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North American species of *Agrostocynips* Diaz (Hymenoptera: Figitidae: Eucoilinae), parasitoids of Agromyzidae (Diptera): bionomics and taxonomy

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Abstract

The genus *Agrostocynips* Diaz is redescribed, as well as two species endemic to the Nearctic: *Agrostocynips diastrophi* (Ashmead) and *A. robusta* (Ashmead). Previous to this study, only Neotropical species of *Agrostocynips* were well diagnosed both taxonomically and biologically. *Agrostocynips* belongs to the *Zaeucoila* group of genera, which are Neotropical eucoilines that principally parasitize Agromyzidae (Diptera); among these genera, species of *Agrostocynips* are some of the few representatives that are found in the Nearctic. Detailed host records and biological notes are provided for the Nearctic species.

Key words: *Agrostocynips*, *Phytomyza*, *Liriomyza*, *Agromyza*, Agromyzidae, Eucoilinae, Figitidae, redescription

Introduction

Eucoiline wasps (Hymenoptera: Figitidae) are primary koinobiont endoparasitoids of cyclorrhaphan Diptera inhabiting a variety of habitats. Eucoilinae contains 85 genera and nearly 1000 species, and is by far the most diverse of all figitid subfamilies (Ronquist 1999). Prior to the revisionary work of Nordlander (Nordlander, 1976, 1978, 1980, 1981, 1982a, 1982b), no stable classification of Eucoilinae existed; Nordlander (1982b) summarized his findings by proposing informal genus groups defined by explicit morphological criteria, a first step towards a more logical and natural classification scheme.

The *Zaeucoila* group, as a whole, are mostly parasitoids of agromyzid Diptera (Buffington, 2002, 2004; Fontal-Cazalla *et al.* 2002; Buffington *et al.* 2007). *Agrostocynips* spp. have been reared from various *Liriomyza* Mik and *Phytomyza* Fallén hosts (Salvo *et al.* 2005; this study). The two chief genera of agromyzids that are of economic concern are *Liriomyza* and *Melangromyza* Hendel. Considerable work has been done looking at parasitoids of *Liriomyza* spp. in Texas (Wene 1955, Harding 1965, Chandler & Gilstrap 1989), Hawaii (Hara 1986, Johnson & Mau 1986, Johnson 1987, Lynch & Johnson 1987, Mason & Johnson 1988, Petcharat & Johnson 1988, Hara & Matayoshi 1990, Rathman *et al.* 1991, Rathman *et al.* 1995), Venezuela (Issa & Marcano 1994) and Japan (Saito *et al.* 1996).

The aim of this paper is to re-describe two common species of Eucoilinae reared from *Agromyza* Fallén spp., *Liriomyza* spp. and *Phytomyza* spp. along the southern and eastern United States, and provide detailed host data for these species. Understanding the co-evolution between parasitoids, agromyzids, and their host plants will help shape our understanding this species-rich community as a whole (Lewis *et al.* 2002). Further, since agromyzids in general can be of agricultural concern (as cited above), we feel these re-descriptions and diagnoses should prove valuable in the search for natural enemies of pestiferous agromyzids.

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This project began with reared adults of *Agrostocynips* collected by SJS and sent to MLB for identification. At the time (1998) *Agrostocynips* was unknown from North America. Notes taken by G. Nordlander (Uppsala, Sweden) and shared with MLB indicated that two types in National Museum of Natural History (USNM), *Chrestosema robusta* Ashmead and *Ganapsis diastrophi* Ashmead, belonged in the *Zaeucoila* group of eucoilines, and their present generic placement was incorrect. Some years later, Buffington (2004) moved these species into *Agrostocynips*. We are pleased to now bring to light the species reared by SJS through redescriptions and biological notes.

Taxonomic notes

The *Zaeucoila* group contains the following genera: *Aegeseucoela*, *Agrostocynips, Dettmeria, Dicerataspis, Lopheucoila, Moneucoela, Moritiella, Penteucoila, Preseucoela, Rhabdeucoela, Tropideucoila,* and *Zaeucoila*. Like the *Gronotoma* group s.s. of genera, genera in the *Zaeucoila* group possess a number of unique and rare morphological features (with respect to other eucoiline genera), such as parapsidal ridges, parapsidal hair lines, a mesoscutal keel, laterodorsal and/or posterior projections of the scutellum, an unusually wide pronotal plate and a distinct genal carina (Buffington, 2002).

