	3.2	5.5	6.5	7.0	5.5	6.3	8.2	5.1	3.6	5.9	5.7	5.7	6.3	4.9	2.7	4.6		–	–	–	–
C. rosei	4.7	4.7	3.9	3.8	4.2	6.2	4.2	5.9	5.7	4.3	4.0	3.3	3.9	5.7	5.5	4.0	4.8	5.5		10.3	8.4	–
C. secundapluvia	2.3	2.1	4.6	5.9	6.5	5.3	5.3	7.0	4.4	2.1	5.1	5.5	5.3	5.5	4.0	2.5	3.4	2.9	5.5		9.2	–
C. stenoptera	4.7	4.4	0.5	3.7	4.2	6.9	2.2	4.5	6.0	4.0	0.7	3.5	0.5	2.7	5.4	4.5	5.4	5.9	3.7	4.9		–
C. triensapluvia	5.0	4.2	6.0	6.5	7.2	5.3	6.1	8.6	4.6	4.2	6.5	6.1	5.9	6.3	6.1	4.8	1.7	5.1	5.7	4.0	5.9	

Table 2.4. 28S-D2 sequences for all Neotropical species of *Cales*. Conserved regions have been removed to emphasize regions with variable sites. Positions of the sequence fragments are indicated using the secondary structure model for Chalcidoidea 28S (Gillespie et al., 2005a), and nomenclature follows the convention of Gillespie et al. (2004, 2005b). Positions that varied within species are highlighted in gray. Numbers of males and females sequenced for each species appear in parentheses following the species names.

	D2-1a	2a	2e	2g′	2b′	3b
C. bicolor (3m, 4f)	AAATCCAAA	ATCCTTTGCGGCGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCGC	CCGGCGCC	GGTGTCGGTCTACGGCCCGTTTCG
C. breviclava (4m)	AAATCCAAA	ATCCTTTGCGTCGTTG	GTGTTCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCGC	CCGGCGGC	GGTGTCGGTCTACGGCCCGTTTCG
C. breviscutellum (3f)	AAACCCAAA	ATCCTTTGCGACGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGTTGGCAC	CCGGCGGC	GGTGTCGGTCTACGGCCCGAATCG
C. brevisensillum (1m)	AAACCCAAA	AACCTTTGCGACGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCGC	CCGACGGC	GATGTCGGTCTACGGCCCGTTACG
C. curvigladius (2f)	AAACCCAAA	AACCTTTGCGATGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCGC	CCGACGGC	GATGTCGGTCAACGGCCCGTTACG
C. fusca (1m)	AAATCCAAA	ATCCTCTGCGTCGTTG	GCG TCGCGCGATGCCCGGGA	TCCTGTGGCACGCCGGTGC	CCGGTGGC	GGTGTCGGTCTACGGCCCGTTACG
C. indistincta (1m, 1f)	AAACCCAAA	ATCCTTTGCGACGCCG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCGC	CCGGCGGC	GGTGTCGGTCTACGGCCCGTTACG
C. longiseta (1m)	AAACCCAAA	ATCCTTTGCGCGATTG	GTG TCACGCGATGTTTGGGA	TCCTATGGCACGCTGGCTC	CTGGTTGC	GGTGTCGGTCTACGGCCCGAATCG
C. mogensenae (2m, 1f)	AAATCCAAA	ATCCTTTGCGTCGTTG	GCG TCGCGCGATGCCCGGGA	TCCTGTGGCACGCCGGCGC	CCGGCGGC	GGTGTCGGTCTACGGCCCGTTACG
C. monteverdensis (1m)	AAATCCAAA	ATCCTTTGCGGCGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCTC	CCGGCGGC	GGTGTCGGTCTACGGCCCGTTACG
C. multisensillum (1f)	AAACCCAAA	ATCCTTTGCGACGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGTTGGCAC	CCGGCTGC	GGTGTCGGTCTACGGCCCGAATCG
C. noacki (12m, 20f)	AAACCCAAA	ATCCTTCGCGACGTTG	GTG TTACGCGATGCTCGGTA	TACTGTGGCACGCTGGCAC	CCGGCGGC	GGTGTCGGTCTACGGCCCGAAACG
C. noyesi (9m, 8f)	AAACCCAAA	ATCCTTTGCGACGTTG	GTG TCACGCGATGYTCGGGA	TCCTGTGGCACGTTGGCRC	CCGRCGGC	GGTGTCGGTCTACGGCCCGAGGCG
C. panamensis (1m)	AAACCCAAA	ATCCTTTGCGACGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGTTGGCAC	CCGGCGGC	GGTGTCGGTCTACGGCCCGTGAAG
C. parvigladius (1m, 1f)	AAATCCAAA	ATCCTTTGCGGCGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCGC	CCGATGCC	GGTGTCGGTCTACGGCCCGTTACG
C. pellonotum (1m)	AAATCCAAA	ATCCTTTGCGGCGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCGC	CCGGCGGC	GGTGTCGGTCTACGGCCCGATACG
C. peruviana (1m)	AAATCCAAA	ATCCTTTGCGTCGTTG	GTG TCGCGCGATGCTCGGGA	TCCTGTGGCACGCCGGCAC	CCGGCGGC	GGTGTCGGTCTACGGCCCGTTACG
C. primapluvia (1f)	AAATCCAAA	ATCCTTTGCGTCGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGTAC	CCGACGGC	GGTGTCGGTCTACGGCCCGAATCG
C. rosei (8m, 6f)	AAACCCAAA	ATCCTTTGCGACGTTG	GTG CCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCAC	CCGGCGGC	GGTGTCGGTCTACGGCCCGAAACG
C. secundapluvia (1f)	AAATCCAAA	ATCCTTTGCGGCGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGCTGGCTC	CCGGCGGC	GGTGTCGGTCTACGGCCCGAATCG
C. stenoptera (1m, 3f)	AAACCCAAA	ATCCTTTGCGRCGTTG	GTG TCACGCGATGCTCGGGA	TCCTGTGGCACGTTGGCAC	CCGGCGGC	GGTGTCGGTCTACGGCCCGAAGCG
C. triensapluvia (1f)	AAATCCAAA	ATCCTCTGCGTCGTTG	GTG TCGCGCGATGCTCGGGA	TCCTGTGGCACGCCGGCAC	CCGGCGGC	GGTGTCGGTCTACGGCCCGATGCG
	*** *****	* *** *** * *	* * ******* ** *	* ** ******* ** *	* * *	* ******** ******** *

Table 2.4. Cont.

Table 2.4. Cont.



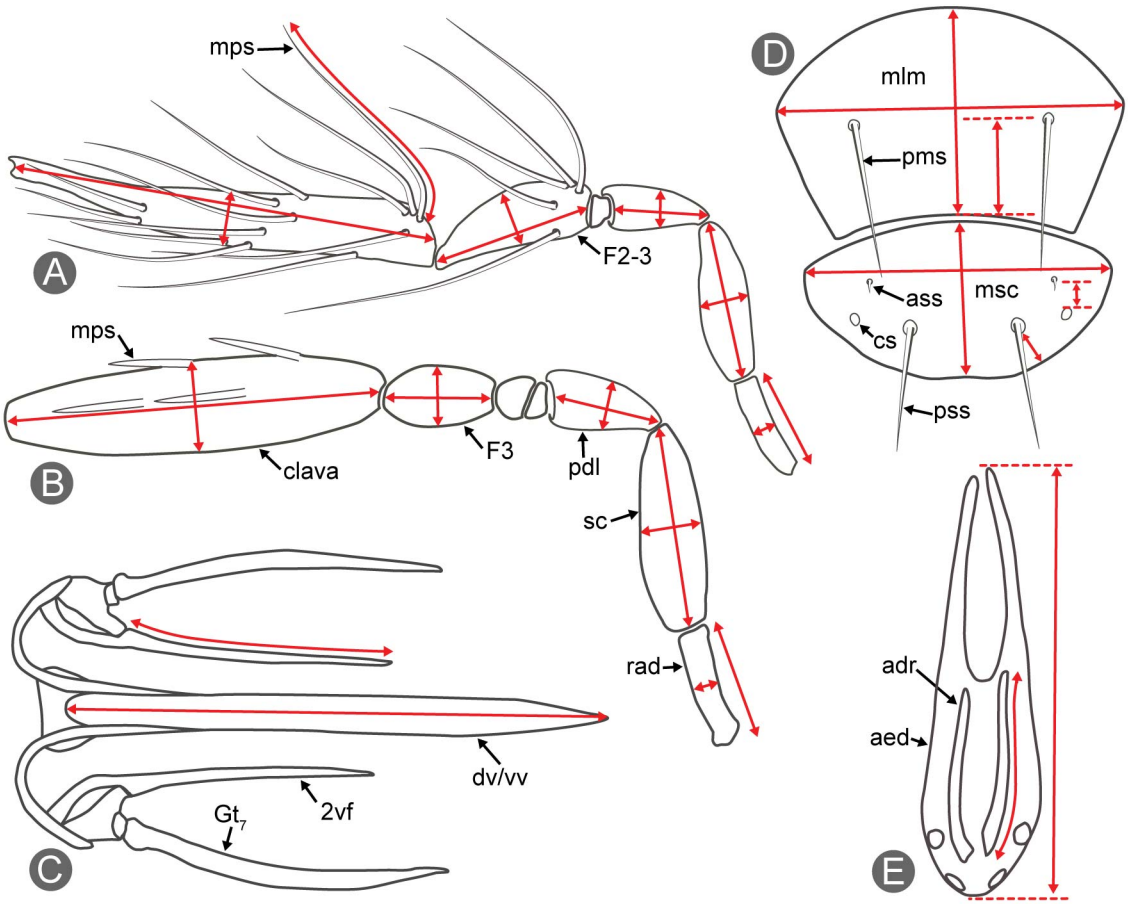


Figure 2.1. Measurements used to characterize the antennae, mesodorsum and genitalia. Red arrows indicate measurements. Length and width measurements are perpendicular to one another, with the width measurement taken at the midpoint of the length measurement for all antennal structure. Width measurements for the midlobe of the mesoscutum and the mesoscutellum are taken at their respective maxima. **A, male antenna**, **mps** = multiporous plate sensillum, **F2-3** = fused second and third flagellomeres. **B, female antenna**, **F3** = third flagellomere, **pdl** = pedicel, **sc** = scape, **rad** = radicle. **C, ovipositor**, **dv** = dorsal valve, **vv** = ventral valve **2vf** = second valvifer, **Gt_7** = 7th gastral tergite (outer plate of ovipositor). **D, mesonotum**, **mlm** = midlobe of the mesoscutum, **pms** = posterior mesoscutal seta, **msc** = mesoscutellum, **ass** = anterior mesoscutellar seta, **cs** = campaniform sensillum of mesoscutellum, **pss** = posterior mesoscutellar seta. **E, aedeagus**, **aed** = aedeagus, **adr** = aedeagal rods.

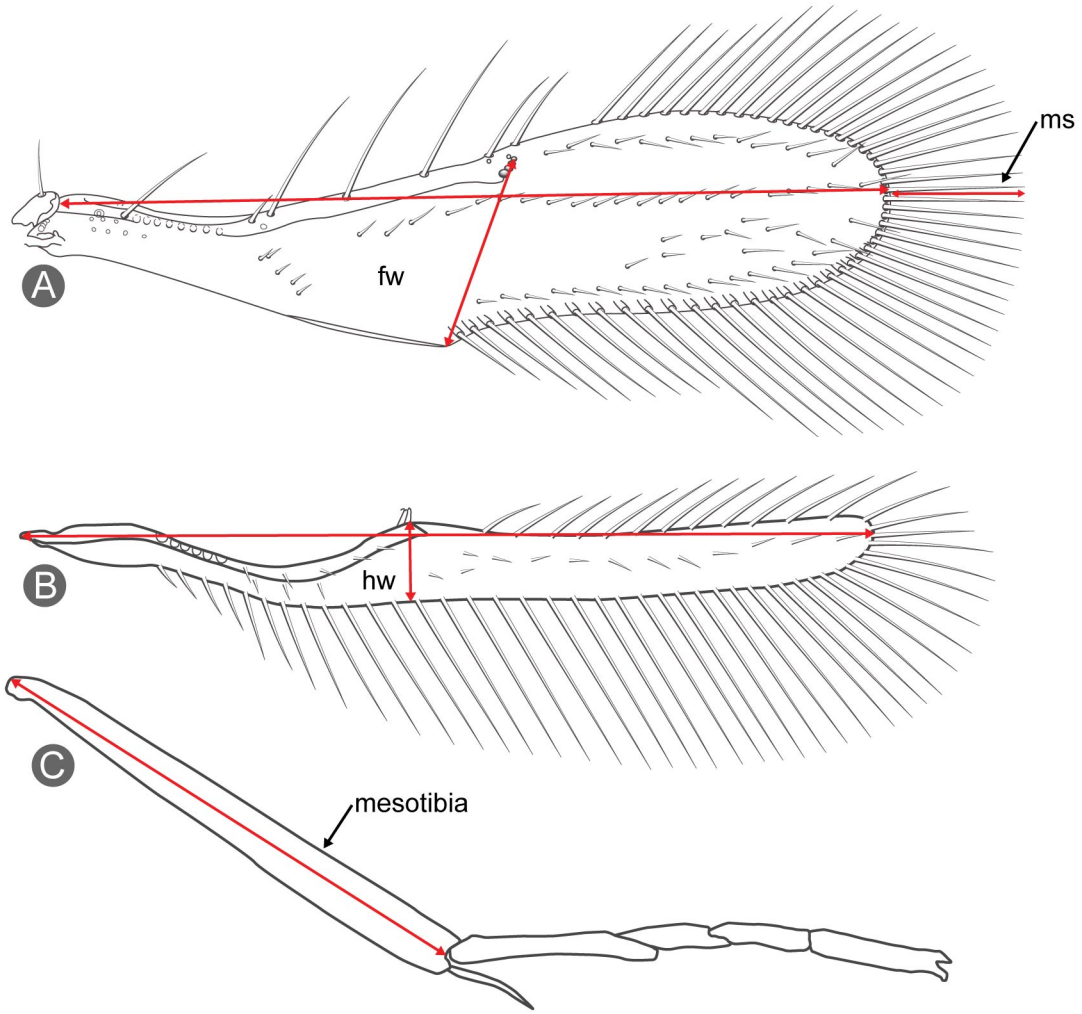


Figure 2.2. Measurements used to characterize the fore wing (**fw**), hind wing (**hw**) and mesotibia. Red arrows indicate measurements. The length measurement for the fore wing is from the proximal end of the submarginal vein to the apex of the wing. The relative length of the marginal setae of the fore wing is assessed by measuring the apical marginal seta (**ms**). The width measurement for the fore wing is from the distal end of the retinaculum to the distal stigmal vein sensillum. The hind wing length is measured from the proximal end of the humeral plate to the wing apex. Width of the hind wing is from the hamuli to the posterior edge of the wing, perpendicular to the length measurement.

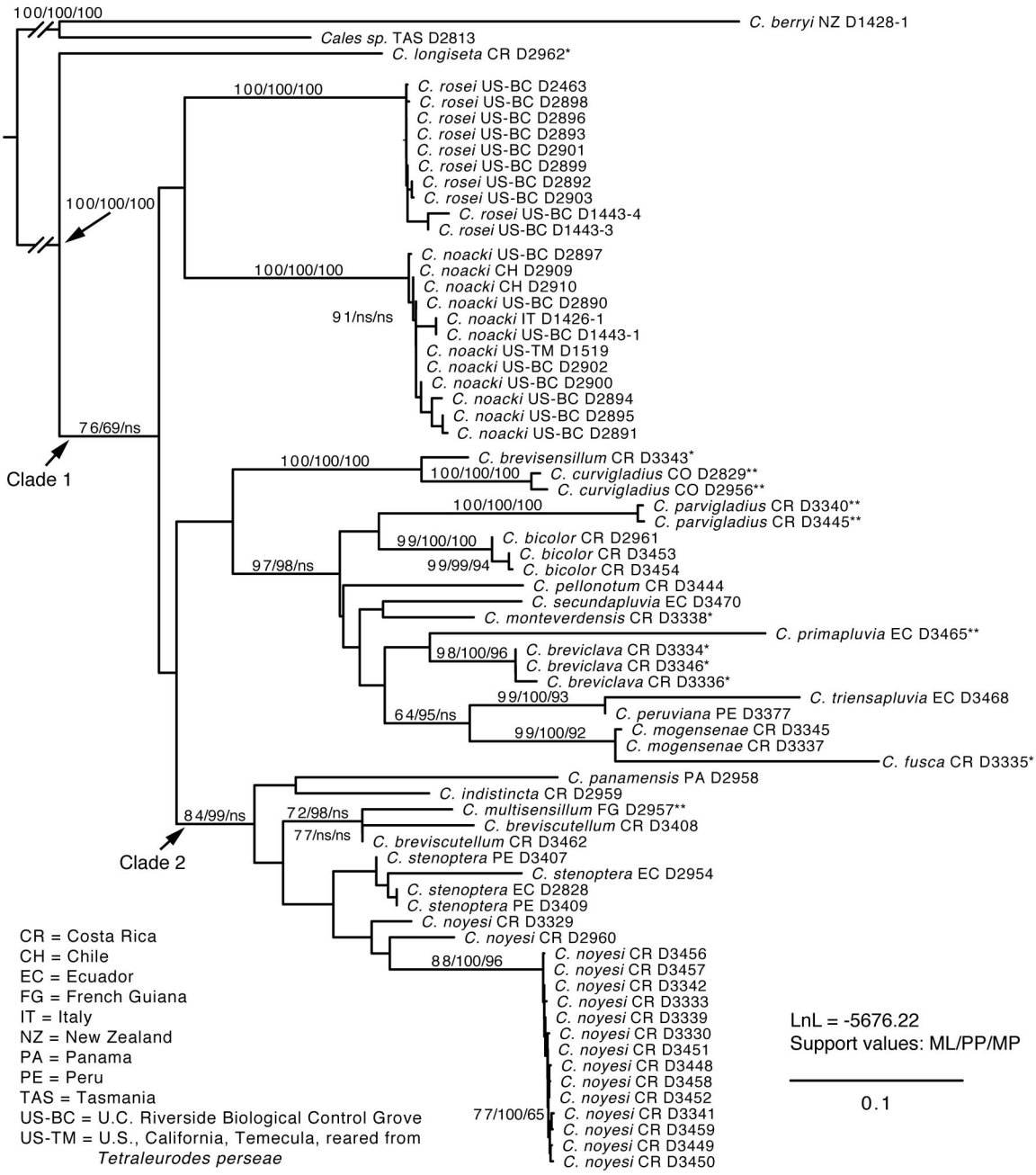


Figure 2.3. Maximum Likelihood analysis of *Cales*. The analysis includes 73 taxa and alignment of 28S-D2-5 rDNA (1124bp) and COI (390bp). Support values are maximum likelihood bootstrap, Bayesian posterior probabilities, and maximum parsimony bootstrap (ML/PP/MP). Values below 60% are not shown. Species with males that can be diagnosed based on morphology alone are indicated by "*". Species with females that can be diagnosed based on morphology alone are indicated by "**".

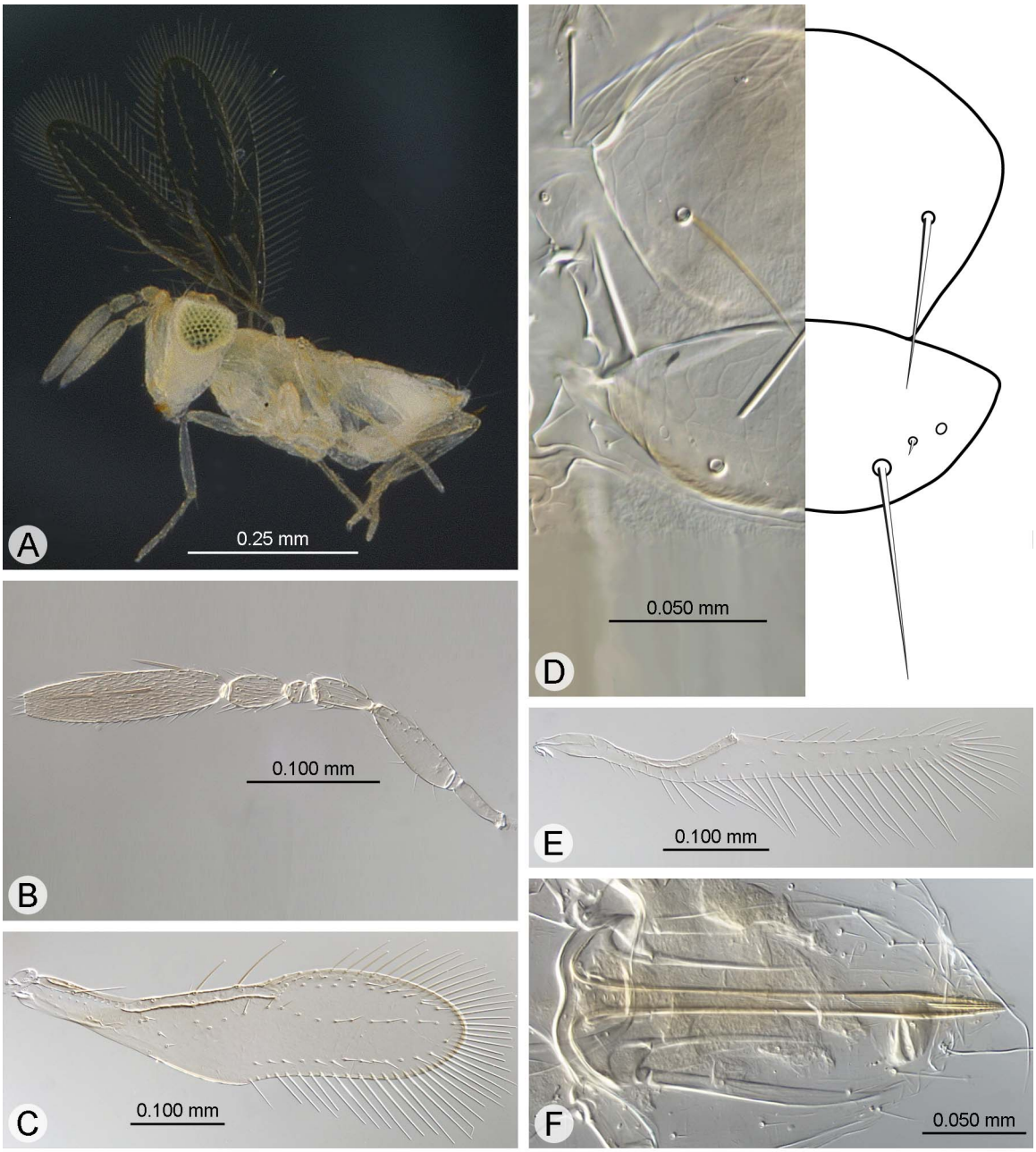


Figure 2.4. *Cales bicolor* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

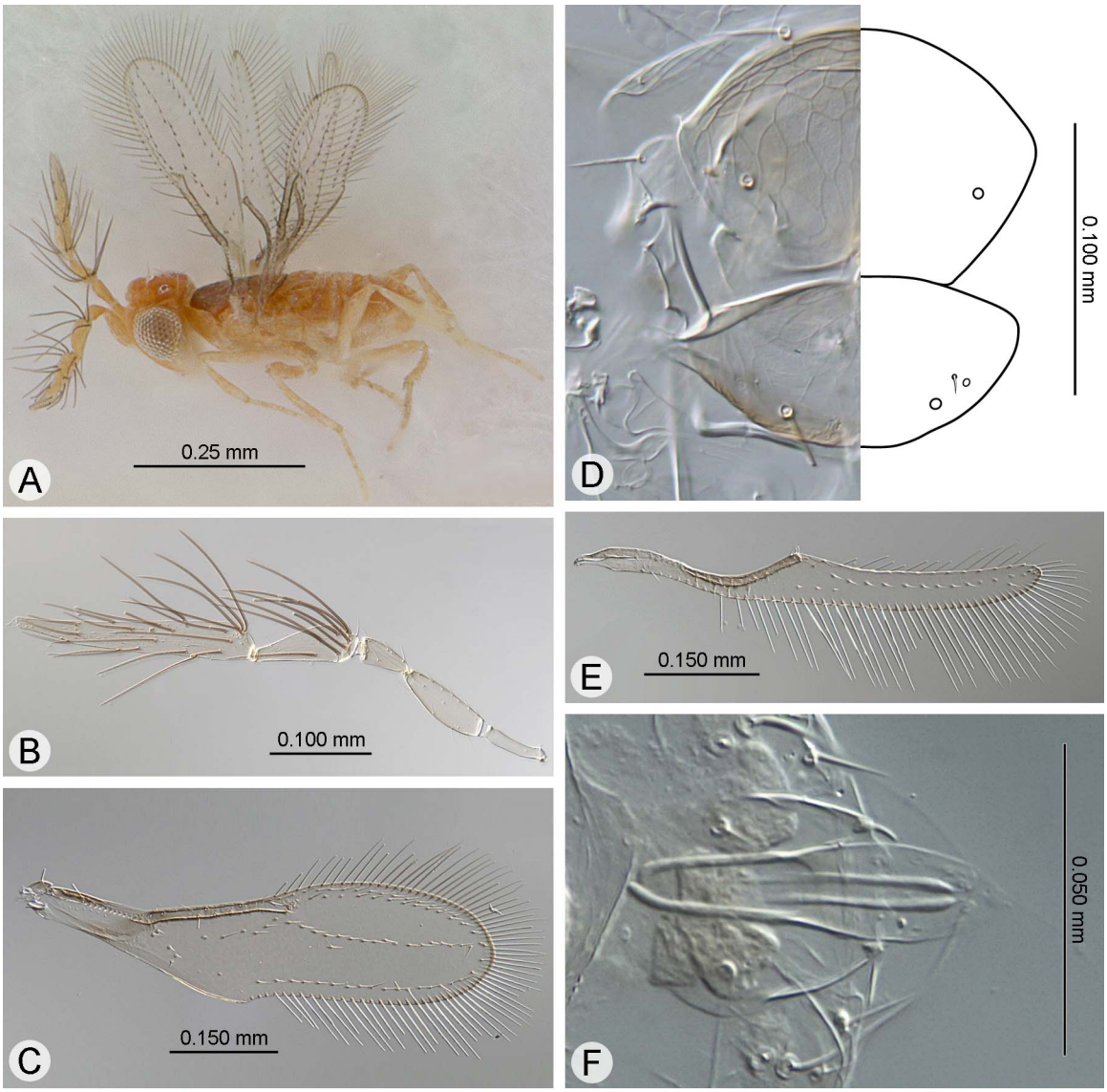


Figure 2.5. *Cales bicolor* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus.

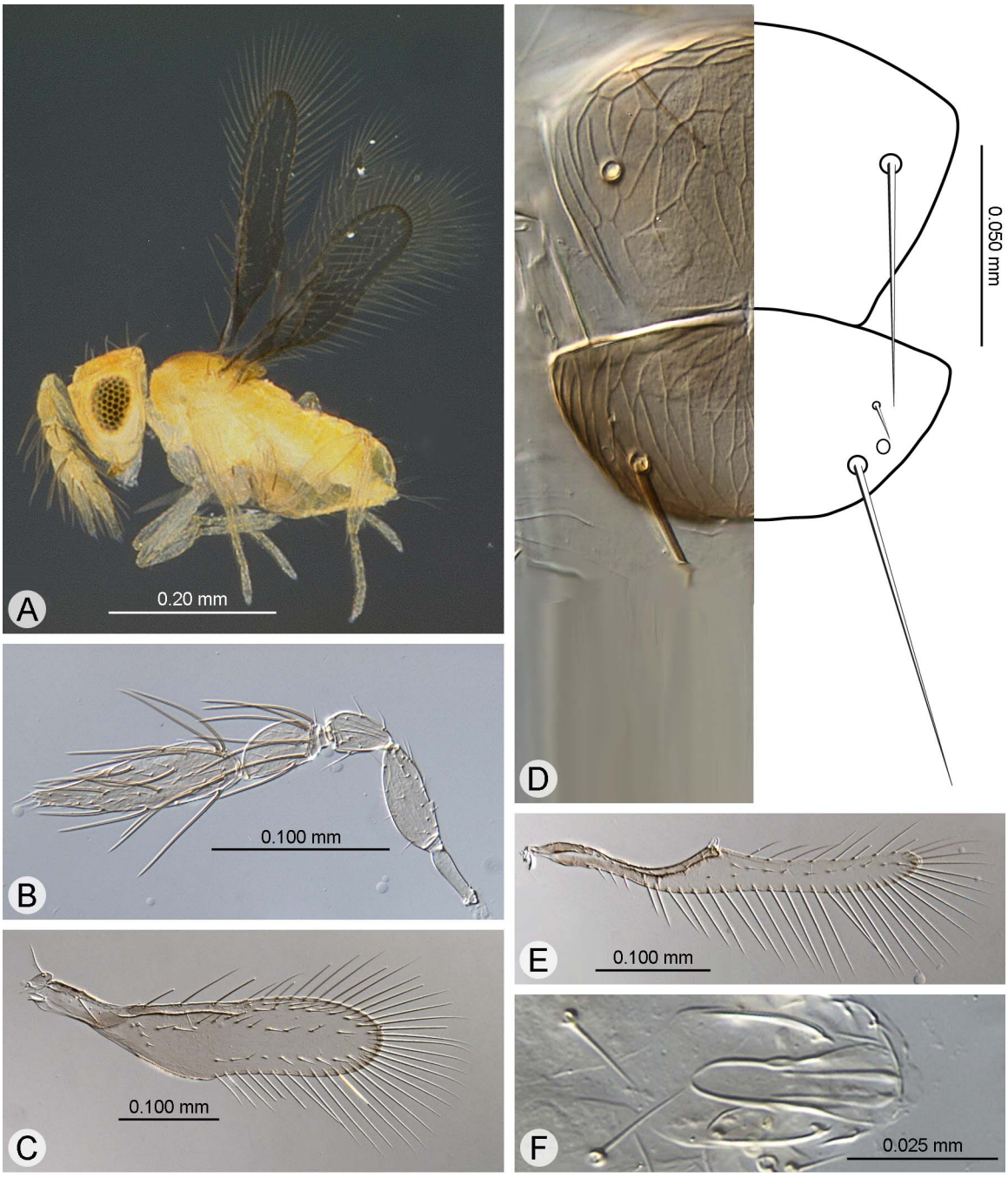


Figure 2.6. *Cales breviclava* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus.

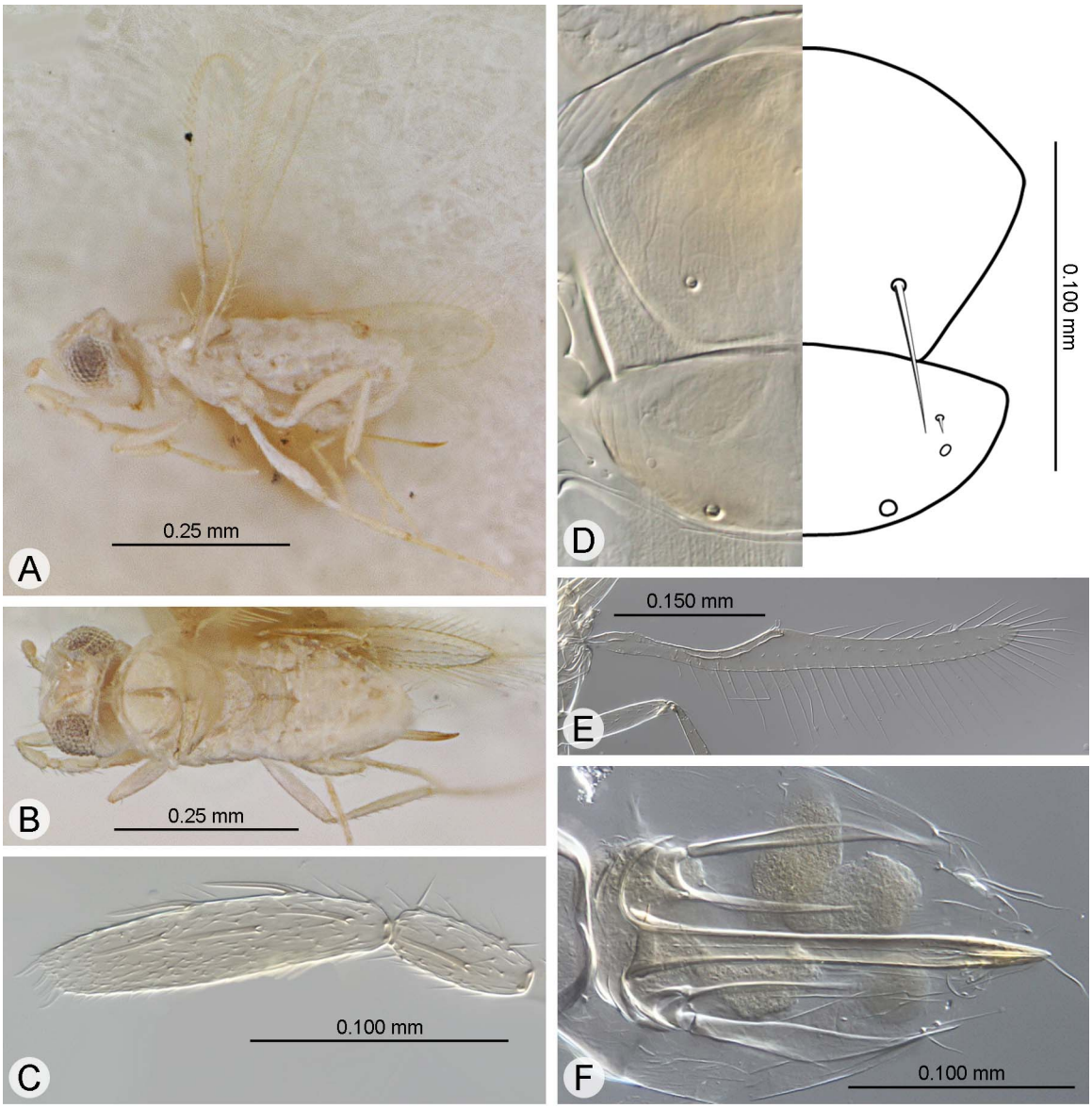


Figure 2.7. *Cales breviscutellum* n. sp., female. **A,** lateral habitus; **B,** dorsal habitus; **C,** F3 and clava of antenna; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

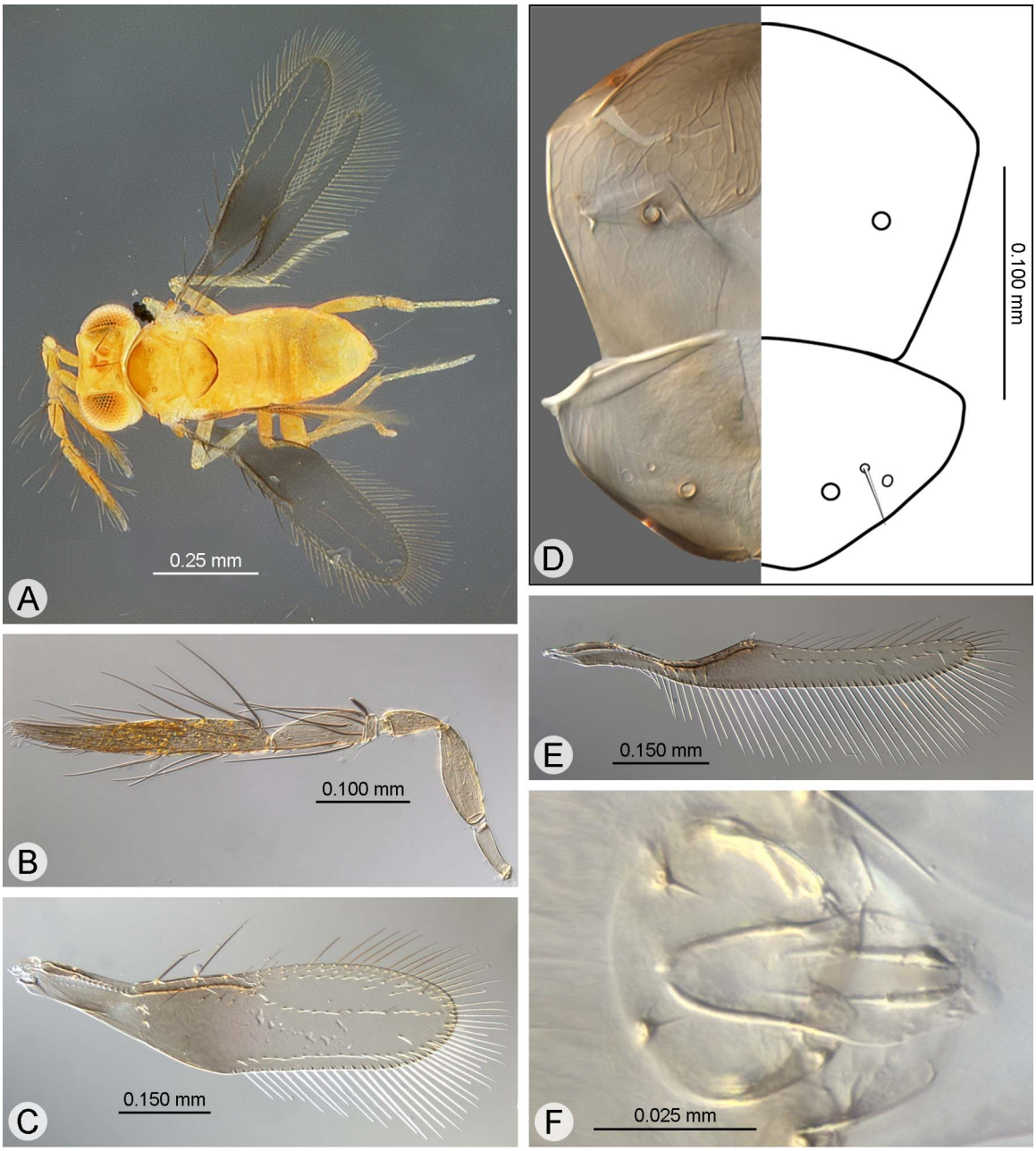


Figure 2.8. *Cales brevisensillum* n. sp., male. **A,** dorsal habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma, torn into several pieces during slide-mounting, so shown digitally reconstructed; **E,** hind wing; **F,** aedeagus.

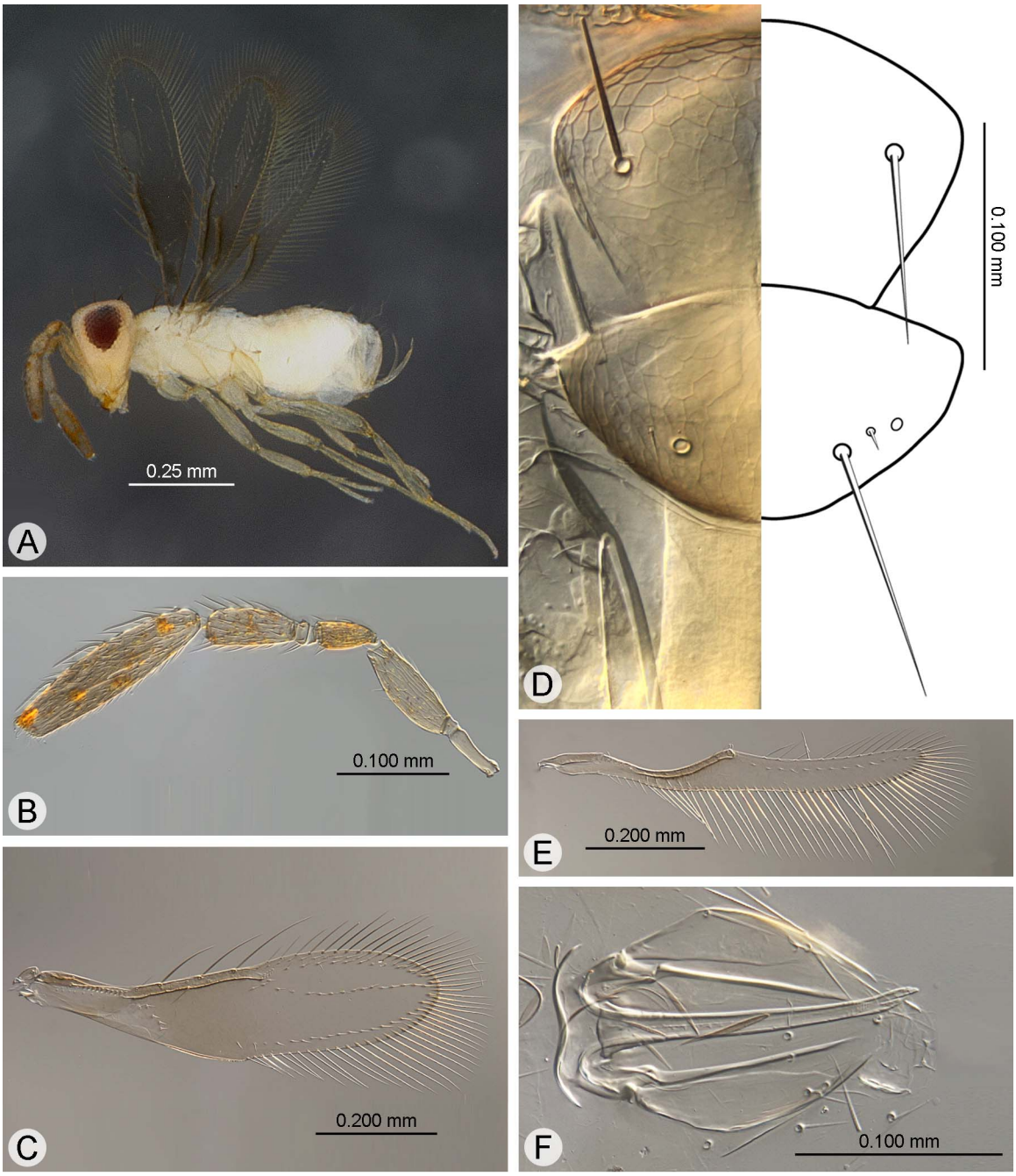


Figure 2.9. *Cales curvigladius* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

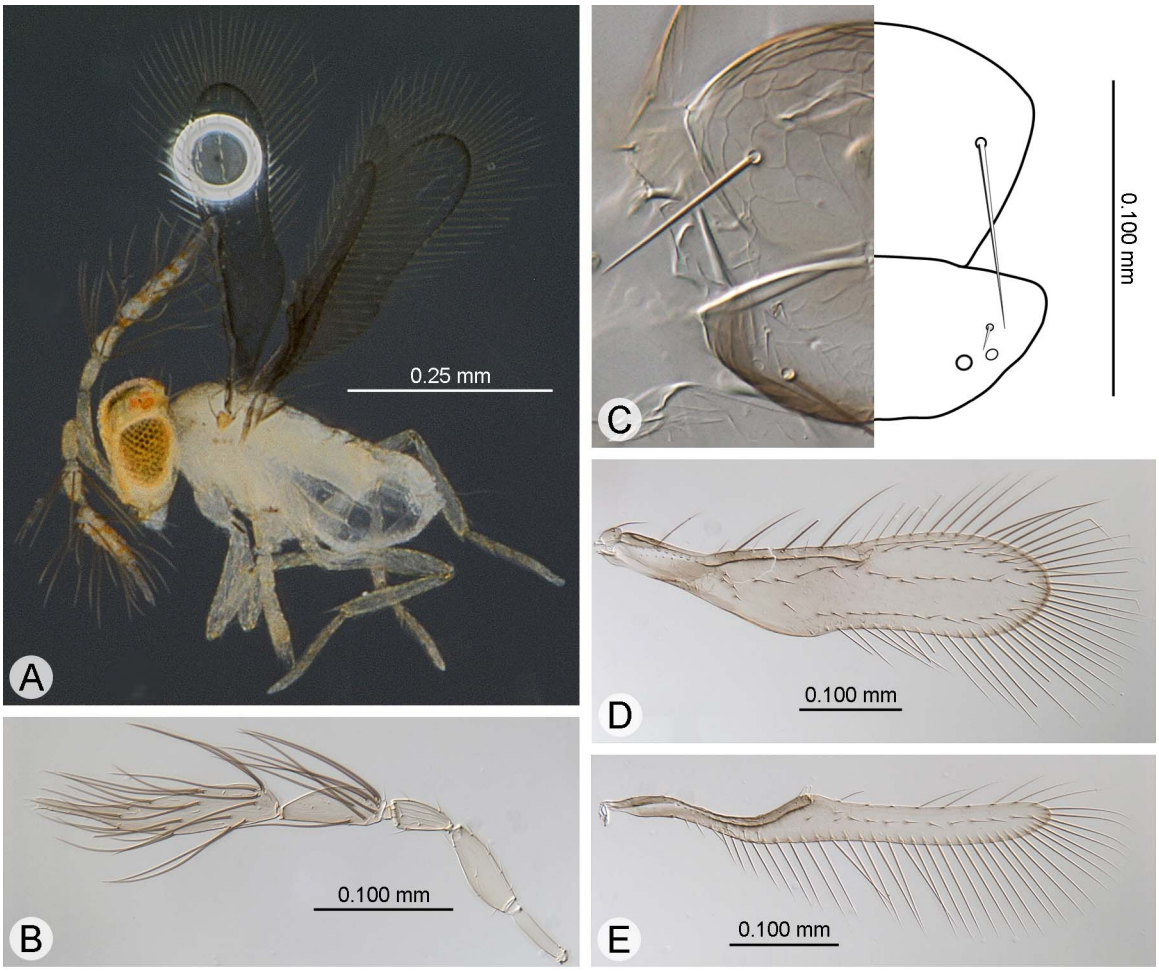


Figure 2.10. *Cales fusca* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** dorsal mesosoma; **D,** fore wing; **E,** hind wing.