The *Zaeucoila* group name was taken from Diaz and Gallardo (1997, 1998) and Gallardo and Diaz (1999). They placed 3 of the Neotropical genera originally included in the *Gronotoma* group by Nordlander (1982b) into a new group called the *Zaeucoila* group (these genera being *Rhabdeucoela, Penteucoila* and *Zaeucoila*); to this group, they added *Agrostocynips* Diaz, *Tropideucoila* Ashmead, *Lopheucoila* Weld, *Dettmeria* Borgmeier and *Moneucoela* Kieffer (Diaz & Gallardo, 1997, 1998; Gallardo & Diaz, 1999). In these works, the genera *Zaeucoila, Agrostocynips, Moneucoela*, *Rhabdeucoela, Dettmeria* and *Lopheucoila* comprised the *Zaeucoila* group (sensu Diaz & Gallardo), differing from the *Zaeucoila* group (*sensu* Buffington, 2002) by the exclusion of *Diceratаspis*. The *Zaeucoila* group (sensu Diaz & Gallardo) was not treated phylogenetically by Diaz and Gallardo (1997, 1998) and Gallardo and Diaz, (1999), but used instead to place their respective studies in context (these being revisionary studies of genera they referred to as the *Zaeucoila* group without providing a diagnosis for the group as a whole). Diaz, working with other authors (De Santis & Diaz, 1975; De Santis *et al.* 1976; Diaz & Valladares, 1979; Diaz & Gallardo, 1997; Diaz & Gallardo, 1998; Gallardo & Diaz, 1999), described and redescribed several genera and species in the *Zaeucoila* group. Buffington (2002, 2004, 2006) has added three new genera to the *Zaeucoila* group, namely *Aegeseucoela* Buffington, *Moritiella* Buffington and *Preseucoela* Buffington.

The *Zaeucoila* group, as a whole, is almost entirely restricted to the Neotropics. MLB has examined specimens of *Agrostocynips* collected from Argentina and Brazil, through Central America, and as far North as southern Canada (see material examined above). It is unknown why species in this particular genus are capable of such an incredibly broad range, when other closely related taxa (e.g. *Zaeucoila*) do not show such patterns. *Aegeseucoela* and *Diceratаspis* have also been collected in the southern Nearctic Region (Buffington, pers. obsv.), but species in these genera are not nearly as widespread as *Agrostocynips* (Buffington, 2002). Southern Mexico (Oaxaca and Chiapas) appears to be the northern limit of the remaining genera in the *Zaeucoila* group; the southern distribution limits and altitudinal limits of the genera in this group remain to be examined. Though Buffington *et al.* (2007) thoroughly sampled the *Zaeucoila* group for phylogenetic data and found this group to be consistently monophyletic, we have preferred to postpone the formal erection of it as a tribe to a more detailed, purely taxonomic work which is currently being prepared (Buffington, in prep.).

Agrostocynips Diaz was described in De Santis *et al.* (1976), with *A. clavatus* Diaz designated as the type. Later, *Zaeucoila enneatoma* Diaz, 1975, was transferred to *Agrostocynips*, resulting in *A. enneatoma* (Diaz) (Diaz & Gallardo, 1997). Buffington (2004) provided the new combinations *Agrostocynips diastrophi* (Ashmead) and *A. robusta* (Ashmead). *Agrostocynips* is undoubtedly closely related to *Zaeucoila* and may eventually prove to be a synonym of it. The incomplete genal carina is a striking feature, most easily viewed when the head is removed from the mesosoma. The incomplete state is distinctive from *Zaeucoila* and *Aegeseu-*

coela, both of which possess the complete state. The reduced orbital furrow is a constant character in *Agrostocynips*, but some species of *Zaeucoila* have gained this state in parallel. The complete reduction of the mesonotal keel is an important feature of *Agrostocynips*, both taxonomically and phylogenetically. Unfortunately, some species in this genus seem to grade between a rather apparent mesoscutal keel (i.e *A. clavatus*) to an extremely reduced mesoscutal keel (i.e. *A. diastrophi*); using other features (e.g. genal carina and orbital furrows) in combination with the mesoscutal keel help to remedy this situation.