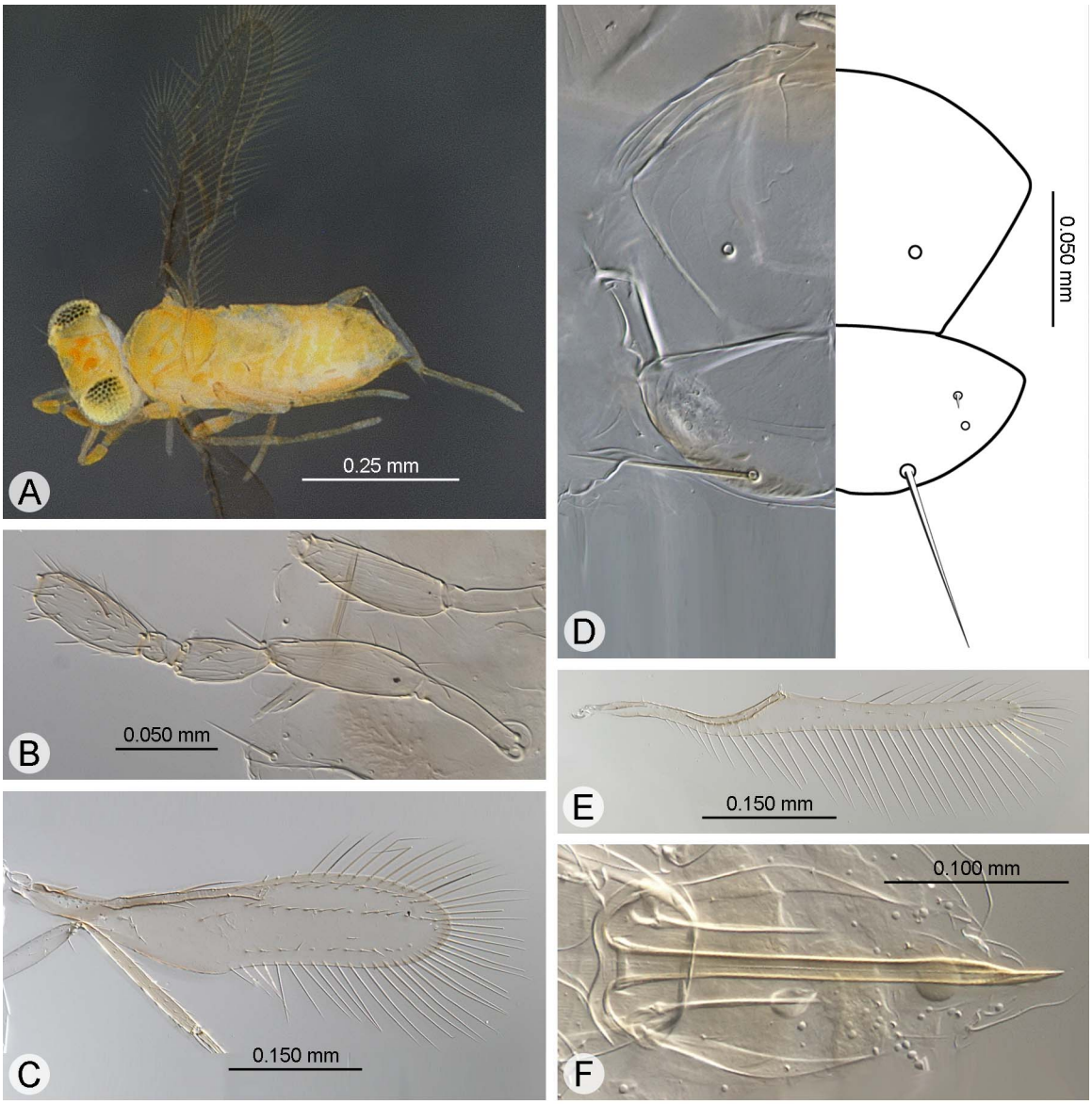


Figure 2.11. *Cales indistincta* n. sp., female. **A,** dorsal habitus; **B,** antenna (clava missing); **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

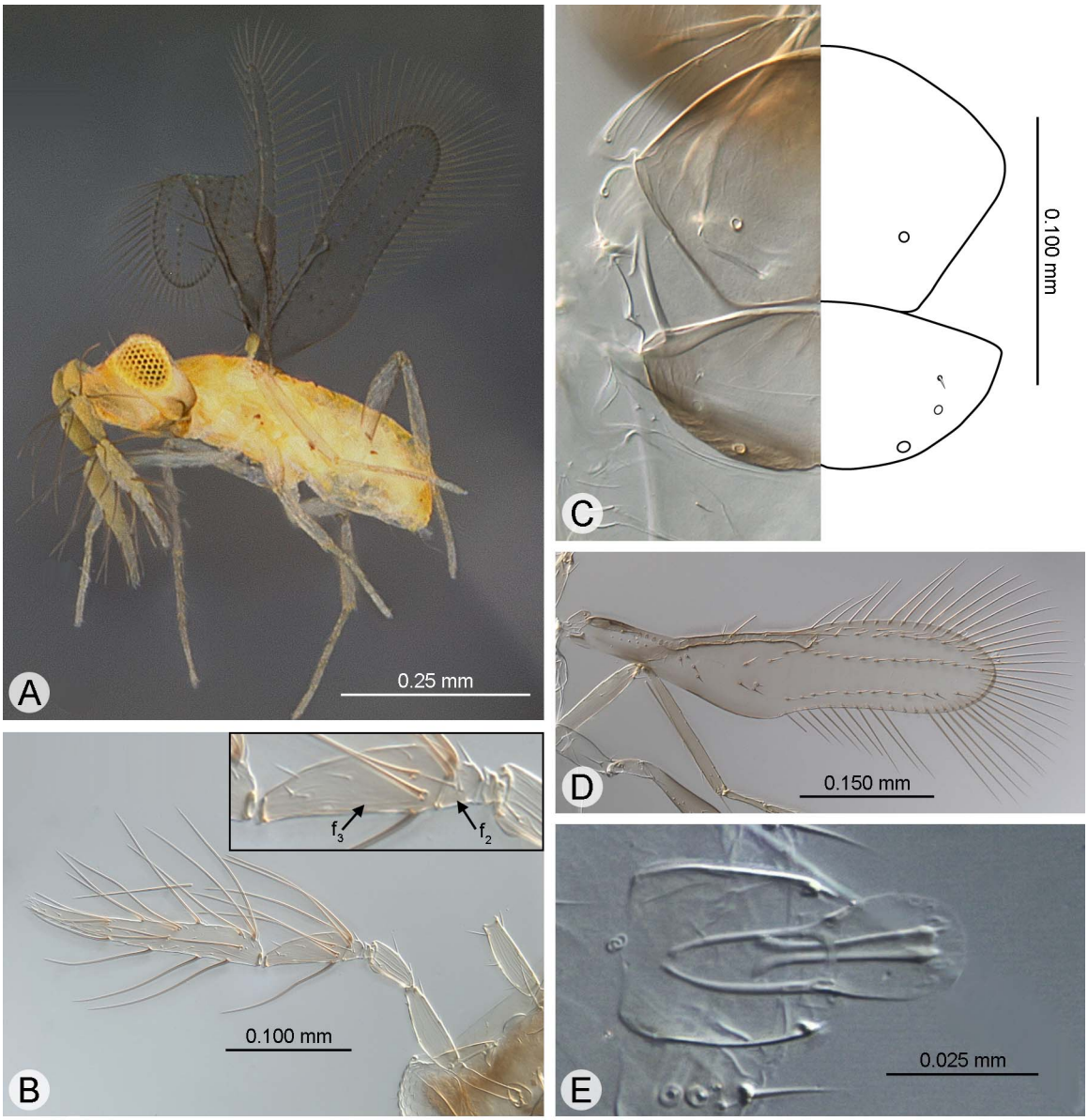


Figure 2.12. *Cales indistincta* n. sp., male. **A,** lateral habitus; **B,** antenna, inset showing F2-3; **C,** dorsal mesosoma; **D,** fore wing; **E,** aedeagus.

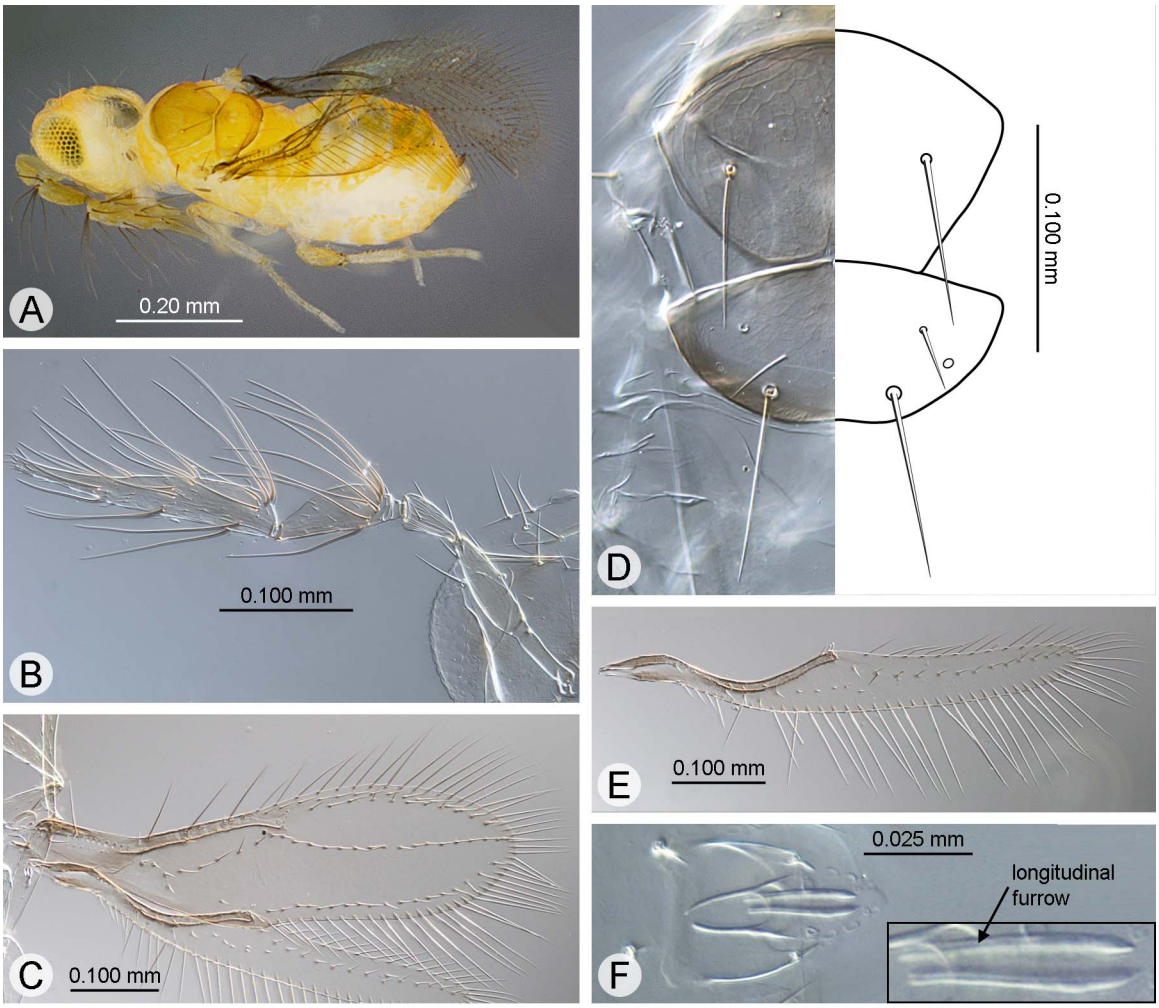


Figure 2.13. *Cales longiseta* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus, inset showing aedeagal rod detail.

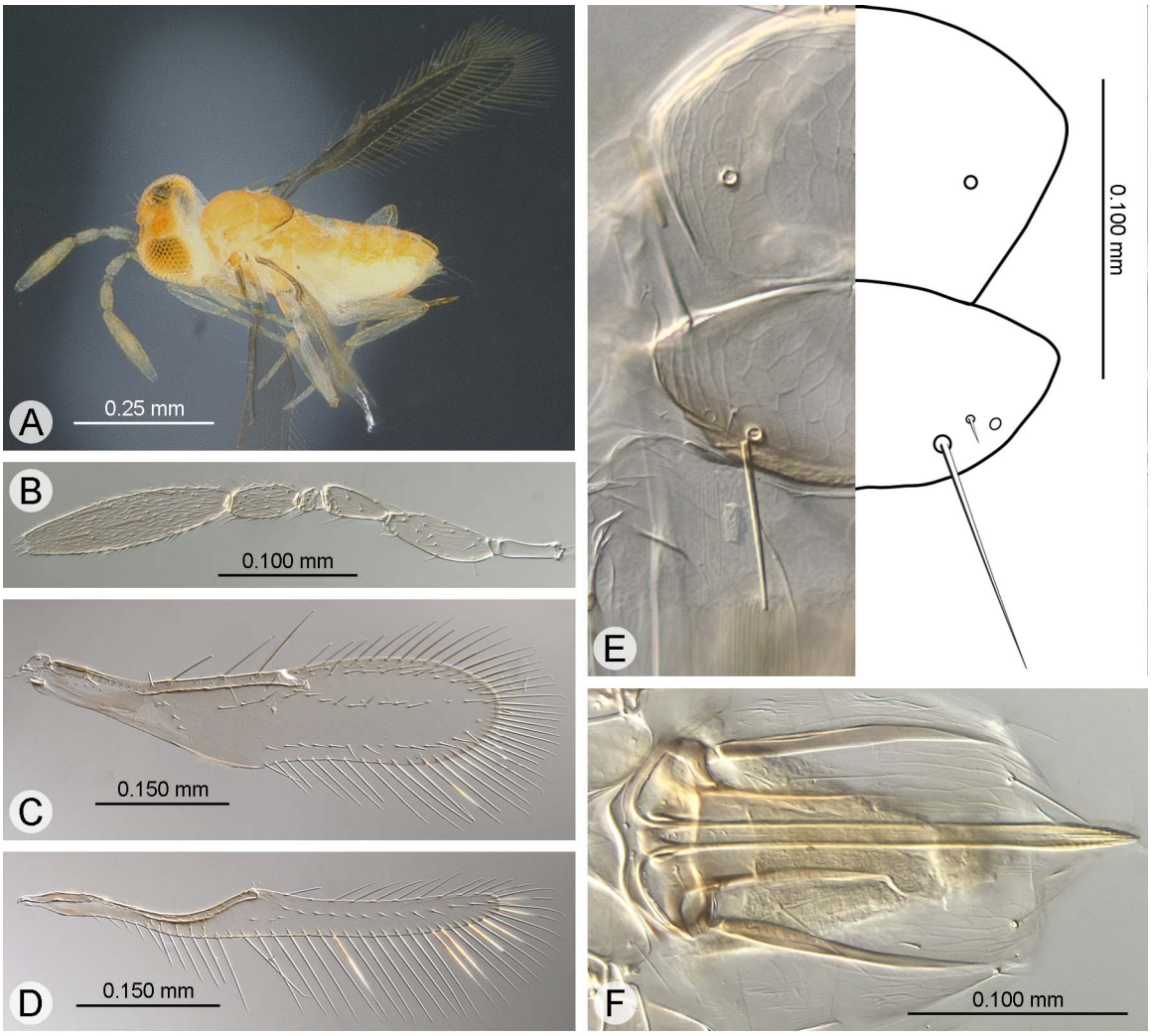


Figure 2.14. *Cales mogensenae* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** hind wing; **E,** dorsal mesosoma; **F,** ovipositor.

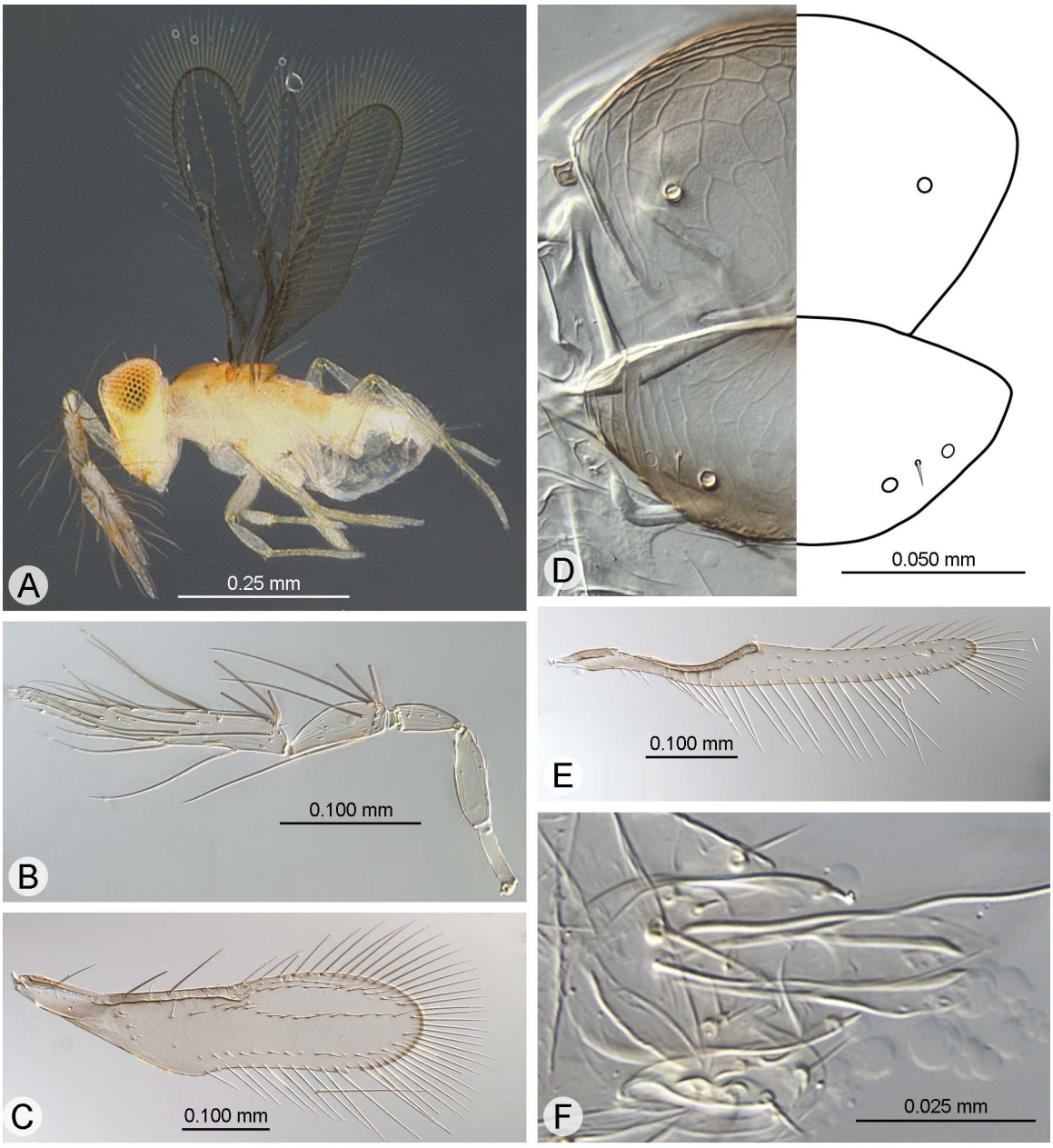


Figure 2.15. *Cales mogensenae* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus.

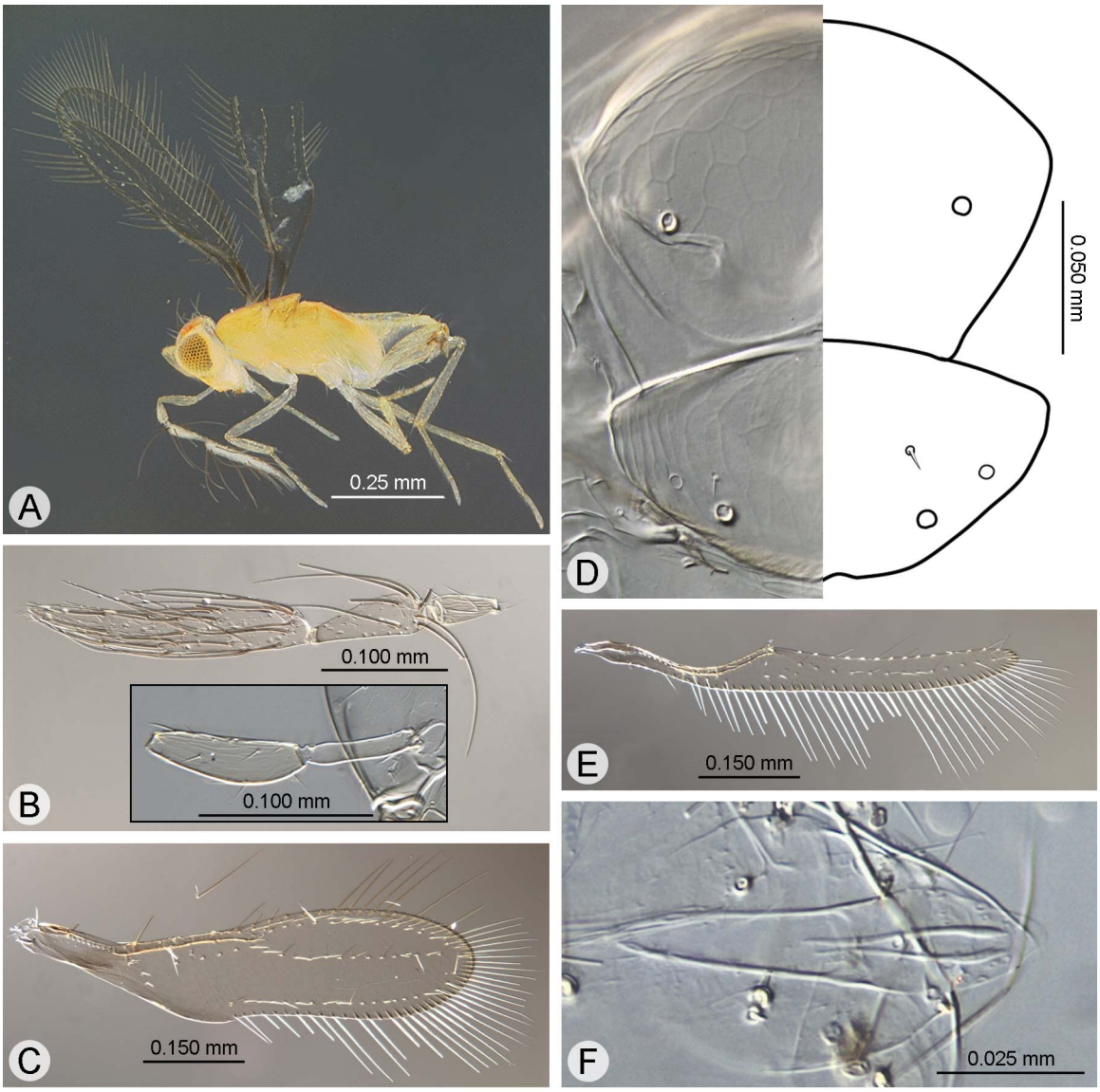


Figure 2.16. *Cales monteverdensis* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus.

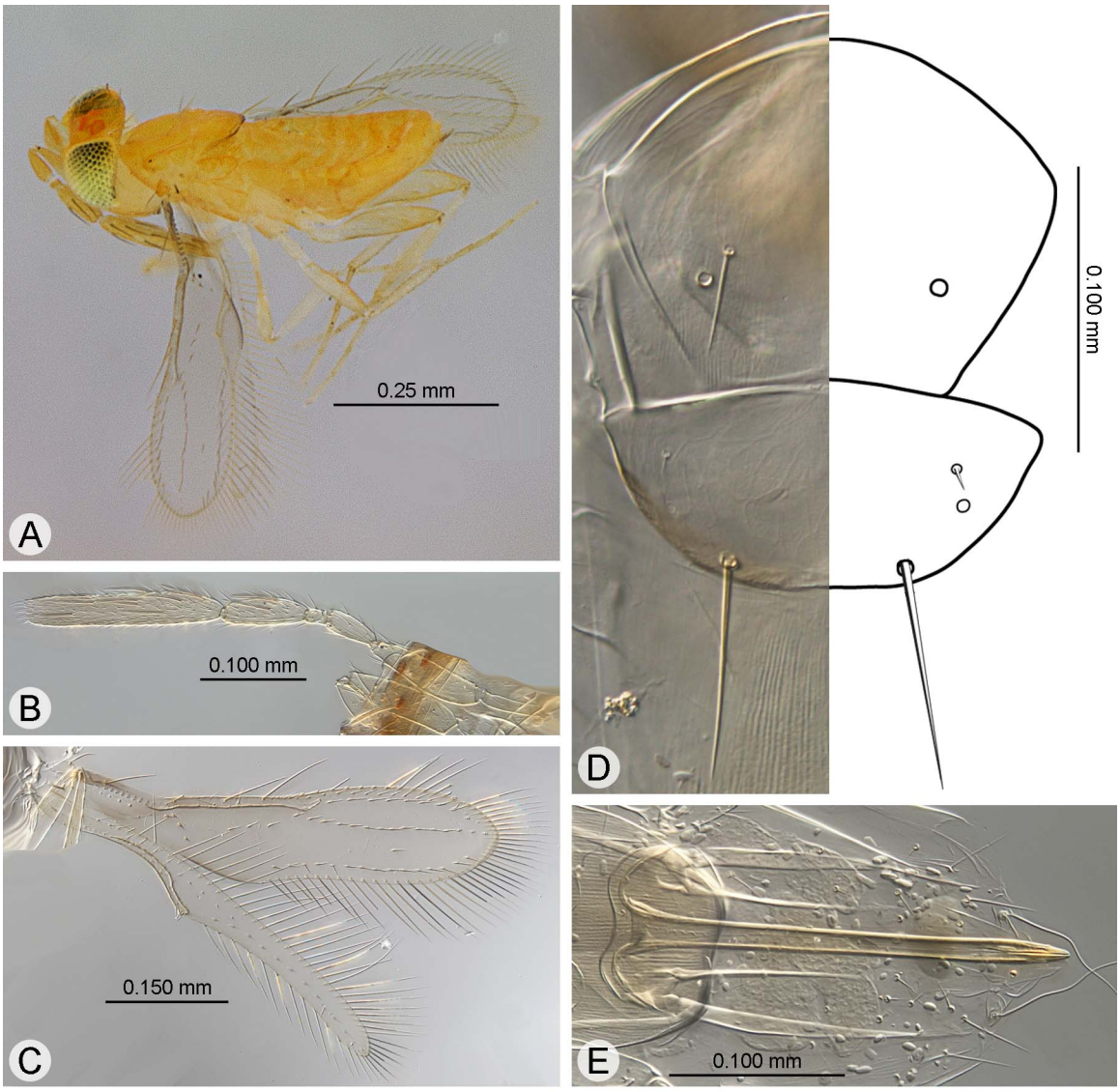


Figure 2.17. *Cales multisensillum* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing and hind wing; **D,** dorsal mesosoma, note aberrant seta on left half of midlobe of mesonotum; **E,** ovipositor.

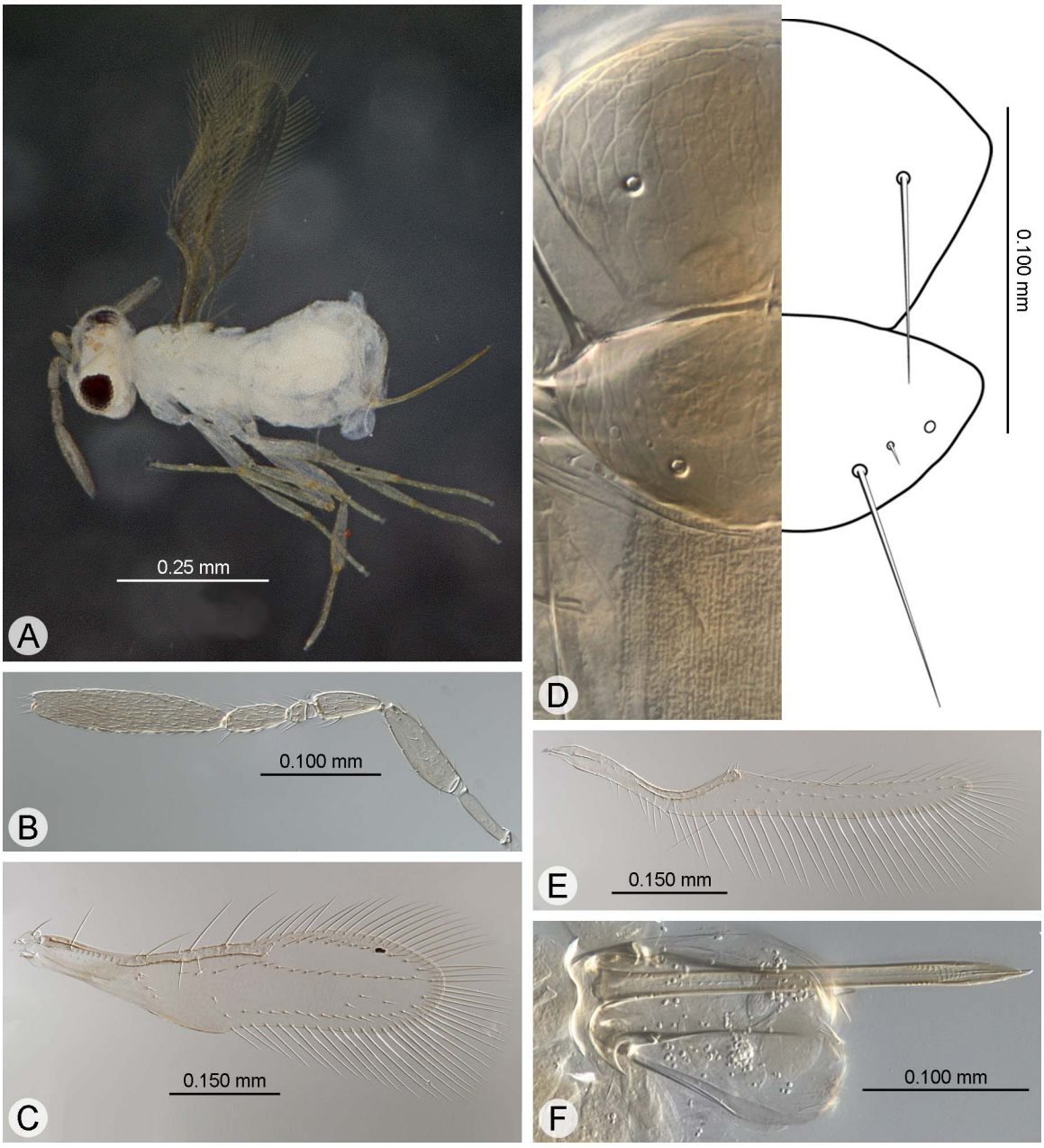


Figure 2.18. *Cales noacki* neotype female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor, mounted obliquely.

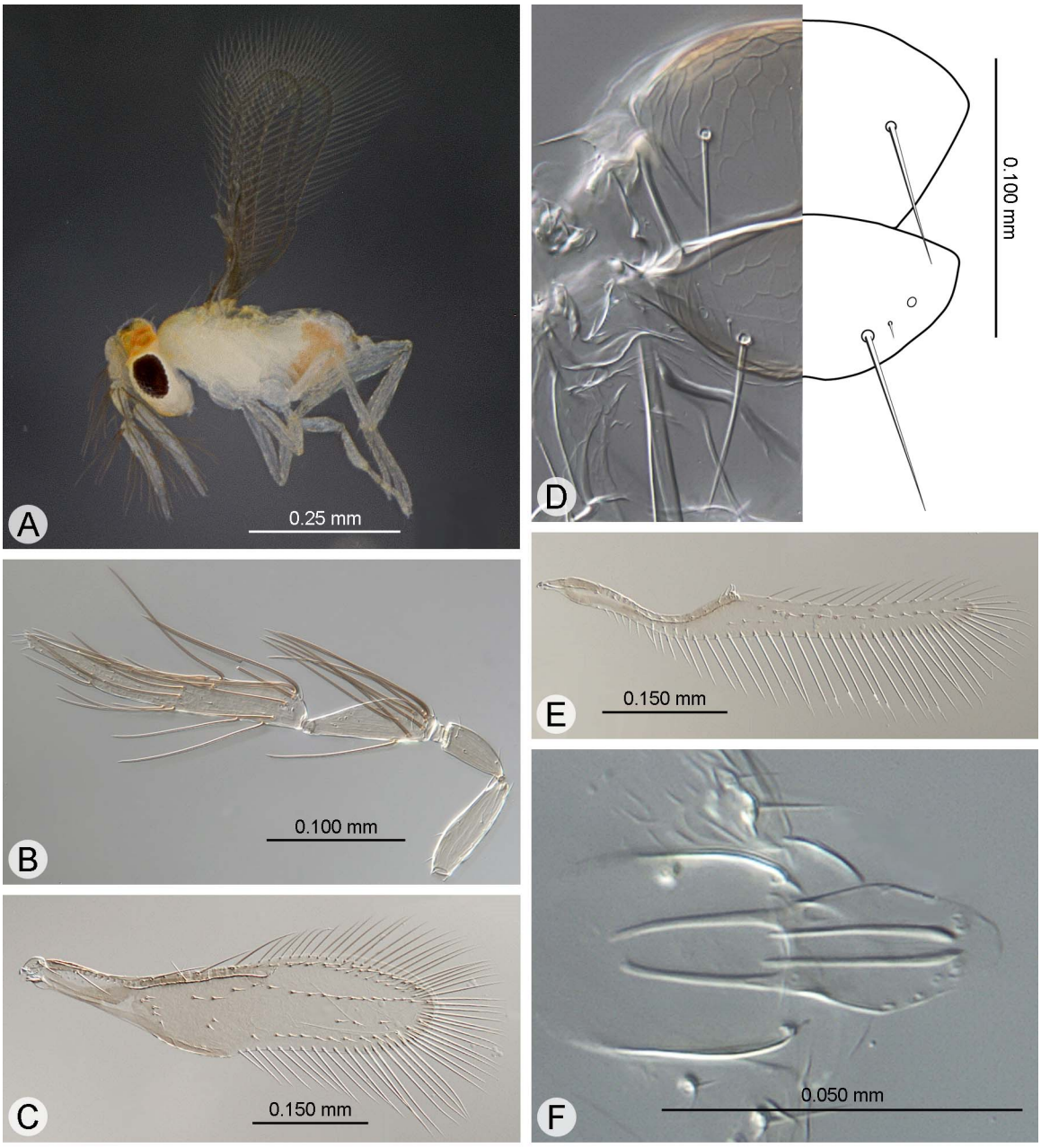


Figure 2.19. *Cales noacki* male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus.

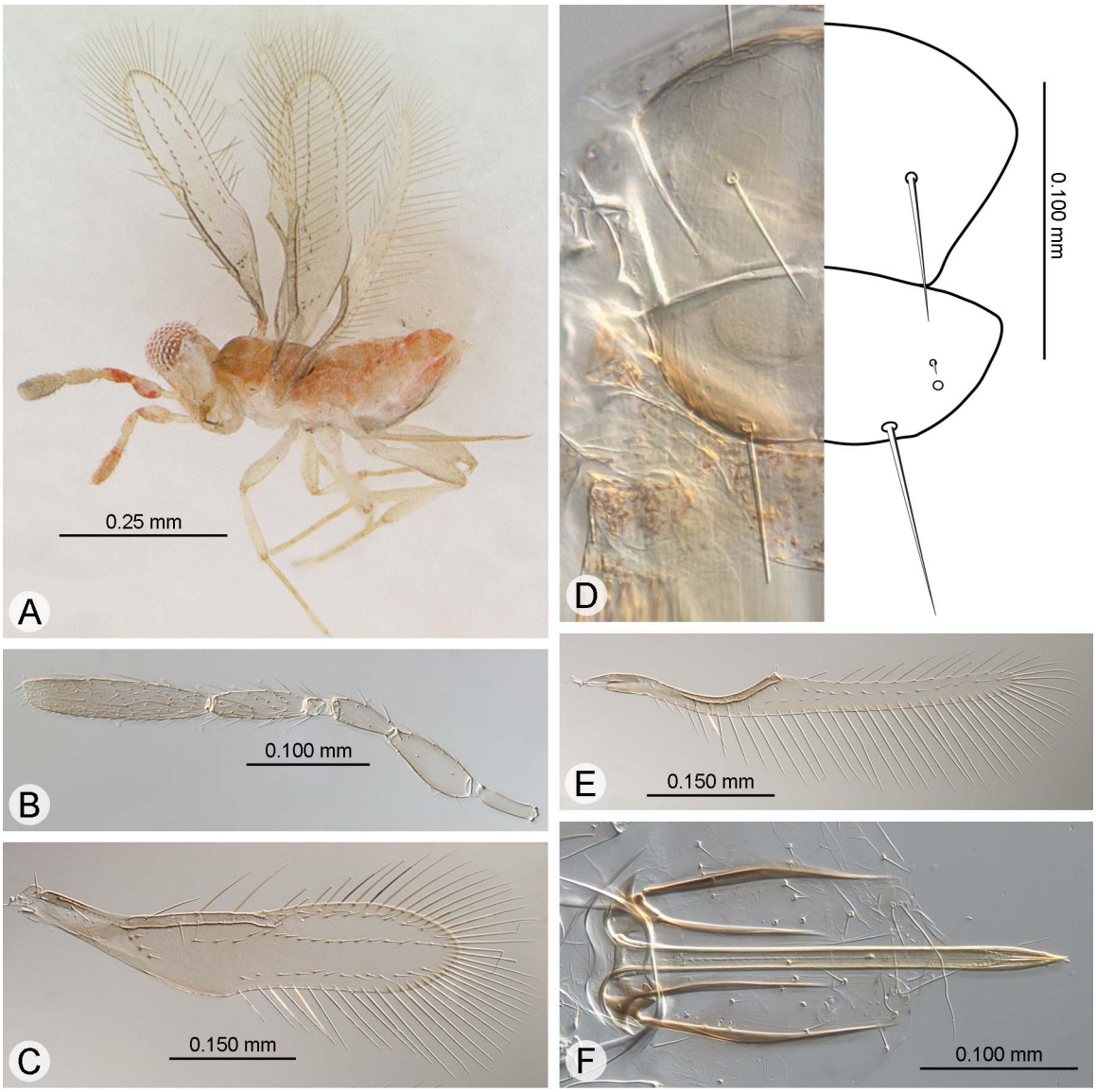


Figure 2.20. *Cales noyesi* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

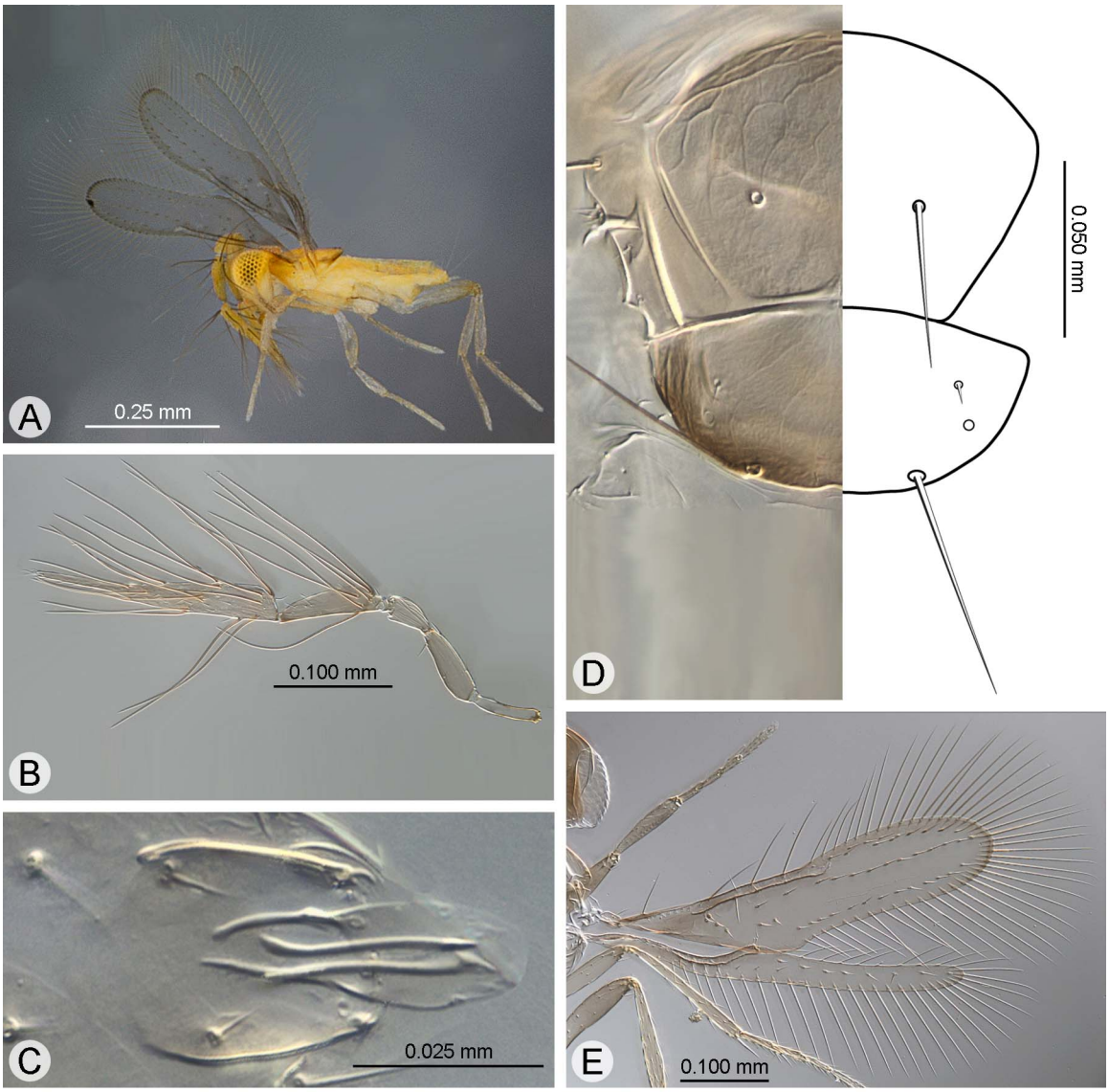


Figure 2.21. *Cales noyesi* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** aedeagus; **D,** dorsal mesosoma; **E,** fore and hind wings.

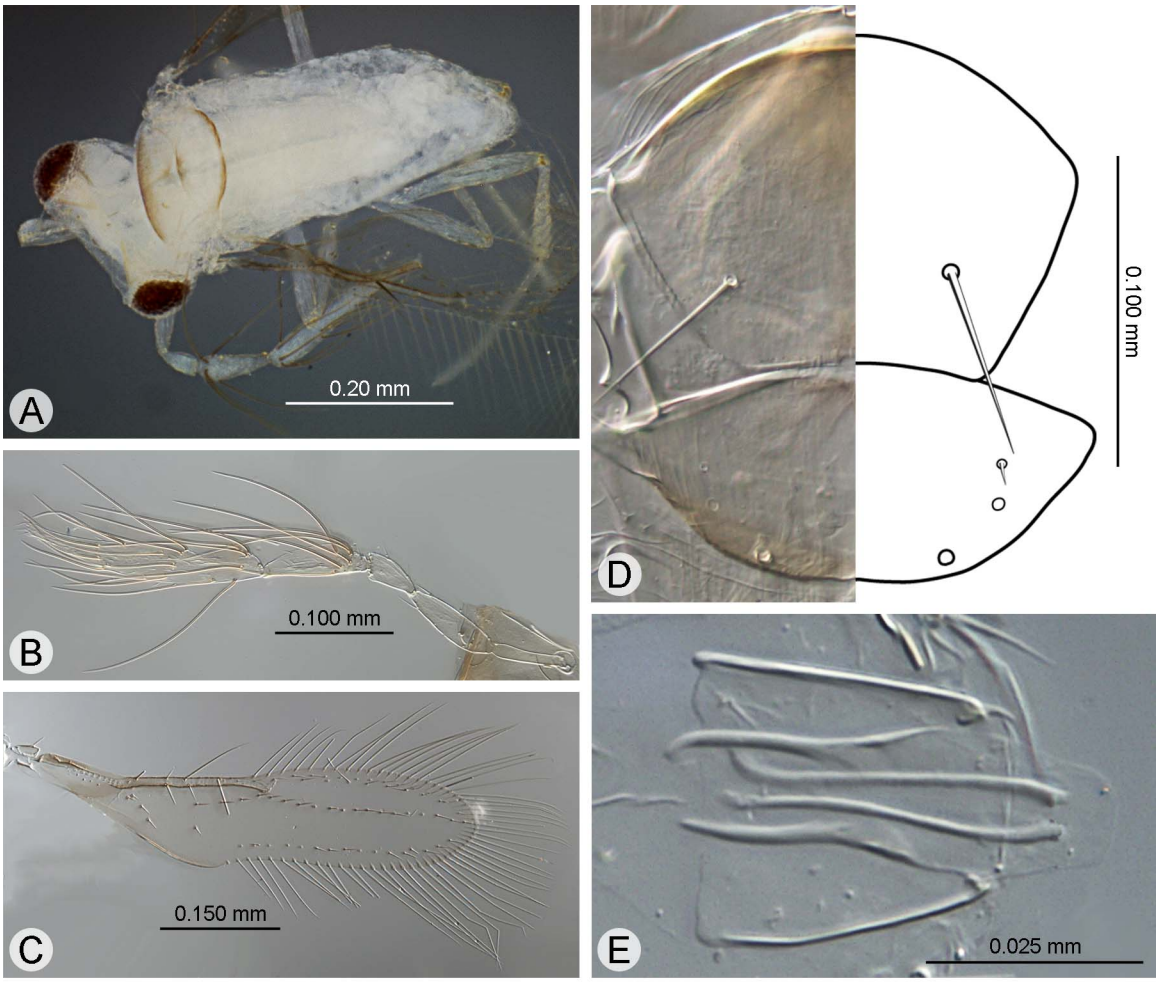


Figure 2.22. *Cales panamensis* n. sp., male. **A,** dorsal habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** aedeagus.

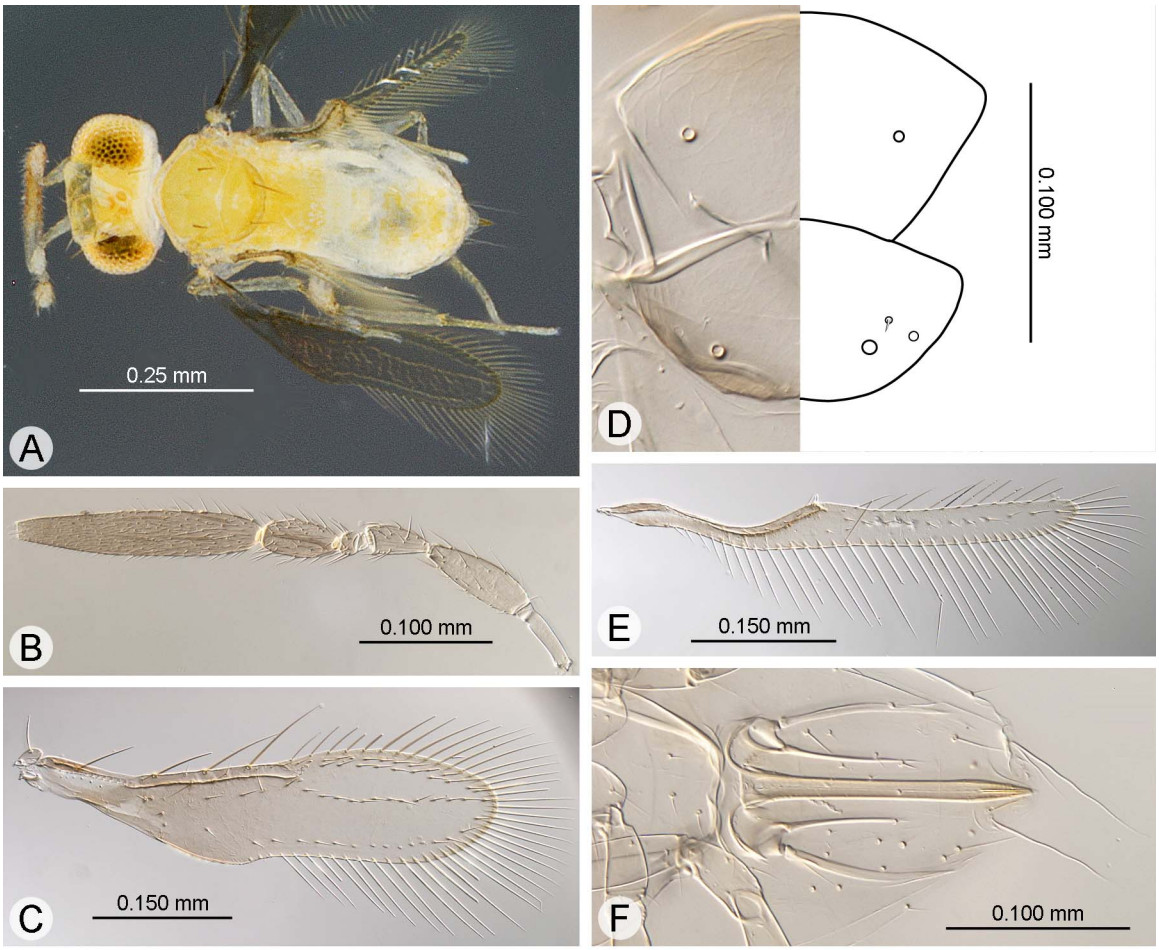


Figure 2.23. *Cales parvigladius* n. sp., female. **A,** dorsal habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

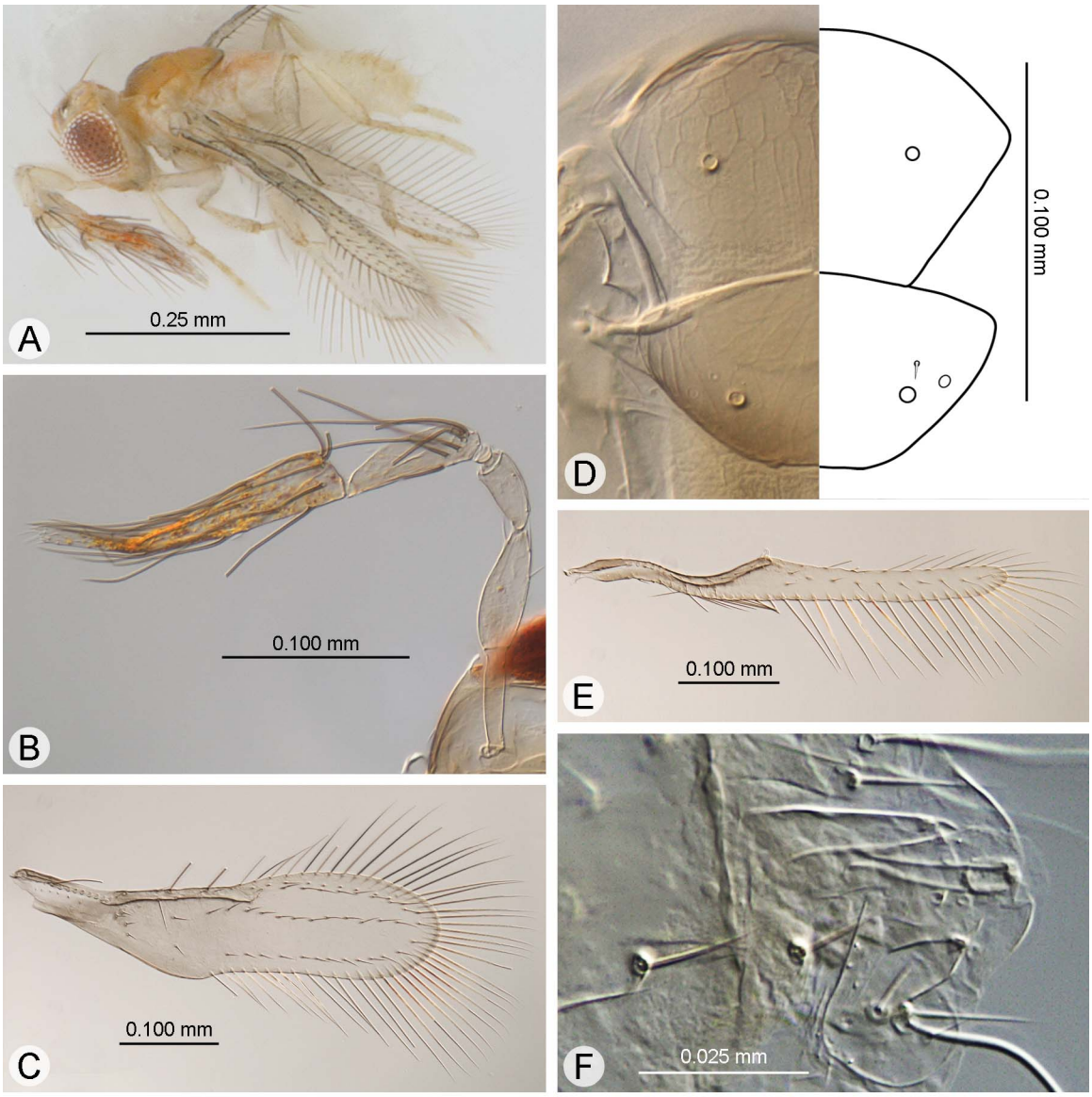


Figure 2.24. *Cales parvigladius* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus.