Material and methods

Parasitoid rearing. As part of ongoing studies of *Phytomyza* leafminers of holly (*Ilex* spp., Aquifoliaceae; Fig. 2F) (Scheffer and Wiegmann 2000, Scheffer 2002, Scheffer and Hawthorne 2007) mined leaves were collected from various holly species from 1995 onward in the eastern coastal plains of the United States. Leafmines from the same host and collecting site were placed into self-sealing plastic bags and stored in a cooler until reaching the laboratory in early February. In the lab, using a dissecting microscope, each leafmine was opened using a fine forceps, and the pupa was removed with a small, moistened paintbrush. Pupae were placed individually into a 0.5 ml Eppendorf tubes and stored together in deli containers lined with moistened filter paper. Every day the deli containers were opened and the tubes checked for emerged flies or parasitoids.

Descriptive format. Morphological terminology follows that of Ronquist and Nordlander (1989), Fontal-Cazalla *et al.* (2002) and Buffington *et al.* (2007); cuticular surface terminology follows that of Harris (1979). Specimens were examined using a Leica Wild M10 with fluorescent lighting. Images for figures were obtained using an EntoVision Imaging Suite, which included a firewire JVC KY-75 3CCD digital camera mounted to either a Leica M16 zoom lens via a Leica z-step microscope stand or to a Leica DMRB compound microscope. This camera fed image data to a desktop computer where Cartograph 5.6.0 (M16 scope) or Archimed 5.5.0 (DMRB scope) (Microvision Instruments, France) was used to capture a fixed number of focal planes (based on magnification); the resulting focal planes were merged into a single, in-focus composite image. Lighting was achieved using either an LED illumination dome with all four quadrants set to 99.6% intensity (Leica scope) or two fiber optic illuminators with light dispersal film (DMRB scope). Scanning electron micrographs of *Agrostocynips diastrophi* (Ashmead) were made by MLB using methods summarized in Fontal-Cazalla *et al.* (2002) and are downloadable from http://www.morphbank.com, collection ID number 195619.

List of depositories

TAMU	Texas A&M Insect Collection, Texas A&M University, College Station, TX, USA.
UCRC	Entomology Research Museum, UC Riverside, Riverside, CA, USA.
USNM	National Museum of Natural History, Washington DC, USA.

***Agrostocynips* Diaz**

Agrostocynips Diaz, 1976: 32. Type-species *Agrostocynips clavatus* Diaz, by original designation.

Diagnosis. Genal carina reduced. Orbital furrows reduced to absent. Mesoscutal keel absent. Scutellar plate with distinct tubercles present. Most easily confused with *Zaeucoila* and *Aegeseucoela*, both of which have complete genal carinae, a mesoscutal keel present (at least anteriorly) and distinct orbital furrows.

Redescription. ***Head.*** Nearly glabrous with scattered setae along lower face, clypeus and gena; ocellar hair patch absent (Fig. 1 C). Ventral 1/4 of lower face with admedian clypeal furrows converging toward the clypeus. Orbital furrows faint to completely reduced, originating from the lateral aspect of torulus and running to dorsal margin of malar sulcus (Fig. 1 C). Malar sulcus compound or simple. Malar space smooth; anteroventral margin with a raised protuberance. Genal carina present only along ventral margin of malar space (Fig. 1 D) (often visible only when head is removed from mesosoma).

Antennae. Female: 13 segments, moniliform (Figs 1 A and 2 A); segments 3-13 of sub-equal size; rhinaria present on segments 4-13. Male: 15 segments, moniliform; rhinaria present on segments 3-15; segments 4-15 sub-equal in size; segment 3 modified, slightly longer than segment 4, curved outwardly, excavated laterally.

Pronotum. Pronotal plate wide, with setae along the dorsal margin (Fig. 1 F); dorsal margin rounded; pronotal fovea open. Pronotal triangle absent. Pronotal impression absent. Lateral pronotal carina absent. Lateral portion of the pronotum smooth and glabrous (Fig 1 E).