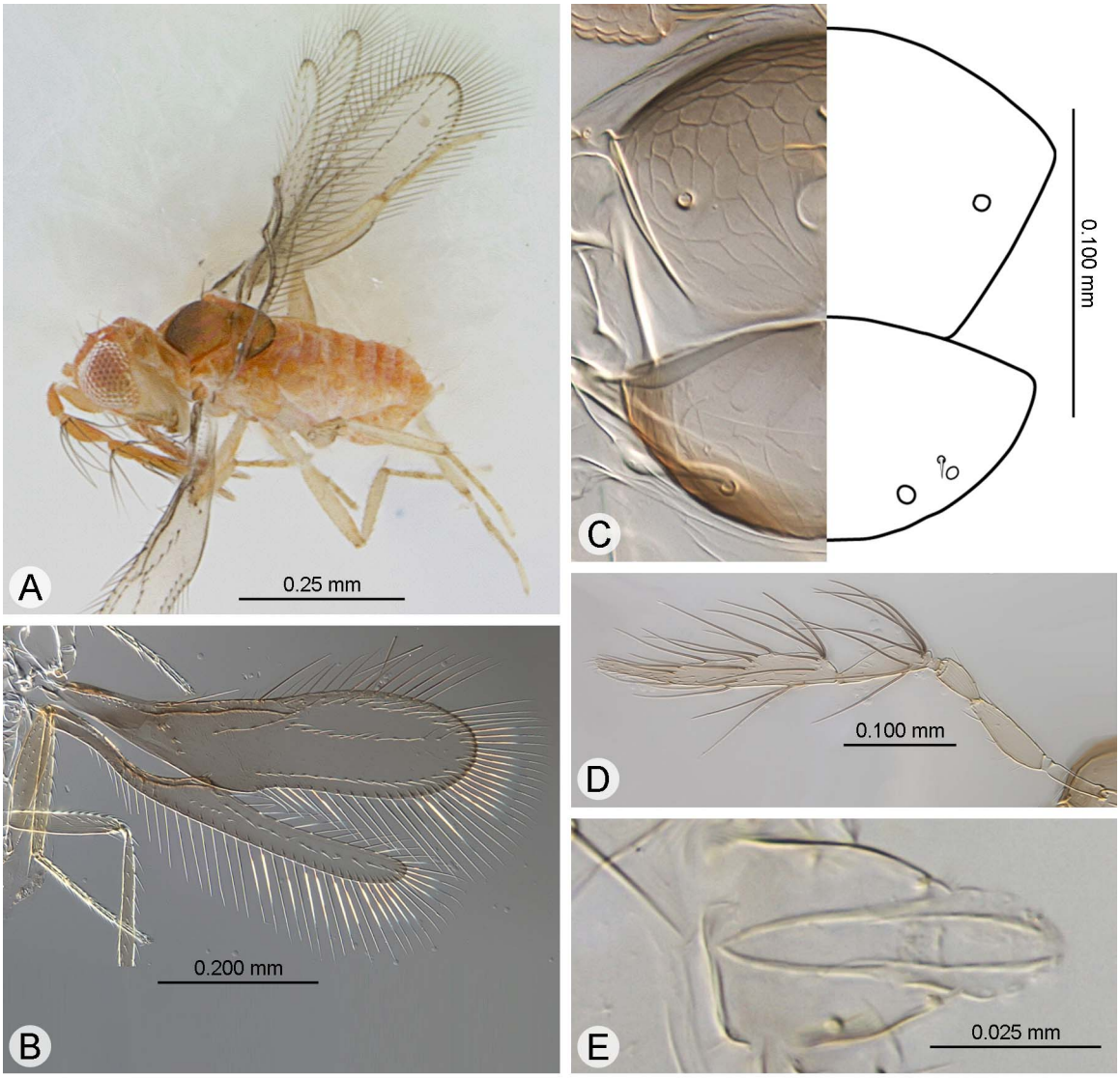


Figure 2.25. *Cales pellonotum* n. sp., male. **A,** lateral habitus; **B,** fore and hind wings; **C,** dorsal mesosoma; **D,** antenna; **E,** aedeagus.

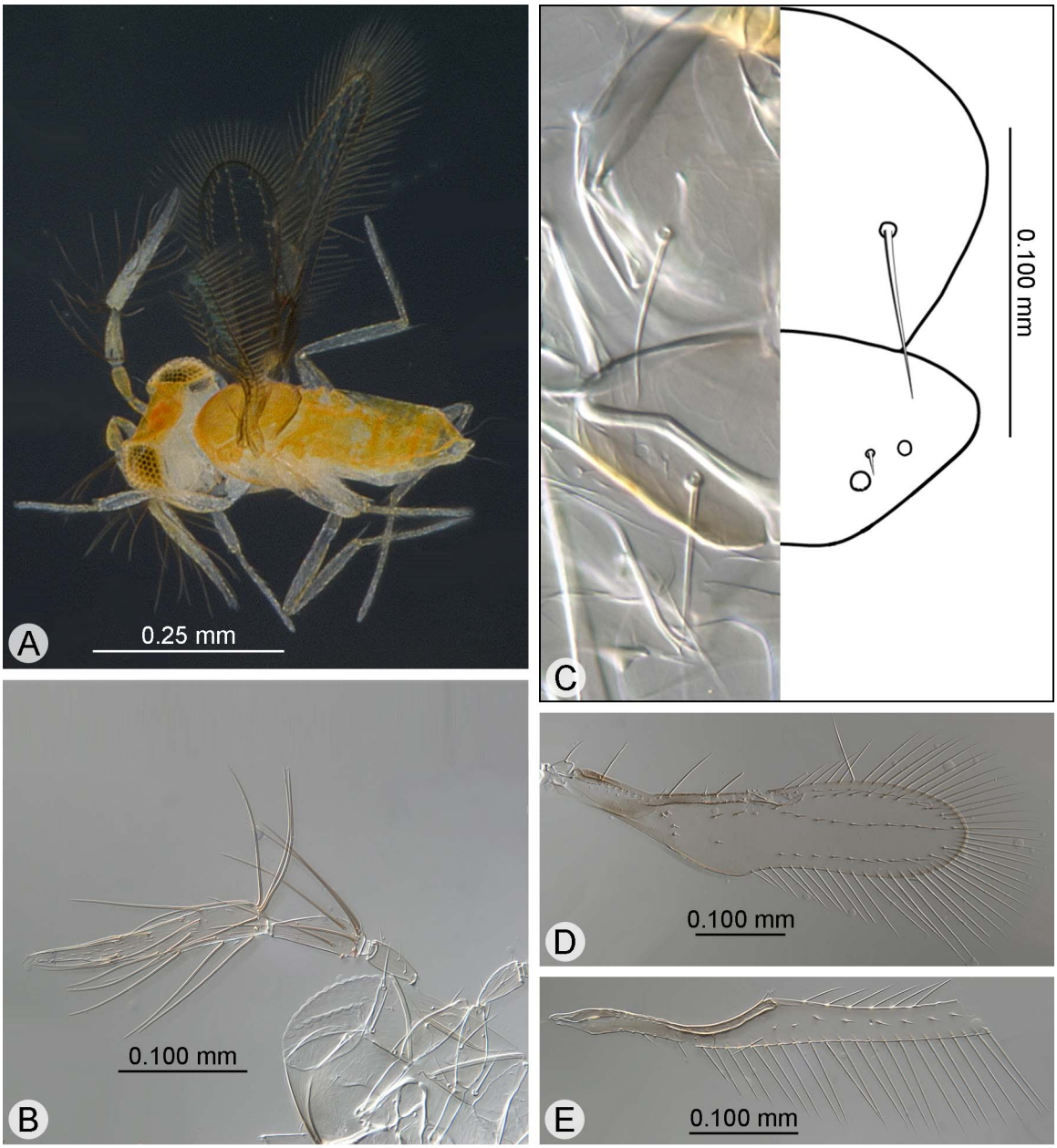


Figure 2.26. *Cales peruviana* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** dorsal mesosoma; **D,** fore wing; **E,** hind wing.

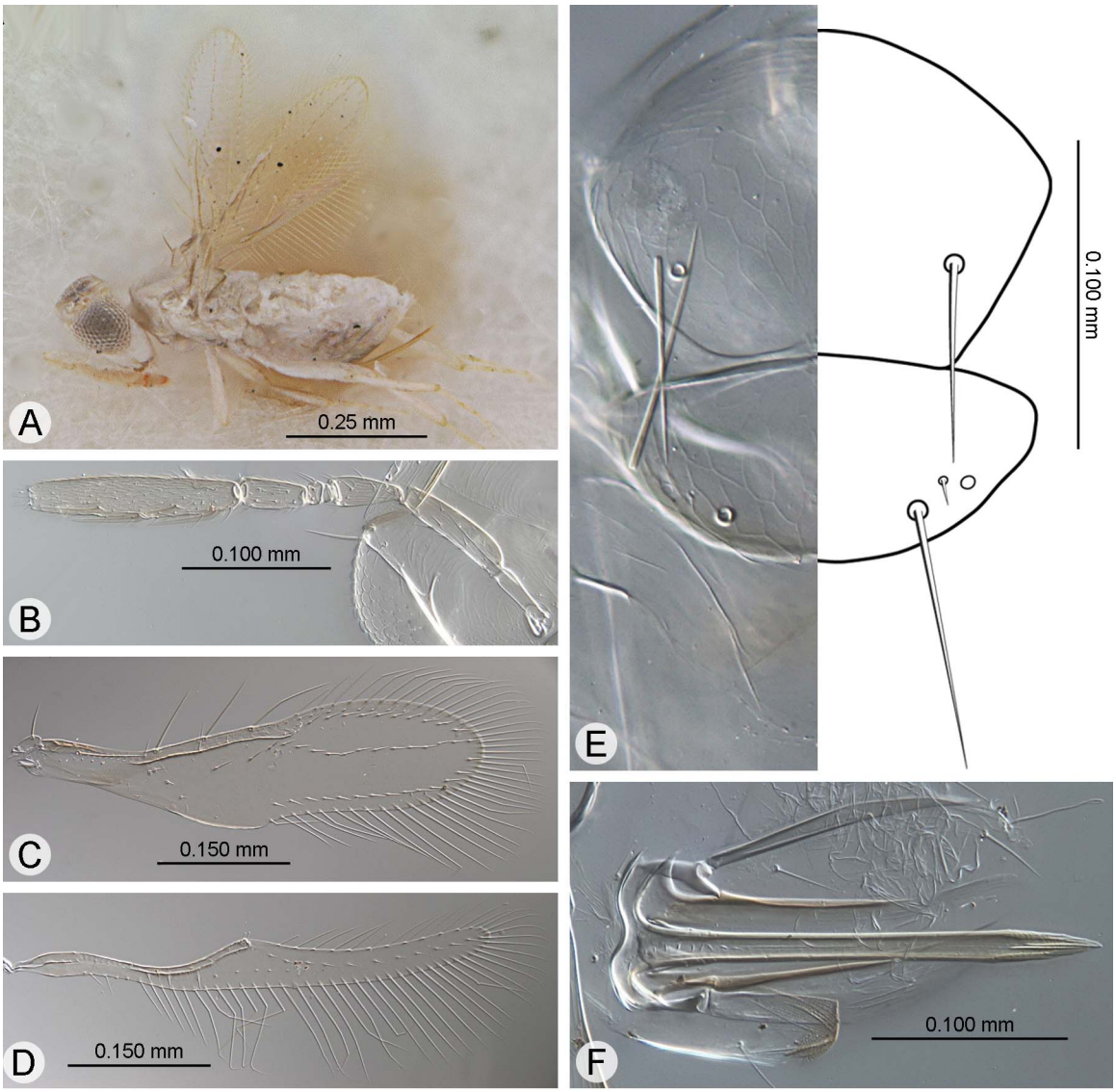


Figure 2.27. *Cales primapluvia* n. sp., female. **A,** lateral habitus; **B,** antenna (flagellum inverted); **C,** fore wing; **D,** hind wing; **E,** dorsal mesosoma; **F,** ovipositor.

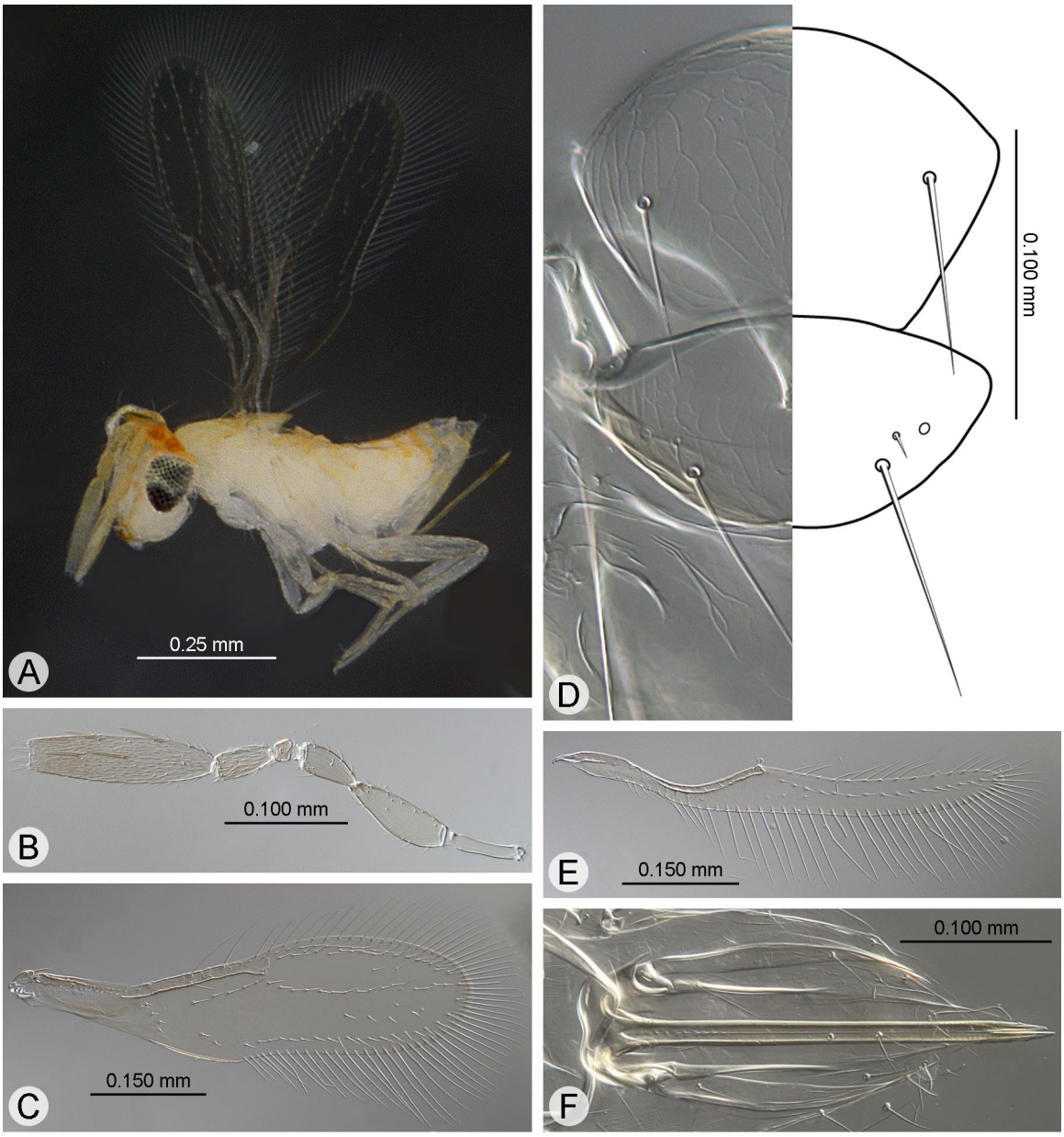


Figure 2.28. *Cales rosei* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

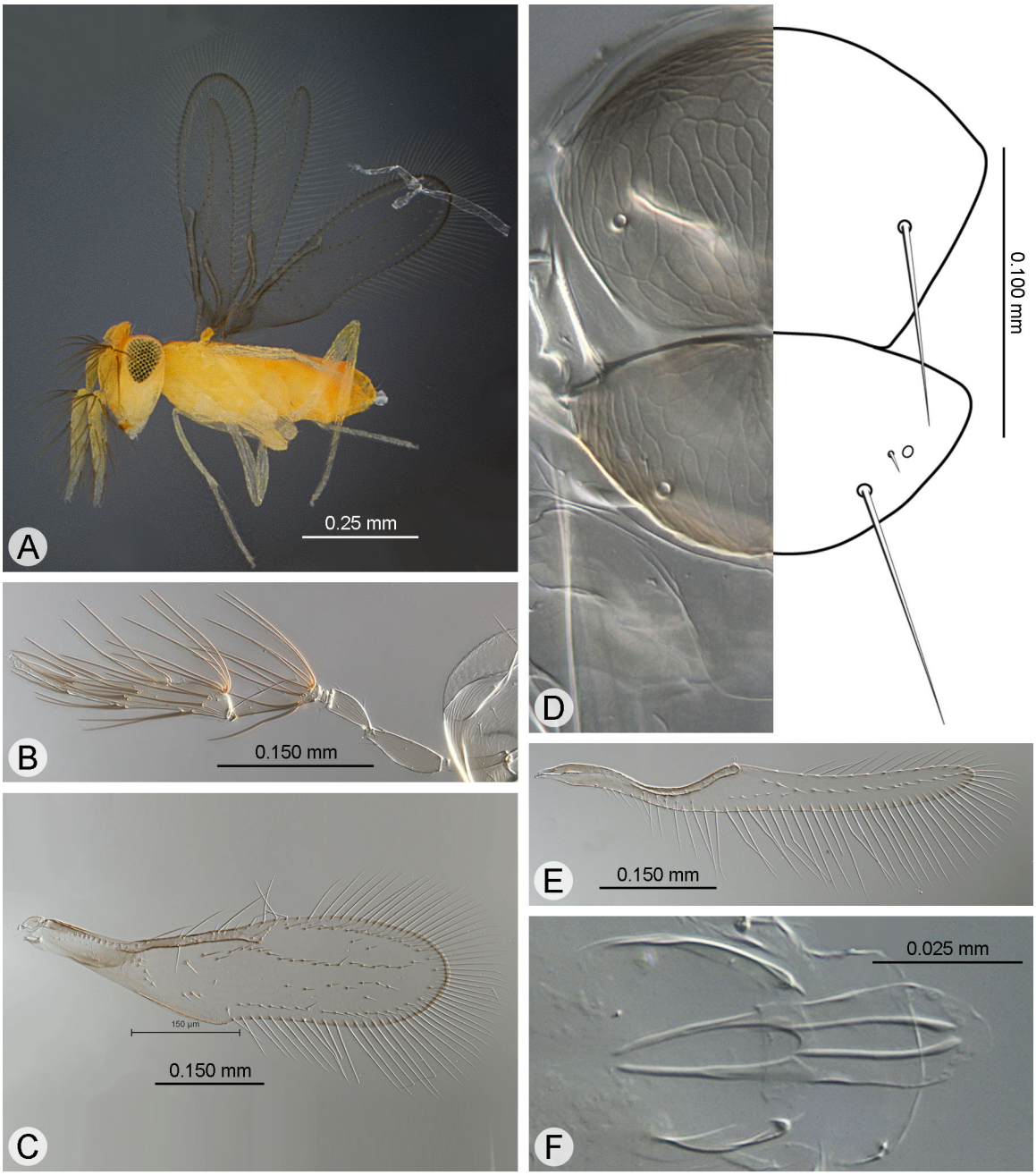


Figure 2.29. *Cales rosei* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** aedeagus.

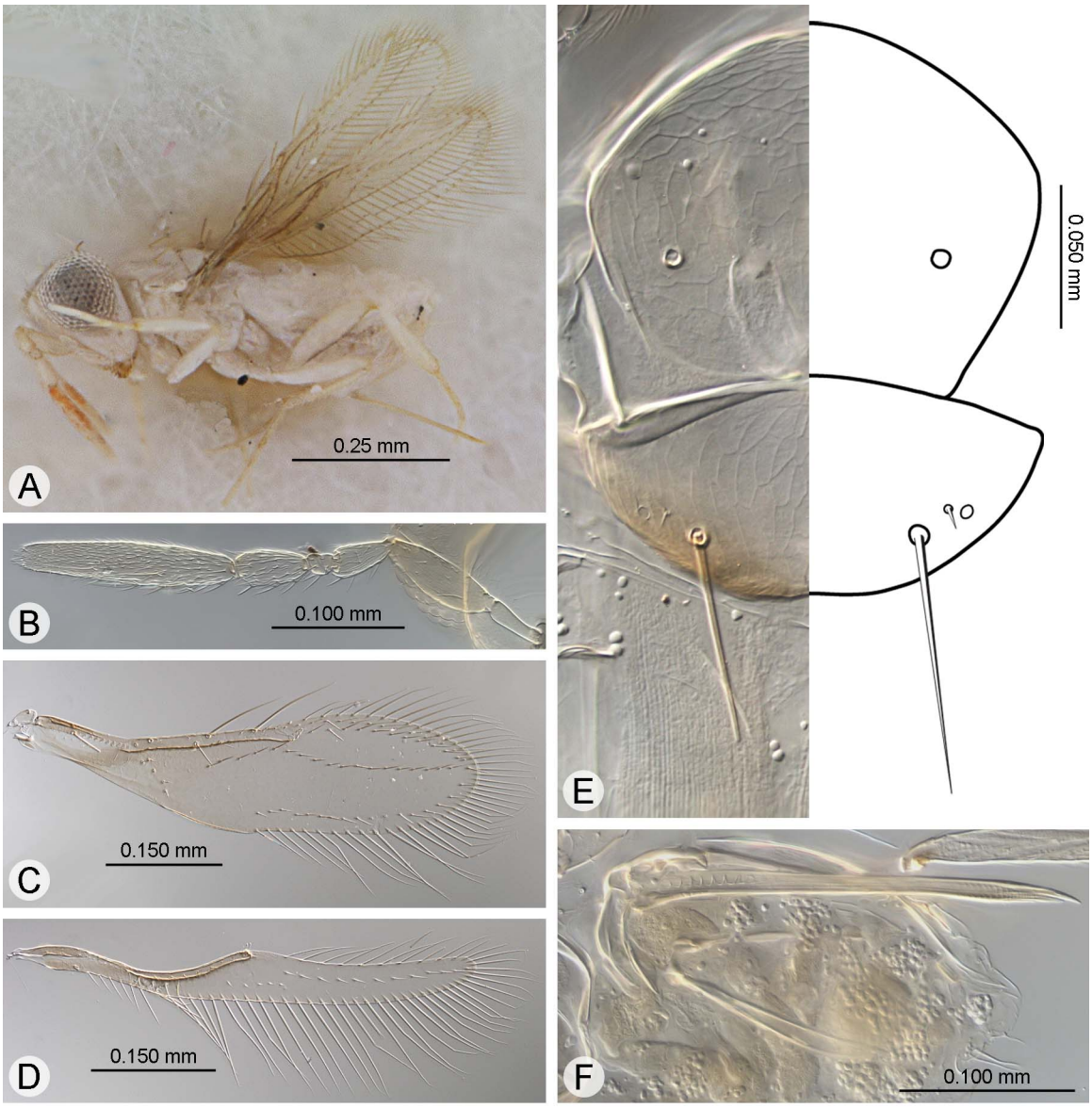


Figure 2.30. *Cales secundapluvia* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** hind wing; **E,** dorsal mesosoma; **F,** ovipositor, mounted obliquely.

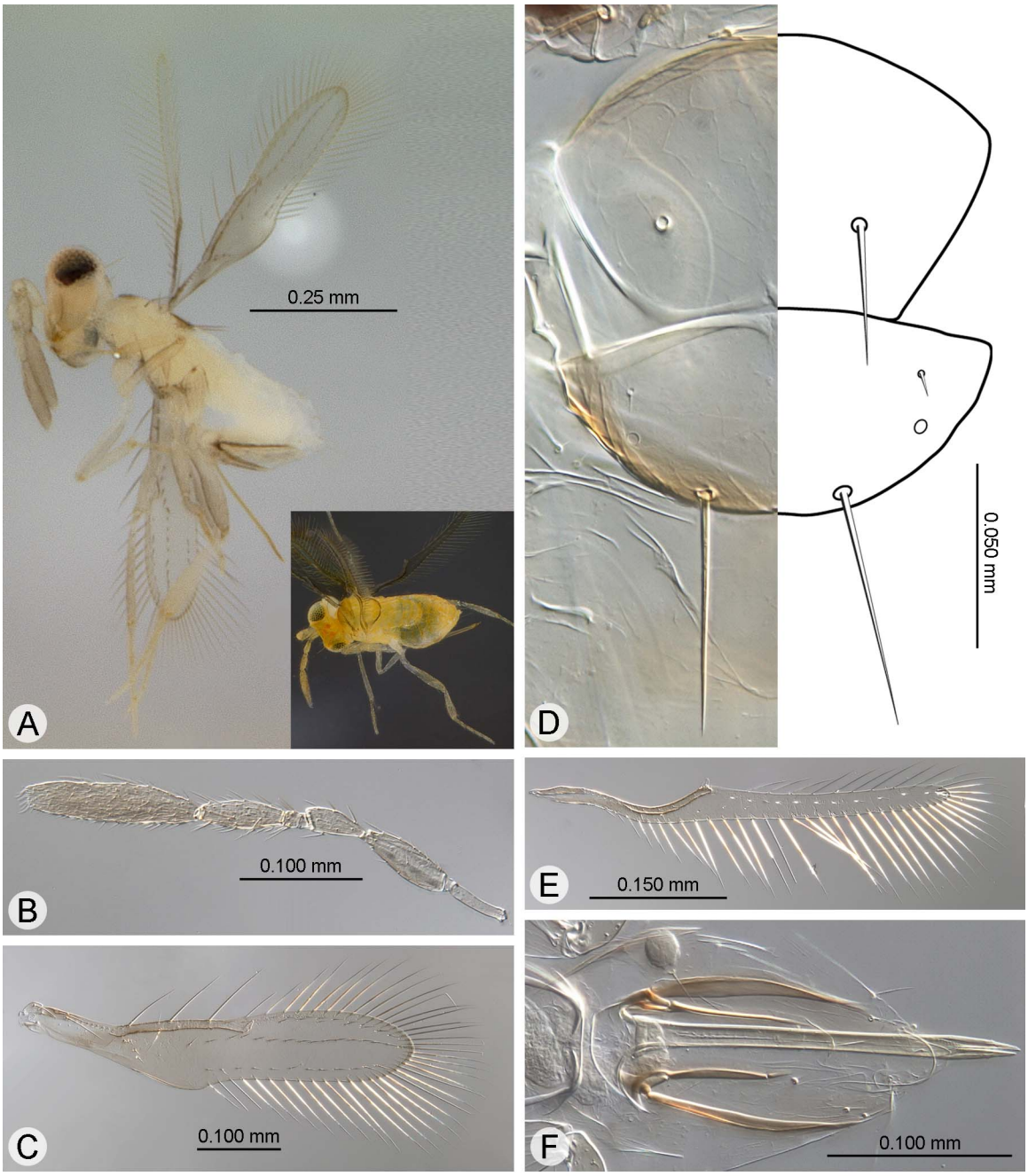


Figure 2.31. *Cales stenoptera* n. sp., female. **A,** lateral habitus, inset showing orange Peruvian specimen; **B,** antenna; **C,** fore wing; **D,** dorsal mesosoma; **E,** hind wing; **F,** ovipositor.

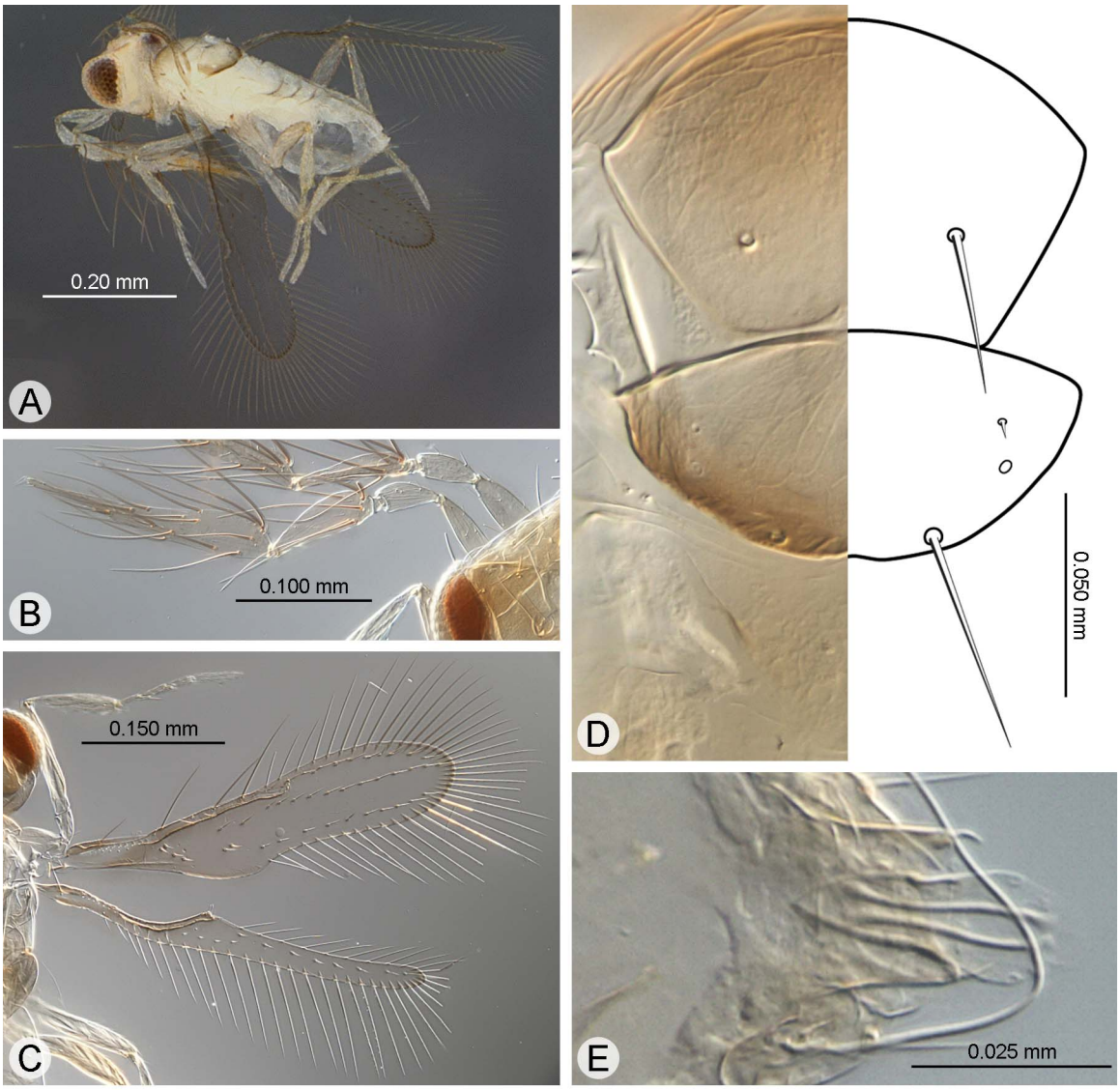


Figure 2.32. *Cales stenoptera* n. sp., male. **A,** lateral habitus; **B,** antenna; **C,** fore and hind wings; **D,** dorsal mesosoma; **E,** aedeagus.

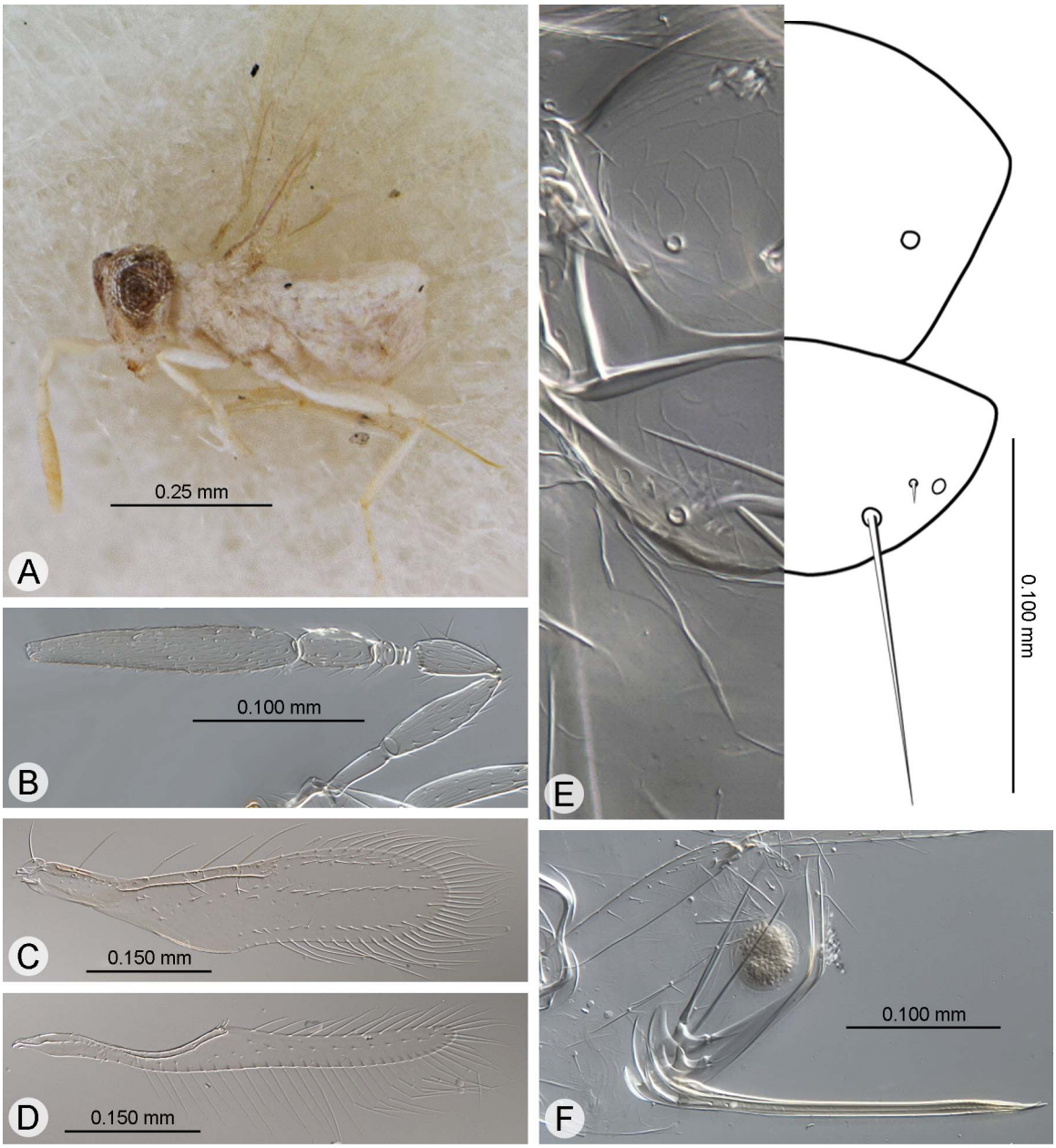


Figure 2.33. *Cales triensapluvia* n. sp., female. **A,** lateral habitus; **B,** antenna; **C,** fore wing; **D,** hind wing; **E,** dorsal mesosoma; **F,** ovipositor, mounted laterally.

CHAPTER 3: The dead can talk: museum specimens show the probable origin of a cryptic species used in biological control

ABSTRACT

The parasitic wasp species *Cales noacki* is an important biological control agent against woolly whitefly, *Aleurothrixus floccosus*, in citrus-growing regions worldwide. We recently discovered two cryptic species of *Cales* on citrus in California: *Cales noacki* and *Cales rosei*. Examination of historical biological control records is combined with a geometric morphometric analysis of fore wing shape to reconstruct aspects of the biological control history of *Cales*. Though it is well established that the *C. noacki* populations originated in Chile, the origin of *C. rosei* has remained unknown. Our analyses indicate that *C. rosei* is most likely descended from populations introduced from Argentina in the mid 1970s, with newly collected specimens from California clustering with Argentinian slide-mounted specimens from the original importation. Our analyses support a Chilean origin of *C. noacki*, the species broadly disseminated for use in biological control efforts. A potential third species was imported from Brazil and Tucumán, Argentina, although it appears not to have established in the field. The implications of these results for future studies on the bionomics of *Cales* and also the utility of geometric morphometric analyses for species identification and description are discussed.

INTRODUCTION

Correct species identification of both the pest and potential natural enemies is a critical early step in biological control programs (DeBach, 1974; Rosen, 1986). Successful biological control is not only dependent on clear delineation of species based on reproductive isolation (Mayr, 1942), but also the suite of behavioral and physiological traits that may vary among and within species (Rosen, 1986). This effort can be severely complicated by the presence of cryptic species in a biological control system. Cryptic species are two or more populations of organisms that are morphologically indistinguishable (or nearly so), but sufficiently reproductively isolated to merit classification as separate species (Brickford et al., 2007). A growing body of literature suggests that cryptic species complexes may be common among insect parasitoids, and may differ substantially in host preferences and other bionomic traits (Campbell et al., 1993; Kazmer et al., 1996; Heimpel et al., 1997; Fernando and Walter, 1997; Stouthamer et al., 1999, 2000; Kankare et al., 2005a,b; Smith et al., 2006, 2007, 2008; Heraty et al., 2007; Bernardo et al., 2008; Kathirithamby, 2009; Desneux et al., 2009; Heraty, 2009). Consequently, cryptic species may have important consequences for the efficacy and safety of biological control programs (DeBach, 1960; DeBach, 1969; Rosen, 1986; Clarke and Walter, 1995; Stouthamer et al., 2000; Pinto et al., 2003; Heraty, 2009).

The woolly whitefly, *Aleurothrixus floccosus* Maskell, is a significant pest in citrus growing regions throughout the world, including California (DeBach and Rose, 1976), the Mediterranean region (European and Mediterranean Plant Protection Organization, 2002), and parts of tropical Africa (Legg et al., 2003). Following the

discovery of the woolly whitefly in a residential area of San Diego in 1966 (Robinson, 1969), Paul DeBach and colleagues conducted surveys in Central and South America to find and import natural enemies (DeBach and Rose, 1976). Their initial efforts resulted in the introduction and release of four parasitic wasp species into California and Baja California: *Amitus spiniferus* (Bréthes) (Hymenoptera: Platygastridae) from Mexico, an unidentified *Encarsia* species (Hymenoptera: Aphelinidae) from El Salvador, *Eretmocerus paulistus* Hempel (Hymenoptera: Aphelinidae) from Mexico and *Cales noacki* Howard (Hymenoptera: Aphelinidae) from Chile. The *Cales, Amitus* and *Eretmocerus* species were successfully established, with *Amitus* and *Cales* becoming the dominant natural enemies of woolly whitefly in California citrus (DeBach and Rose, 1976; Rose and Woolley, 1984; Miklasiewicz and Walker, 1990). Shipments from California *Cales* colonies were subsequently sent to Spain, France and the Canary Islands between 1970 and 1972 (DeBach and Rosen 1976). Contemporaneously with the work of DeBach and Rose, biological control workers from France were conducting their own foreign explorations for parasitoids of woolly whitefly, successfully importing and rearing *Cales* from Chile (Onillon & Onillon, 1972; Onillon 1974).

Cales has historically been a taxonomically challenging genus, with its placement within Aphelinidae only recently affirmed by Heraty et al. (2012). Recent molecular and morphological studies have indicated that *Cales* is far more diverse than previously thought (Mottern and Heraty, in prep., b). Their revision increased the number of described Neotropical species from one (*C. noacki*) to 23, and focused on species that have the *C. noacki* phenotype (c.f. Mottern et al. 2011). These species are exclusively

Neotropical in distribution, with the exception of introduced populations in citrus-growing regions of the world. Molecular analyses indicated that at least two species of *Cales* were present in its biological control range: *C. noacki* and *Cales rosei* Mottern. While nearly indistinguishable morphologically, the two species have a distinctly different molecular signature, differing by 2.2–2.3% in 1109 bp of 28SD2–5 rDNA and 9.2–9.7% in 390 bp of cytochrome oxidase subunit I (COI) mtDNA (Mottern and Heraty, in prep., b). By contrast, there is very little within-species divergence (0.0–0.09% for 28SD2–5 and 0.0–3.3% for COI). This divergence in 28S between the two species is comparable to that expected between distantly related species (Campbell et al., 1993; Babcock et al., 2001), and exceeds the genetic divergence associated with reproductive incompatibility found in cryptic species complexes of chalcidoid parasitoids (Triapitsyn et al., 2006; Heraty et al., 2007).

The distribution of *C. rosei* was somewhat perplexing as its unique haplotype was only recovered from specimens collected in an unsprayed "biological control" citrus orchard at the University of California, Riverside (UCR-bc), where it exists in both temporal and spatial sympatry with *C. noacki* and attacks the same host. Though almost certainly introduced in the 1970s along with the more common *C. noacki* (a genetic match to more recently collected specimens from Chile and established populations in Italy), the native range of *C. rosei* remained unclear.

Here, we use two sources of data to determine the likely origin of *C. rosei*: 1) importation and release records from the Insectary and Quarantine facility at the University of California, Riverside (UCR-I&Q), and 2) geometric morphometric analysis

of fore wing shape in both slide-mounted specimens from the original importations, and recent collections of *Cales* specimens that have been determined based on molecular phylogenetic analysis. We discuss the relevance of these results for future studies on the ecology and systematics of *Cales* in both its native and biological control ranges, and also discuss the utility of geometric morphometric analyses for species description and identification.

MATERIALS AND METHODS

Biological Control records

Importations – In order to determine the timing and geographic origins of all *Cales* imported into the UCR-I&Q, we examined the UCR Sender's and Receiver's Reports from explorations for entomophagous insects. This process was facilitated by an electronic database maintained by the UCR Entomology Research Museum that could be queried for the taxon of interest. For records prior to 1971, which have not been entered in the database, we examined hard-copy files maintained at the UCR-I&Q. These records are somewhat variable in the data they contain, but generally include shipping and receiving dates, collector, country of origin, host identifications, natural enemy identifications, approximate number of natural enemy individuals, and a ship-receive (SR) number that remained associated with laboratory colonies derived from the original shipments.

Releases – Unfortunately, there is currently no electronic database of biological control releases originating from the UCR-I&Q. Therefore, we examined hard copies of the reports of natural enemy releases that were prepared two times per year and submitted

to the California State Department of Food and Agriculture, County Agricultural Commissioners, and other parties who may be interested in biological control programs in California. These reports include the dates of release, species of natural enemies, number of natural enemies released, country of origin, the release locality, and usually the SR number for the original source population. These records exist in two forms, the *Colonization of Beneficial Organisms* report that is submitted to state agencies, and the *Parasite/Predator Release Report* that contains greater detail regarding release dates, release localities, and the source populations.

Geometric morphometrics

We used geometric morphometric analyses of fore wing shape to examine *Cales* populations used for biological control of woolly whitefly. The use of fore wings is a natural choice for such analyses because they are relatively easy to slide-mount within a single plane of focus, minimizing error due to tilting within the mounting medium. Also, many specimens that were destructively sampled during DNA extraction could be included in the analysis because their wings were retained and mounted.

The analysis included a total of 158 specimens collected during the 1970's as part of a biological control program against the woolly whitefly in citrus (DeBach and Rose, 1976). Collection localities are Brazil (São Paulo), Argentina (Buenos Aires, Sáenz-Peña, and Tucumán), and Chile (La Cruz) (Table 3.1). These specimens are original material (reared from woolly whitefly collected from the field) from source populations used for subsequent *Cales* introductions into California and Baja California, Mexico. Though originally all identified as "*C. noacki*" their actual species composition was unknown

prior to this study because no sequenceable material exists to place them with *C. noacki* or *C. rosei* (or other undescribed species). Hence, these are the "unknowns" that we aim to identify based on comparison of wing shape with molecular voucher specimens of *C. noacki* and *C. rosei*. The female *Diaspidophilus pallidus* Brèthes syntype is included to determine if the subsequent synonymization of this species with *C. noacki* is supported by wing morphometric analysis. The *C. noacki* holotype (U.S. National Museum type no. 10309; described from a single female collected in Campinas, Brazil) has been lost (M. Gates, Pers. Comm.). Therefore, our *C. noacki* concept is based on the neotype designation of Mottern and Heraty (in prep).

Molecular vouchers included a total of 42 specimens identified as either *C. noacki* or *C. rosei* based on molecular phylogenetic analyses of ribosomal and mitochondrial DNA (Mottern and Heraty, in prep., b; also see Table 3.1). Specimens include *C. noacki* collected in 2010 from Oasis de Pica, Chile; *C. rosei* and *C. noacki* from UCR-bc, and *C. noacki* from Campania, Italy. The specimens from UCR-bc are presumably descended from introductions into California by DeBach and Rose in the 1970's. The specimens from Italy are either descended from populations sent to Europe from California or material sent directly from Chile by European biological control workers.

Preliminary morphometric analyses indicated that males and females differed in wing shape, at least for some species (data not shown), so males and females were analyzed separately. Five landmarks around the periphery of the fore wing were chosen to capture overall wing shape, plus two landmarks to set the scale (Fig. 3.4). These landmarks were chosen because they are easy to locate, even on slide mounts with

darkened mounting medium or artifacts, and are sufficient to capture the overall shape of the wing. One wing from each specimen was photographed using a Zeiss Axioskop 2 mounted with a 1.4 megapixel CCD camera (model# LW1165C, Lumenera Corp., Ottawa, Ontario, Canada). Individual images were captured and stacked using Archimed software v5.4.1 (Microvision Instruments, Évry, France). No wing is precisely within a single plane of focus, so some judgment is required to determine if a given wing has been mounted flat enough for inclusion in the analysis. Generally, if more than 4 photos were required to capture a focused wing outline (excluding setae) at 20X magnification, the wing was excluded.