Mesoscutum**.** Smooth and glabrous; no sculpture present (Fig. 1 F). Parascutal impression incomplete, narrow. Notauli, mesoscutal keel, parapsidal ridges and parapsidal hair line absent (Fig. 1 F).

Mesopectus**.** Upper part and lower part of mesopleuron smooth and glabrous (Fig. 1 E). Dorsal margin of mesopleural triangle well defined, rounded ventrally. Mesopleural carina simple. Lower part of mesopleuron bounded by distinct precoxal carina; surcoxal depression present, smooth.

Scutellum**.** Scutellar plate large to medium; midpit placed between center point of plate and posterior margin of plate; rim of plate translucent; prominent tubercles commonly found along the entire rim (often resembling "sawblade teeth" in lateral view) (Figs 1 B, E–F, 2B). Dorsal surface of scutellum reticulate, margined laterally and posteriorly; rounded laterally and posteriorly; laterodorsal and posterior projections of the scutellum absent. Lateral bars as long as wide; ventral lobe present. Scutellar fovea oval, smooth and deep.

Metapectal-Propodeal Complex. Anterior 3/4 of metapectus glabrous, posterior 1/4 setose (Fig. 1 E). Spiracular groove with a well defined dorsal margin, reduced ventral margin. Posterior margin of metapectus ridged. Metapleural ridge reduced to absent; submetapleural ridge absent. Anterior impressions of metepimeron and metepisternum present. Anteroventral cavity oval, setose. Propodeum covered in both long and short setae. Lateral propodeal carinae semi-parallel, bowed at junction with the auxiliary propodeal carinae; auxiliary propodeal carinae reduced. Nucha glabrous, crenulate.

Wings. Hyaline, with base of wing rarely darkened; setose (Fig. 2 D–E). R_1 complete; marginal cell as long as deep. Apical fringe present, medium in length.

Legs. Fore- and mid-coxa sub-equal in size, hind-coxa twice the size of either fore- or mid-coxa (Fig. 2 B). Fore coxa variously setose; mid and hind coxa with distinct lateral and posterior dorsoventral setal bands. Femora and tibiae with sparse setal lines; tarsomeres with dense appressed setae. Length of hind tarsomere 1 equal to the combined length of remaining hind tarsal segments.

Metasoma**.** Female: sub-equal in size to mesosoma (Figs. 1 A and 2 A). Base of syntergum with hairy ring present, composed of dense, short setae and longer, thin setae; remainder of metasoma glabrous. Micropunctures present on posterior 1/3 to 1/4 of syntergum, and on remaining terga. Terga posterior to syntergum abruptly directed ventrally, resulting in a near 90 degree angle between syntergum and terga. Male: As in female.

Distribution. Neotropical Region: Argentina, Chile, though Central America and into Central Mexico; Nearctic Region: Continental United States and into Southern Canada (British Columbia).

Biology. *Agrostocynips clavatus* has been recorded in the neotropics from several agromyzid species in the genera *Melanagromyza* (De Santis *et al.* 1976) and *Liriomyza* (Diaz & Valladares, 1979; Salvo, pers. comm.). Nearctic species of *Agrostocynips* have been reared from agromyzids in *Agromyza* (on *Panicum* (Poaceae)), *Liriomyza* (several host plants) and from *Phytomyza* (on *Ilex cassine* and *I. myrtifolia*).

Included species

clavatus Diaz, 1976: 32. Holotype in MLP (not seen).

diastrophi (Ashmead), Buffington (2004). *Ganaspis diastrophi* Ashmead, 1896: 184-185. Holotype in USNM.

enneatoma (Diaz), Diaz & Gallardo (1997). *Zaeucoila enneatoma* Diaz, 1975: 1999. Holotype in MLP (not seen).

robusta (Ashmead), Buffington (2004). *Chrestosema robusta* Ashmead, 1894: 68. Holotype in USNM.