Landmarks were plotted on each image using the program tpsDig v.2.16 (Rohlf, 2010). The resulting coordinates were then subjected to Procrustes transformation using the program CoordGen6f (Sheets, 2002) to exclude size and rotational effects. These rescaled data were then used to calculate shape variables (partial warp scores) that were subjected to ordination analyses (Zelditch et. al, 2004). Principal components analyses (PCA) and Canonical variates analyses (CVA) were conducted using PCAGen6N and CVAGen6j (Sheets, 2002), respectively. Tests for distinct eigenvalues between successive PCs were conducted using the method of Anderson (1958). To test the significance of the differences between groups, multivariate analyses of variance (MANOVA) of the partial Procrustes distances were conducted using Goodall's F, as implemented in the program TwoGroup6h (Sheets, 2002).

RESULTS

Biological control records

Importations – The contents of UCR-I&Q records regarding the importation of *Cales* are summarized in Table 3.2. Importations all occurred between March 1970 and May 1976. Collection localities included at least one site in La Cruz, Chile, at least three sites in Argentina, one site in Lima, Peru, and at least one site in São Paulo Brazil (Fig. 3.1). Two of the Argentinian sites (Buenos Aires and Sáenz-Peña) are close together (within 20 km of each other), as Sáenz-Peña borders Buenos Aires proper. The third Argentinian locality in Tucumán is separated from the other Argentinian localities by over 1000 km. All importations list *A. floccosus* as the host insect and varieties of citrus as the host plants, with at least some collections from "street trees" in urban areas. At least seven of the 17 shipments successfully founded laboratory colonies and served as source populations for biological control releases.

Releases – Recorded biological control releases of *Cales* in California and Baja California are summarized in Table 3.3. Releases all occurred between April 1970 and December 1982. Notably, less than two weeks elapsed between the first importation of *Cales* into the United States on March 30, 1970 and the first release into the field by Paul DeBach in Tijuana on April 8, 1970. Recorded releases occurred in San Diego, Orange, Los Angeles, Santa Barbara, and Ventura counties in Southern California and also Tijuana and Ensenada in Baja California, Mexico.

Wing geometric morphometrics

Principal component analyses resulted in one distinct eigenvalue for males (χ^2=37.30, df=2, p<0.0001) and one distinct eigenvalue for females (χ^2=33.54, df=2, p<0.0001), indicating that only one axis (PC1) explains a greater percentage of the variance than expected by chance in each analysis. For both males (Fig. 3.2) and females (Fig. 3.3), specimens identified as *C. rosei* using molecular data cluster with specimens from the Argentinian biological control importations, suggesting that the *C. rosei* present in Riverside (UCR) are descended from Argentinian collections from Buenos Aires/Sáenz-Peña (Figs 3.2 and 3.3, blue circles). Similarly, specimens from the biological control range identified as *C. noacki* using molecular data cluster with the biological control importations from Chile (Figs 3.2 and 3.3, solid red circles). The Chilean origin of *C. noacki* is further supported by the inclusion of two males and two females from Chile identified as *C. noacki* based on molecular data (Figs 3.2 and 3.3, gray circles with white dots). Despite originating from Pica, which is over 1300 km north of La Cruz (Fig. 3.1), these specimens cluster with the other *C. noacki* specimens, though sample size is obviously small. Specimens from Italy (Fig. 3.3, black circles with white dots) cluster more closely other *C. noacki* specimens, though the sample size is small, and they appear to be intermediate between *C. rosei* and *C. noacki*. Specimens from Brazil (Figs 3.2 and 3.3, green circles) and Tucumán, Argentina (Figs 3.2 and 3.3, orange circles) were also included in the analyses. Qualitative examination of the PCA plots suggests that these two populations cluster together, and some separation exists between them and *C. rosei* along PC1 and from *C. noacki* along PC2, especially in males (Fig.

3.2). However the eigenvalue for PC2 was not significant for males (χ^2 =0.11, df=2, p=0.16) or females (χ^2=3.35, df=2, p=0.19), so PCA offers no compelling evidence that the Brazilian and Tucumán specimens are distinct from *C. noacki*. In the PCA and all subsequent analyses, the *C. noacki* (=*Diaspidophilus pallidus*) female syntype groups with the other *C. noacki* specimens (Fig. 3.4), indicating that, based on the shape of the forewing, the synonymy between *D. pallidus* and *C. noacki* is valid.

Plots of the relative positions of the mean landmark coordinates and their vectors from the PCA analyses (Fig. 3.4) are instructive for examining which landmarks contribute most to the variation in wing shape. The vectors with the greatest magnitude are associated with the base of the wing, the base of the fourth marginal seta of the anterior edge of the wing, and the apex of the wing. Of these, the fourth marginal seta exhibits the most variation, suggesting that in some specimens the anterior start of the fore wing disc is shifted toward the base of the wing (see dashed line, Fig. 3.5 for relative change in position of the anterior fourth marginal seta).

To determine if we could develop a method of discriminating among groups based on one or two linear measurements rather than a multivariate analysis, we generated Hubbs-Hubbs plots based on overall wing length (Fig. 3.6A) and the ratio of wing length to the distance to the fourth anterior marginal seta (Fig. 3.6B). Though the ratio method is superior for distinguishing between *C. rosei* and *C. noacki* plus the Brazilian and Tucumán populations, the within-group variation is too high to identify individual specimens based on wing measurements.

We next used CVA to determine if wing shape data could be used to discriminate among the three main *Cales* groups (*C. noacki*, *C. rosei*, and Brazil+Tucumán). As in PCA, CVA results were similar for males (Fig. 3.7) and females (Fig. 3.8). The CVA for males had two distinct canonical variates (CV1, Λ=0.1062, χ^2=225.35, df=12, p<0.0001; CV2, Λ=0.5129, χ^2=67.10, df=5, p<0.0001), as did the CVA for females (CV1, Λ=0.1627, χ^2=160.69, df=12, p<0.0001; CV2, Λ=0.5660, 50.37, df=5, p<0.0001), indicating that all three groups could be distinguished based on fore wing shape.

Though CVA can statistically determine that some groups differ from others, it cannot determine which particular groups are different. Therefore, we employed MANOVA to test for differences between pairs of groups. Significant distances between means existed among each of the three groups for both males and females (Table 3.4). Our results support *C. noacki* and *C. rosei* as morphologically distinct species, and also suggest that the Brazil+Tucumán specimens may represent a third undescribed species.

CONCLUSIONS

To our knowledge, the only published mention of the source populations of *Cales* used for biological control in California citrus is DeBach and Rose (1976), in which they list only Chile as the origin. The lack of any follow-up publication regarding their rearing and release program, combined with the fact that many additional importations and releases were made after this DeBach and Rose publication, has resulted in the mistaken "conventional wisdom" that the species of *Cales* used for biological control in California originated in Chile. In fact, the examination of records from the UCR-I&Q reveals that *Cales* (all identified as *C. noacki* at the time) were imported from Chile, Argentina, Peru,

and Brazil, and that these source populations all resulted in successful lab colonies that ultimately contributed to field releases.

Quarantine records did not indicate any releases of *Cales* spp. into the biological control groves at UC Riverside or in other parts of Riverside County, although records of other natural enemy releases into these localities are present. Therefore, the presence of *Cales* spp. in Riverside is either due to range expansion from neighboring counties where the wasps were released, or unreported releases. Unreported releases could have occurred if populations were consigned to other researchers in the UCR Department of Entomology and subsequently released or sent to other institutions. Releases made from colonies established from such consignments would not be listed UCR-I&Q records (S. Triapitsyn, pers. comm.). Similarly, shipments to Europe, though reported in DeBach and Rosen (1976), were apparently not reported in UCR-I&Q records.

The biological control history of *Cales* represents a case where a combination of molecular analyses, morphology, and biogeography are essential, not just theoretically desirable, for species identification and delimitation. The combination of thorough record keeping on the part of UCR-I&Q, the existence of good-quality slide mounted specimens from the original importations, a recent molecular phylogenetic analysis of *Cales*, and a geometric morphometric analysis of fore wings allows us to explore in more detail the biological control history of *Cales*. The mysterious population of *C. rosei*, which has thus far only been collected from the biological control grove at UCR Riverside, is most likely descended from collections made in the Buenos Aires/Sáenz-Peña area of Argentina by Rose in 1976. This analysis allows us to identify Rose's original slide-mounted material

as *C. rosei*, despite a lack of genetic data for these specimens. Though we are confident with this identification, it should be noted that for the sake of this analysis, we assumed that all of the specimens from the Buenos Aires/Sáenz-Peña area are the same species. Given the apparent ubiquity of cryptic species among parasitic Hymenoptera and the fact that specimens were collected from multiple localities, it is possible that the Argentinian specimens themselves are composed of multiple species.

Our analyses also provide support for a Chilean origin of *C. noacki* (as defined by Mottern and Heraty, in prep., b). We can now identify specimens collected by Rose and DeBach from the La Cruz area of Chile in 1970 as *C. noacki*. It remains unclear if populations from Brazil, Peru and Tucumán, Argentina represent other distinct species, and it is unknown if any of these were successfully established in California.

Cales rosei and *C. noacki* are probably one of the few sibling species pairs that have been demonstrated to exist in both temporal and spatial sympatry. Not only are they sympatric, but they are also each bisexual and attack the same host. It is unclear what mechanisms the two species are using for partitioning of host resources, or if there is any niche partitioning at all. We have found no evidence of *Wolbachia* that might suggest symbiont-mediated reproductive incompatibility. However, we have observed recently-emerged males engaged in a peculiar behavior where they "puff" out the elongate sensilla of their antenna when approaching females, suggesting chemical recognition of species. Future studies on the bionomics of *Cales* will be complicated by the presence of this cryptic species complex. Iso-female lines of each species (or genetic sequencing of each

specimen used in the study) will be necessary in order to establish treatments of known species composition.

Geometric morphometric analysis is a powerful tool, especially when combined with the standard set of morphological and molecular techniques available to systematists. Shape variation is often subtle and, even if perceptible, difficult to articulate in a clear and meaningful way. Multivariate analyses of shape reduce the many covarying components that form a complex biological shape into only those components that explain the most variation. By combing these analyses with molecular data, we have been able to resurrect the scientific value of unsequenceable museum specimens that given our new taxonomic hypothesis could not even be identified to species.

There are some important considerations when embarking on a geometric morphometric analysis for species identification and description. First, a large number of specimens are needed for a meaningful analysis. This study underscores the importance of keeping and properly curating a reasonably large sample of voucher specimens. If DeBach, Rose and their colleagues had not kept and slide-mounted a portion of the original material from their importations, there would have been no specimens to compare against the newly collected specimens from the biological control range. Second, landmark coordinates are not individually interpretable after a Procrustes fit, so it is not possible to generate a set of coordinates for an "unknown" wing and determine where it clusters with a "training set" of known wings. However, shape analyses have tremendous potential utility for character discovery and description when dealing with cryptic or nearly cryptic species. Individual landmarks can be assigned a direction and

magnitude of change as the scores along a given ordinal axis increase. This can draw attention to the areas of the structure that are changing the most, and help refine the search for characters that can be described with simple measurements or qualitative statements. Combined with molecular analyses, these techniques may render the ubiquitous and troublesome cryptic species much less cryptic.

Table 3.1. *Cales* specimens used for geometric morphometric analysis of fore wing shape. Specimens from source populations marked with "*" have been determined to species based on 18S, 28S D2-5 rDNA and partial COI (Mottern and Heraty, in prep., b)

Source	Ship-Receive no. (S-R)	Species	No. ♂	No. ♀
Brazil: São Paulo State: São Paulo	76-33	*Cales* sp.	21	17
Argentina: *Cales noacki* (=*Diaspidophilus pallidus*), ♀ syntype	n/a	*C. noacki*	0	1
Argentina: Buenos Aires Prov.: Sáenz-Peña	76-12-2, 76-14, 18, 19	*C. rosei*	33	23
Argentina: Tucumán Prov.: Tucumán	76-26A, 27A, 29A, 31, 32A	*Cales* sp.	13	13
Chile: Región de Valparaíso: de Quillota Prov.: La Cruz	70-19, 27	*C. noacki*	21	16
***Chile:** Región de Tarapacá: Oasis de Pica	n/a	*C. noacki*	2	2
***Italy:** Campania: Portici	n/a	*C. noacki*	0	10
***U.S.A.:** CA: UC Riverside Biological Control Grove	n/a	*C. noacki* and *C. rosei*	16	12

Table 3.2. Importations of *Cales* spp. from South America to the UCR-I&Q. Importations with ship-received numbers marked with "*****" were either never released, or release records from these populations did not include ship-receive numbers. Importations with bolded ship-receive numbers are source populations for specimens used in the morphometric wing analyses.

Importation date	Collection locality	No. received	Quarantine S-R No.
30-Mar-1970	La Cruz, Chile	630	**70-19**
22-Apr-1970	La Cruz, Chile	357	**70-27***
8-Sep-1971	Chile	1296	71-61B*
28-Jun-1972	La Cruz, Chile	704	72-29A*
1-Oct-1974	Lima, Peru	3	74-84B
29-Apr-1976	Buenos Aires, Argentina	987	76-12-01A, **02A**
29-Apr-1976	Buenos Aires, Argentina	35	76-13A (all dead on arrival)
29-Apr-1976	Sáenz-Peña, Argentina	195	**76-14A**
11-May-1976	Sáenz-Peña, Argentina	636	**76-18A**
11-May-1976	Buenos Aires, Argentina	743	**76-19A**
13-May-1976	Tucuman, Argentina	235	**76-25*, 26A*, 27A*, 29A, 31*, 32A**
15-May-1976	Sãu Paulo, Brazil	70	**76-33B**, 34B

Table 3.3. Summary of *Cales* releases in California and Baja California, Mexico. Release marked with "*" was listed as originating from colonies of Chilean wasps, but all associated ship-receive numbers referred to either Argentinian or Brazilian collections.

Release report period	No. released	Country of origin	Release site(s)
Jul-Dec, 1970	155	Chile	Tijuana, Baja California, Mexico
Jan-Jun, 1971	750	Chile	San Diego, San Diego Co., CA
Jul-Dec, 1971	350	Chile	San Diego, San Diego Co., CA
Jul-Dec, 1971	3	Chile	Tijuana, Baja California, Mexico
Jul-Dec, 1972	805	Chile	San Diego, San Diego Co., CA
Jan-Jun, 1974	252	Chile	Del Mar and Carlsbad, San Diego Co., CA
Jul-Dec, 1974	505	Chile	Pico Rivera, Los Angeles Co., CA
Jul-Dec, 1974	23,740	Chile	Solana Beach, Del Mar, El Camino Real and Carlsbad, San Diego, Co., CA
Jul-Dec, 1974	83	Peru	San Diego Co., CA
Jul-Dec, 1975	11,286	Chile	Pico Rivera, Compton, and Gardena, Los Angeles Co., CA
Jul-Dec, 1975	16,365	Chile	Santa Ana, Orange Co., CA
Jul-Dec, 1975	5,010	Chile	San Diego Co., CA
Jan-Jun, 1976	4,370	Argentina/Brazil	Santa Ana, Orange Co., CA*
Jan-Jun, 1976	215	Argentina	Pico Rivera and Whittier, Los Angeles Co., CA
Jul-Dec, 1976	10,940	Argentina/Chile	Pico Rivera and Whittier, Los Angeles Co., CA
Jul-Dec, 1976	11,415	Argentina	Orange Co., CA
Jul-Dec, 1977	2,480	Argentina	Los Angeles Co., CA
Jul-Dec, 1977	870	Argentina	Orange Co., CA
Jul-Dec, 1979	4,400	Chile	Santa Barbara Co., CA
Jul-Dec, 1981	55	Chile	San Diego Co., CA
Jul-Dec, 1981	620	Chile	Ventura Co., CA
Jul-Dec, 1982	10,800	Chile	Ventura Co., CA

Table 3.4. MANOVA table showing pairwise comparisons showing differences between groups based on the partial Procrustes differences (Goodall's F).

MALES	F	DFn, DFd	p (α = 0.05)
C. rosei vs. *C. noacki*	57.21	6, 420	< 0.0001
C. rosei vs. Brazil+Tucumán	57.94	6, 432	< 0.0001
C. noacki vs. Brazil+Tucumán	11.64	6, 384	< 0.0001
FEMALES			
C. rosei vs. *C. noacki*	30.44	6, 372	< 0.0001
C. rosei vs. Brazil+Tucumán	54.72	6, 336	< 0.0001
C. noacki vs. Brazil+Tucumán	11.35	6, 384	< 0.0001

Figure 3.1. Source locality map for all *Cales* imported into UCR-I&Q from South America, plus the recent collection from Oasis de Pica, Chile. Specimens from Oasis de Pica, La Cruz, Buenos Aires/Sáenz-Peña, Tucumán and São Paulo are included in the wing morphometric analyses.

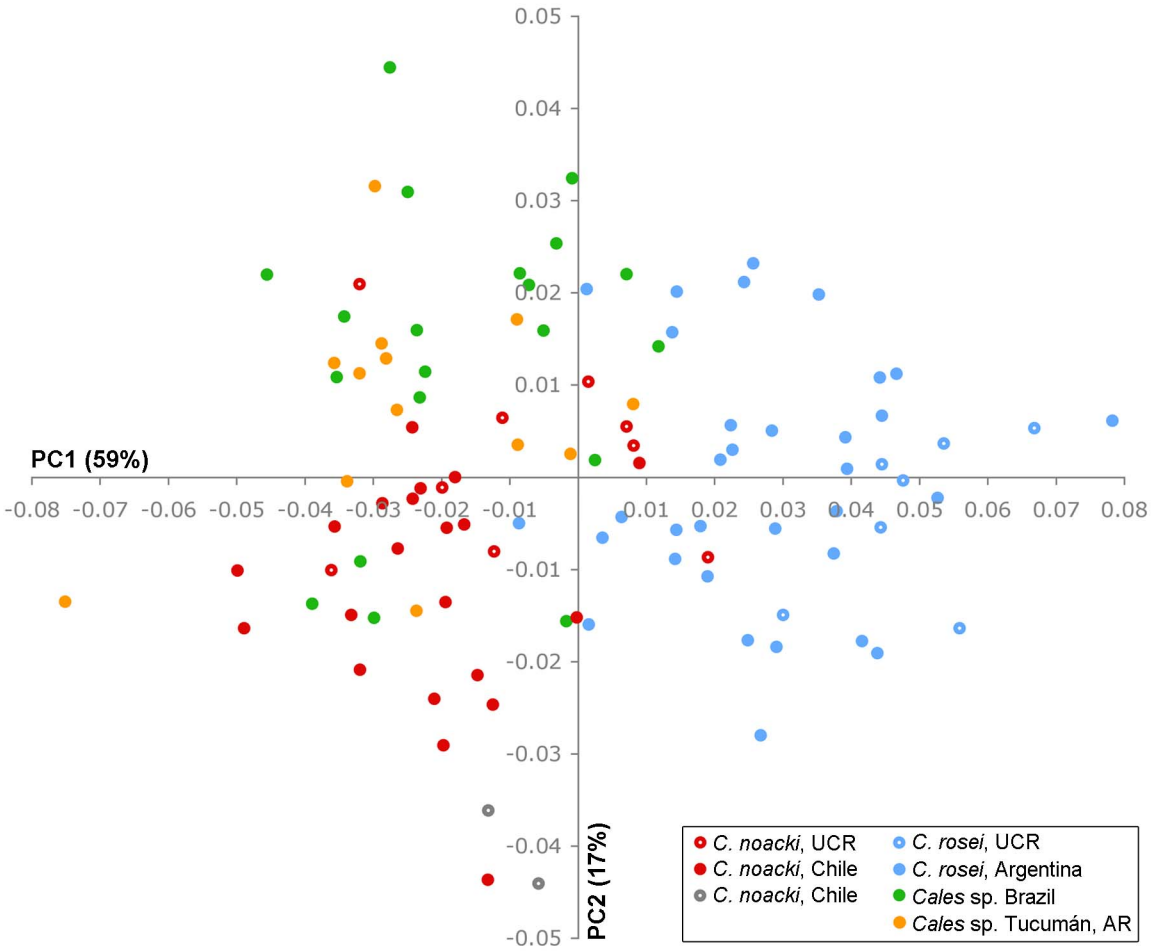


Figure 3.2. Principal component analysis of male wing shape. Numbers in parentheses following axis labels indicate the percent of variation explained by each component. The eigenvalue for PC1 is statistically distinct. A white dot in the center of the symbol indicates that the specimen has been determined to species based upon molecular data. Solid circles are slide-mounted specimens from historical biological control collections.