Agrostocynips diastrophi (Ashmead)

Figures 1 A–F, 2 E

Diagnosis. Differs from *A. clavatus* by the lack of an indication of the mesoscutal keel at the anterior margin of the mesoscutum (Fig. 1 F) (distinctly present in all specimens examined of *A. clavatus*); from *A. robusta* by the larger and more elongate scutellar plate (Fig. 1 E–F) (shorter and truncated posteriorly in *A. robusta*, Fig. 2 C), as well as the presence of 7-8 perimeter teeth on the dorsal surface of the scutellar plate (Fig. 1 E–F) (4-6 present in *A. robusta* (Fig. 2 B); further differentiated from *A. robusta* by the more pronounced orbital furrows on the inner margins of the eyes (Fig. 1 C) (rarely present in *A. robusta*).

Rediscription. As in description of genus, with orbital furrows distinctly developed, running from lateral aspect of torulus to dorsal margin of malar sulcus; scutellar plate with 7-8 perimeter tubercals present on dorsal surface, usually in pairs, occasionally non-paired posteriorly; posterior margin of scutellar plate broadly rounded.

Material examined. *Holotype*. [first label] West Point, Neb[raska], [second label, folded] *Diastrophus cuscataiformis* [in Ashmead's hand], [third label] Type No. 3280 U.S.N.M., [fourth label] *Ganaspis diastrophi* Ashmead, type [in Ashmead's hand]. The holotype is a male, in poor condition, consisting of only the metasoma and hind legs glued to a card point. Deposited in USNM. *Additional material.* Several specimens from the following US states (deposited in UCRC and USNM): Arkansas, Florida, Illinois, Iowa, Kansas, Louisiana, Maryland, Michigan, Minnesota, Missouri, Oklahoma, Pennsylvania, South Carolina, Texas, Virginia and West Virginia.

Biology. Reared from the agromyzids *Phytomyza* sp., *Phytomyza bipunctata* Loew (host plant not recorded), as well as an unknown species of *Agromyza* on *Panicum* (switchgrass, Poaceae). A specimen examined in the USNM perported to be reared from *Phytomyza illicola* requires confirmation. At the time of that collection (1919), it was not known that the linear miner and the blotch miner on *I. opaca* were two different species (see Kulp 1968). Further, no figitids have been reported from studies of parasitoids of *P. ilicicola* in Kentucky (Potter and Gordon 1985), Delaware (Kahn and Cornell 1989), Georgia (Braman and Pendley 1993), and various locations in the eastern U.S. by the junior author (SJS, unpub. data).

Distribution. Southeastern United States (see *material examined*) and Northeastern Mexico (data not shown).

Agrostocynips robusta (Ashmead)

Figure 2 A–D

Diagnosis. Differs from *A. clavatus* by the lack of an indication of the mesoscutal keel at the anterior margin of the mesoscutum (distinctly present in all specimens examined of *A. clavatus*); from *A. diastrophi* by the smaller and posteriorly truncate scutellar plate (Fig. 2 C) (longer and rounded posteriorly in *A. diastrophi*, Fig. 1 E–F), as well as the presence of 4-6 perimeter teeth on the dorsal surface of the scutellar plate (Fig. 2 B) (7-8 present in *A. diastrophi*, Fig. 1 E); further differentiated from *A. diastrophi* by the weak to absent orbital furrows on the inner margins of the eyes (nearly always well developed in *A. diastrophi*).