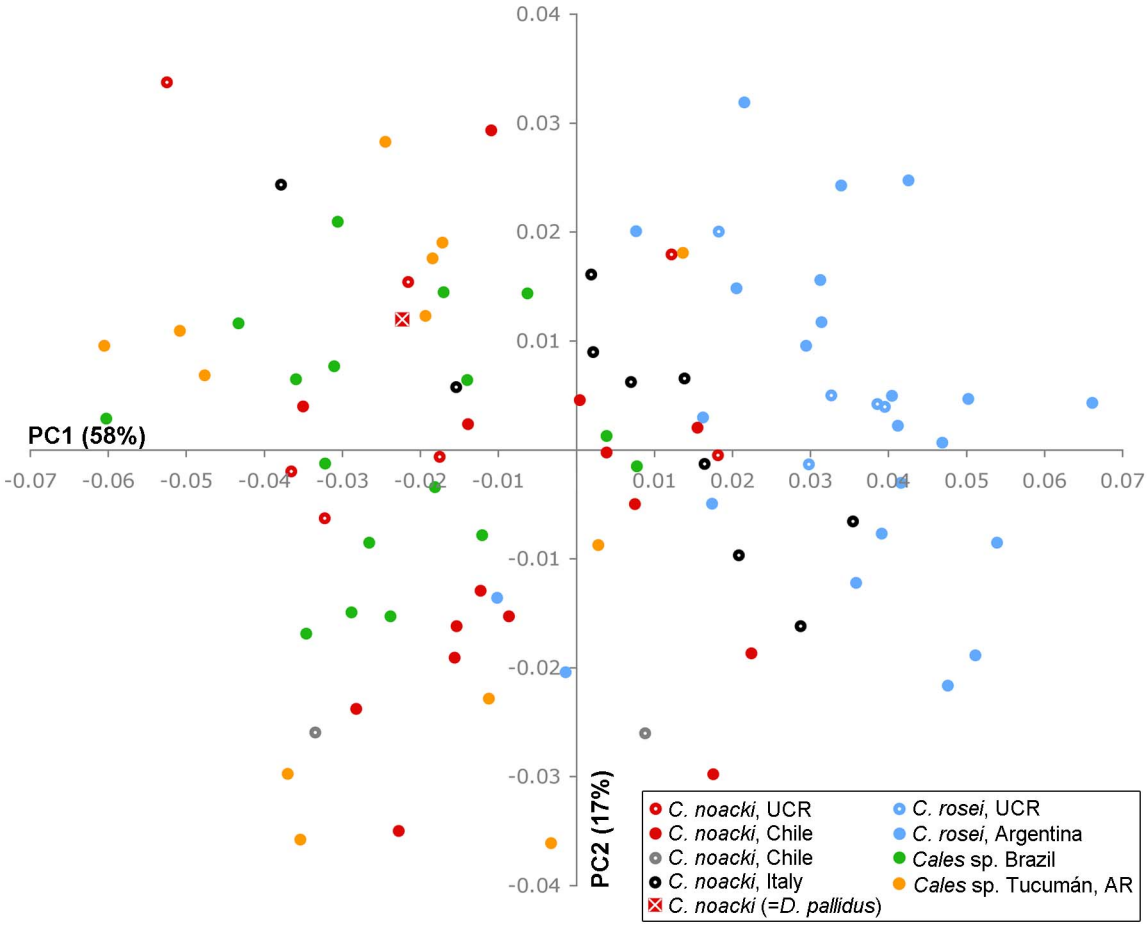


Figure 3.3. Principal component analysis of female wing shape. Numbers in parentheses following axis labels indicate the percent of variation explained by each component. The eigenvalue for PC1 is statistically distinct. A white dot in the center of the symbol indicates that the specimen has been determined to species based upon molecular data. Solid circles are slide-mounted specimens from historical biological control collections

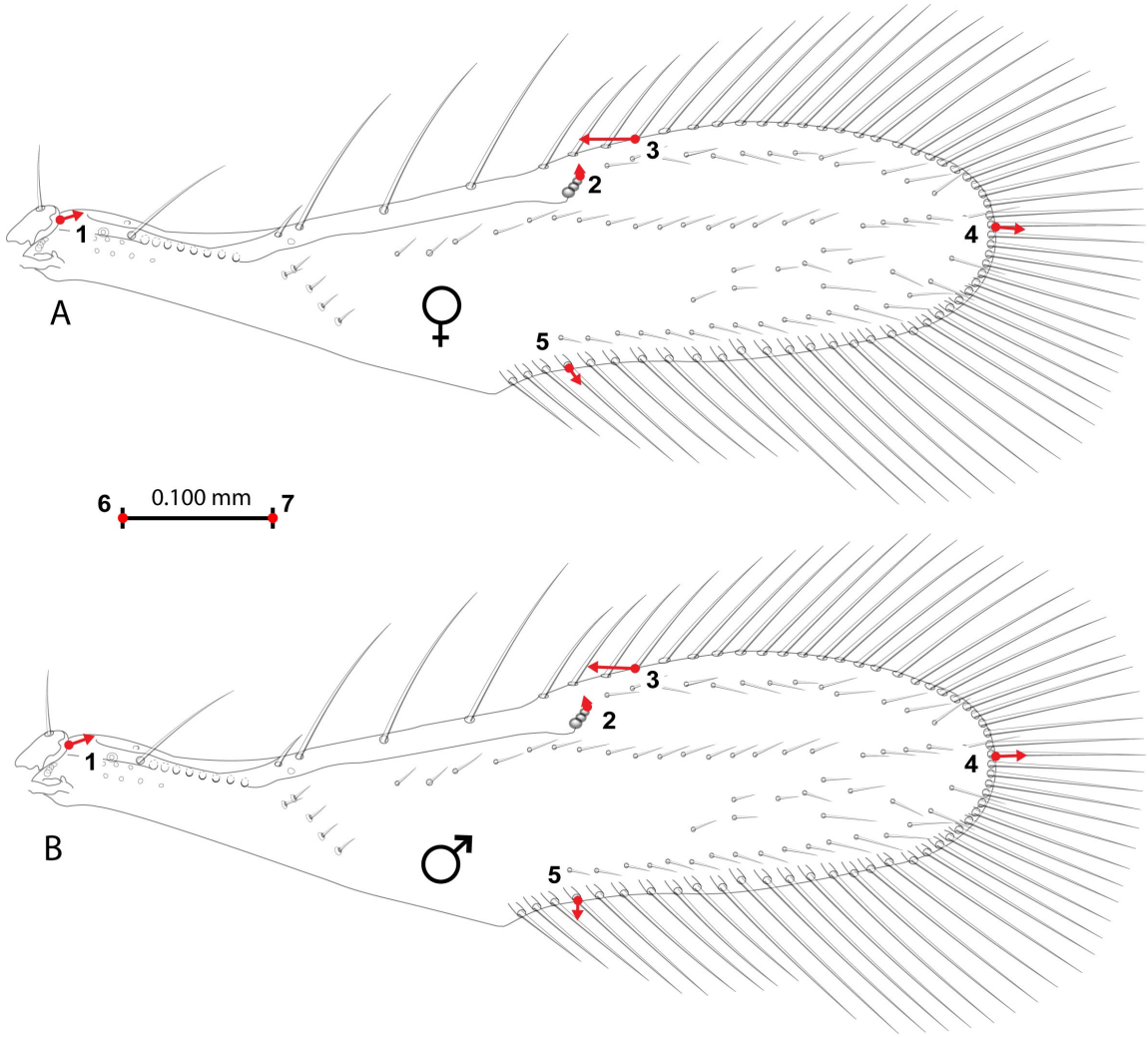


Figure 3.4. Generalized male and female wings showing landmarks used for analysis of wing shape. **Landmark 1** marks the proximal end of the submarginal vein. **Landmark 2** is the distal-most stigmal vein sensillum. **Landmark 3** marks the base of fourth marginal seta of the anterior edge of the fore wing disc. **Landmark 4** marks the apex of the wing. **Landmark 5** marks the fourth marginal seta of the posterior edge of the fore wing disc. **Landmarks 6 and 7** set the scale used during the Proscrustes transformation to remove size effects from the images. Red dots are the relative locations of the mean landmark coordinates based on PCA analysis of fore wing shape. Arrows indicate the relative change in position of the landmarks as the score for PC1 increases.

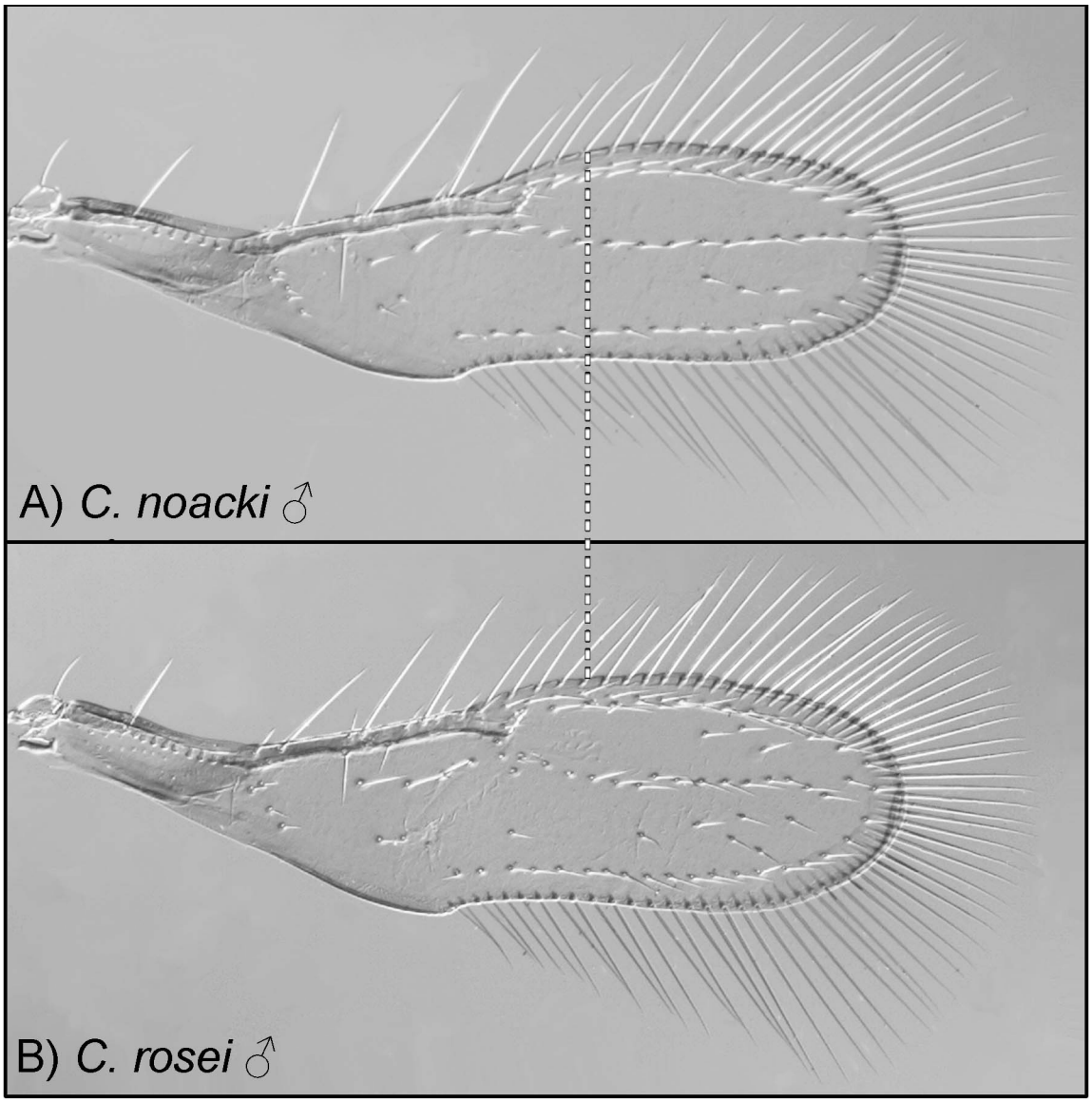


Figure 3.5. Fore wings of A) *C. noacki* male and B) *C. rosei* male. Wings have been rescaled so that they are the same length. Dotted line indicates position of the fourth marginal seta in *C. noacki*, and the relative shift of the seta toward the base of the wing in *C. rosei*. Apparent differences in setal characteristics such as the length of the marginal fringe relative to the wing length and the number of setae on the disc are variable within (Mottern and Heraty unpub.).

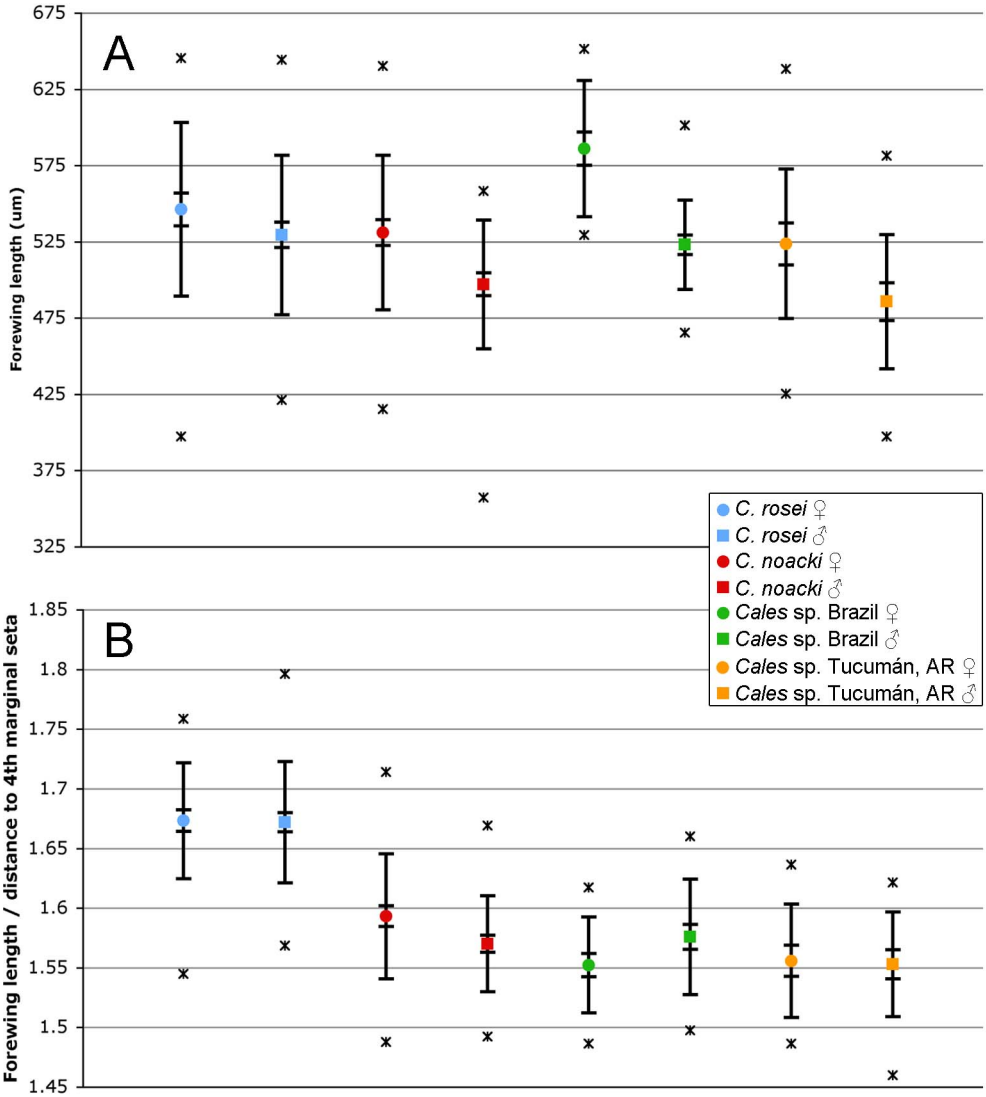


Figure 3.6. Hubbs-Hubbs diagrams based on A) overall wing length as defined by the distance between landmark 1 and landmark 4, and B) the ratio of overall wing length to the distance from landmark 1 to landmark 3 (the fourth marginal seta). Means are indicated by colored markers; squares for males and circles for females. Standard error is indicated by internal error bars, and standard deviation is indicated by external error bars. The minimum and maximum values are marked with "*".

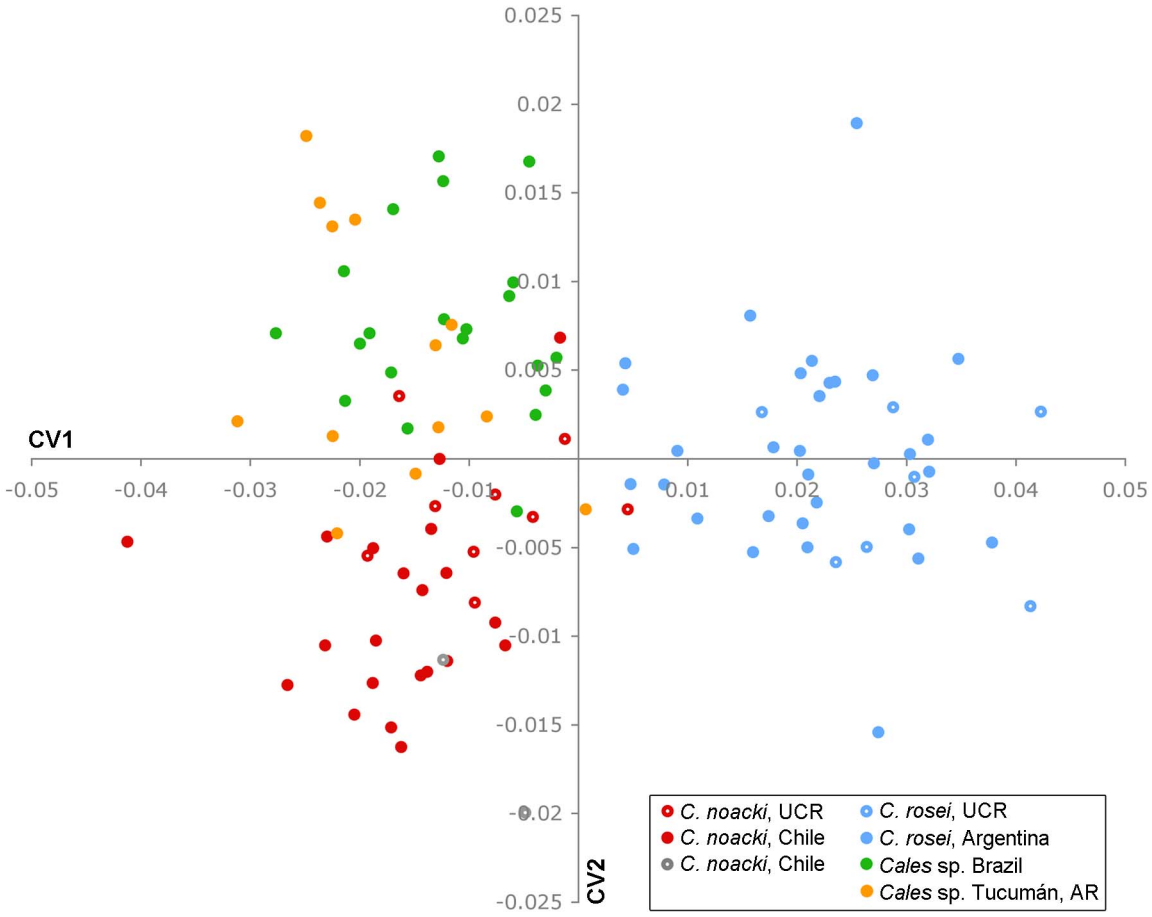


Figure 3.7. Canonical variates analysis of male fore wing shape. CV1 and CV2 are statistically distinct. A white dot in the center of the symbol indicates that the specimen has been determined to species based upon molecular data. Solid circles are slide-mounted specimens from historical biological control collections. Though the specimens from Tucumán, Argentina are colored differently on the graph, they were grouped with the Brazilian specimens for the sake of the canonical variates analysis.

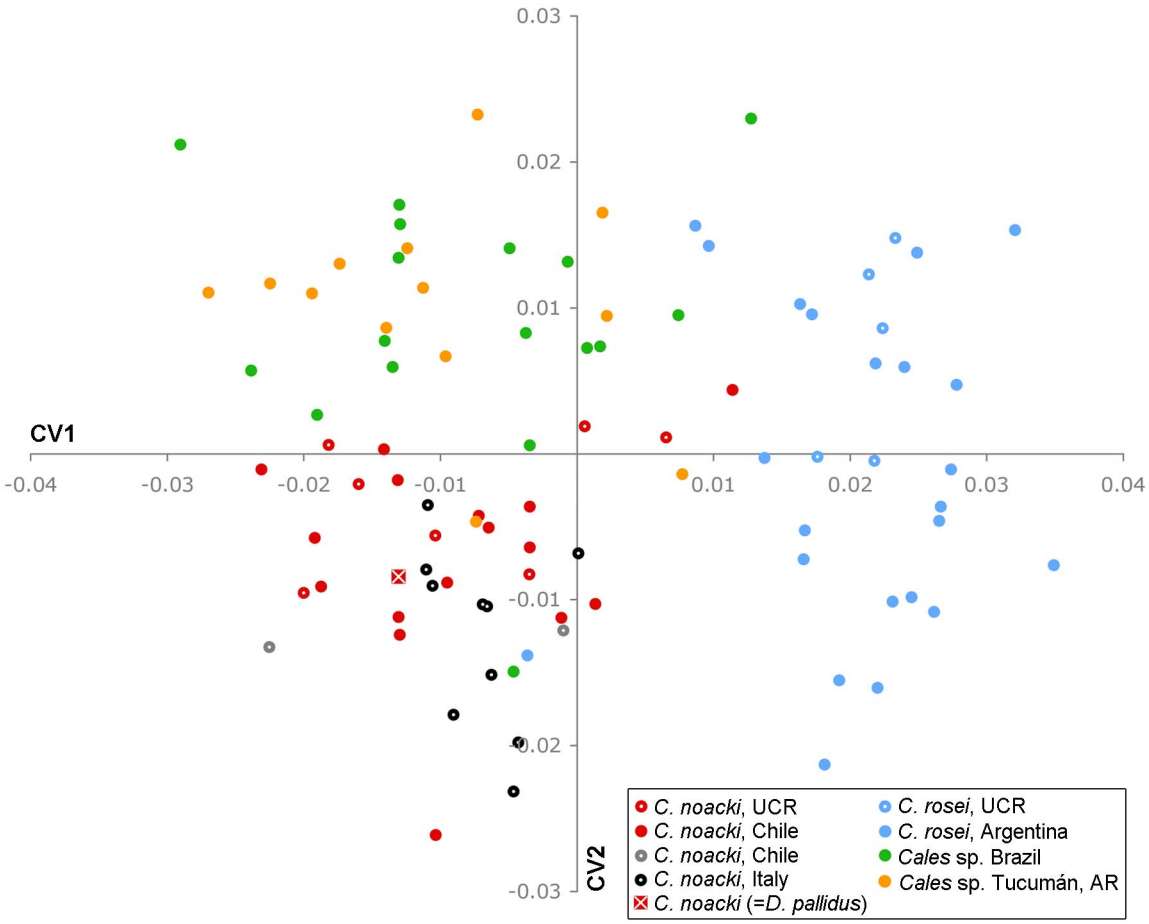


Figure 3.8. Canonical variates analysis of female fore wing shape. CV1 and CV2 are statistically distinct. A white dot in the center of the symbol indicates that the specimen has been determined to species based upon molecular data. Solid circles are slide-mounted specimens from historical biological control collections. Though the specimens from Tucumán, Argentina are colored differently on the graph, they were grouped with the Brazilian specimens for the sake of the canonical variates analysis.

CONCLUSION

Cales is an unusual group of parasitoid wasps that have proven difficult to place within the broader context of Chalcidoidea, even in the current era of molecular systematics. Morphologically, species of Cales most closely resemble members of the aphelinid subfamilies Coccophaginae and Aphelininae, though molecular analysis alone do not support this relationship, nor is it firmly established that Coccophaginae and Aphelininae themselves form a monophyletic group. The most recent analysis (Heraty et al., 2012) combines morphology and molecular data, and places *Cales* within Aphelinidae (*sensu* Heraty et al, 2012). The morphological study presented here generally supports this conclusion, though the morphological evidence must be interpreted cautiously. Many aspects of *Cales* morphology exhibit reductions from the likely plesiomorphic states, including reduced number of tarsomeres, reduced number of antennal segments, sparse wing discal setation and reduced mesonotal setation. These may be derived features that are correlated with a reduction in body size, and therefore may be independently derived in small-bodied chalcidoid groups, including many of the groups traditionally associated with *Cales*. A more thorough survey of various character systems across all of Chalcidoidea may yield additional morphological evidence (for or against current concepts), and phylogenomic techniques offer many new avenues for molecular approaches to chalcidoid phylogenetics.

The revision of Neotropical *Cales* has revealed a surprising diversity within the group, with the total number of described species increasing from one to 22. It is also noteworthy that much of this species diversity is centered in Costa Rica, a likely artifact

of disproportionate sampling intensity in that country. Sampling in additional localities in Central and South America using techniques that are optimized for small soft-bodied Chalcidoidea (screen sweeping) will likely yield many more species.

Despite their molecular diversity, *Cales* are remarkably morphologically uniform. Only eight of the twenty-two Neotropical species can be diagnosed based on morphology alone; all others require sequencing of either the 28S-D2 or COI genes to be identified with certainty. This cryptic species phenomenon is probably common among the parasitic Hymenoptera, and it poses some challenges for species discovery and description. The traditional "taxonomic workflow" of collecting specimens, mounting them in the appropriate medium, then placing them in a research collection for future study is no longer adequate. DNA extraction and storage (or storage of the entire specimen in a manner that preserves DNA for future extraction) must be incorporated into the specimen workflow. Also, DNA-based diagnostic techniques will be necessary for species descriptions and diagnoses. Traditional alpha-taxonomic studies based on morphology alone are likely to greatly underestimate species diversity.

The cryptic species phenomenon may also have important consequences for biological control. The woolly whitefly parasitoids *C. noacki* and *C. rosei* are able to coexist on the same host and host plant in both spatial and temporal sympatry. It is unclear how resources might be partitioned by these two species, and also unclear if the presence of both species enhances or detracts from their effectiveness as biological control agents. Future studies of *Cales* biology or ecology in a biological control context

will need to take into consideration the cryptic nature of the wasps, with study groups drawn from iso-female lines of genetically-determined species.

Despite the clear need for fresh material capable of yielding DNA sequences, the study of fore wing shape indicates that all usefulness is not lost for specimens that cannot be sequenced. A comparison of the fore wing shape between molecularly-determined *C. rosei* specimens from the UC Riverside Biological Control Grove and slide-mounted specimens from the original importations from South America suggests that Argentina (near Buenos Aires) was the likely source locality for *C. rosei*. These studies further confirm that *C. noacki* most likely originated from collection localities in Chile. Examination of importation and release records from the biological control efforts against the woolly whitefly reveal another intriguing possibility: *C. noacki* and *C. rosei* may not be the only species present within the *Cales* biological control range. Specimens were also collected from Tucumán, Argentina, Sãu Paulo, Brazil and Lima, Peru. Quarantine records indicate that some of these importations resulted in successful laboratory colonies and releases. Specimens from Tucumán and Brazil were included in the wing morphometric analysis, and although inconclusive, the multivariate analyses suggested that these specimens may have their own distinct wing shape. Broader sampling of *Cales* within its biological control range, as well as a more detailed understanding of species diversity in Central and South America, will be needed to generate a complete picture of *Cales* diversity and biogeography.

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