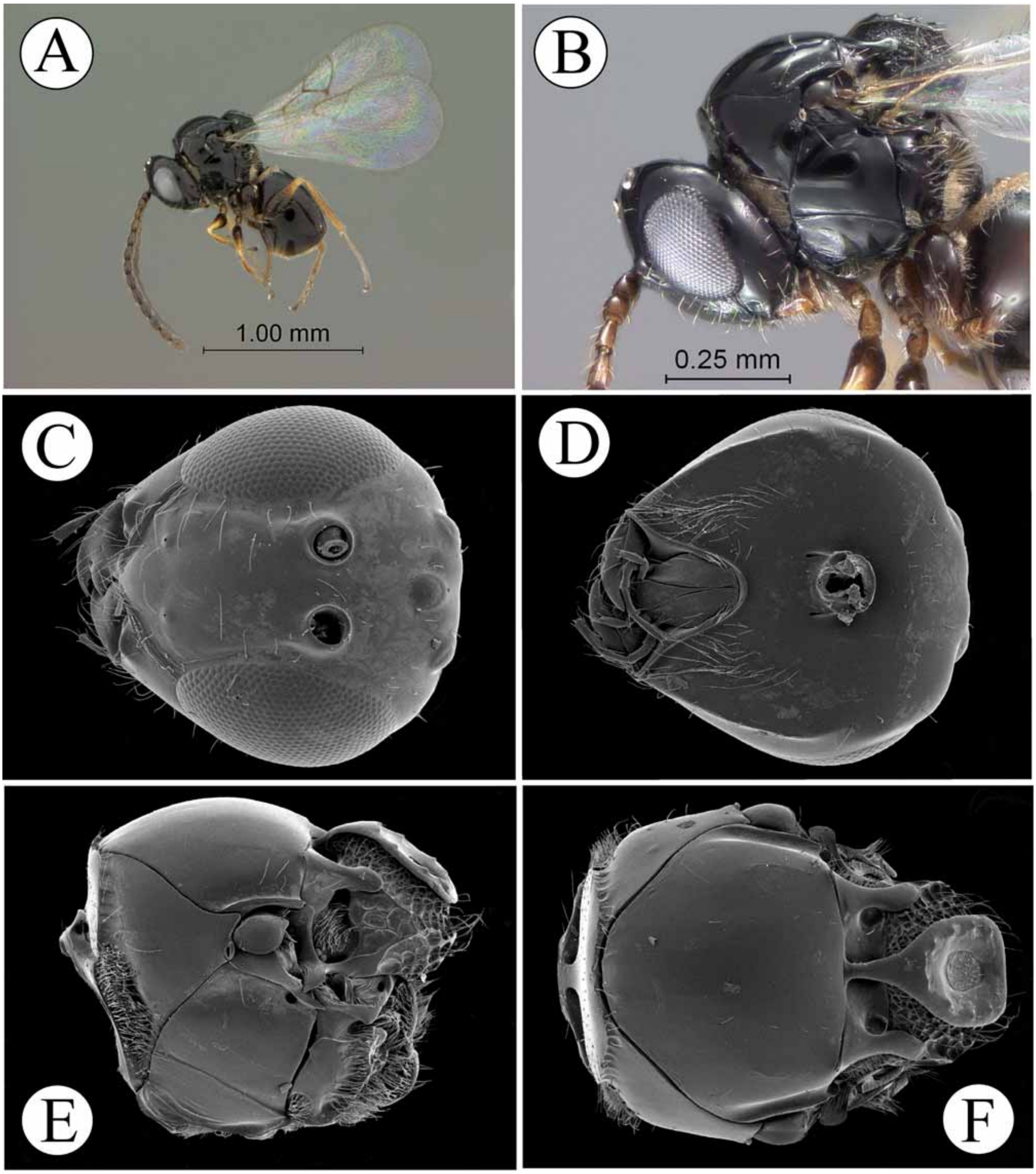

FIGURE 1. A–F, *Agrostocynips diastrophi* (Ashmead). A, habitus, female; B, close-up of head and mesosoma, female; C, SEM of female head, anterior view; D, SEM of female head, posterior view; E, SEM of female mesosoma, lateral view; F, SEM of female mesosoma, dorsal view.

Redescription. As in description of genus, with: orbital furrows poorly developed, often entirely absent; scutellar plate with 4-6 perimeter tubercals present on dorsal surface, always in pairs; posterior margin of scutellar plate shortened, truncated.

Material examined. *Holotype*. [first label] St. Vincent, W[est] I[ndies], H.H. Smith, [second label] Type No. 2336 USNM [third label] *Chrestosem robusta* Ashm. [in Ashmead's hand], [fourth label] *Agrostocynips* *robusta* (Ahm.), Det. By M.L. Buffington 2003. The holotype is a male, in poor condition, consisting of only the metasoma and hind legs glued to a card point. Deposited in USNM. *Additional material*. Several specimens from the following US states and Canadian province (deposited in TAMU, UCRC and USNM): **CANADA:** Ontario. **U.S.A.:** Alabama, Arizona, Colorado, Florida, Georgia, Indiana, Iowa, Louisiana, Maryland, Massachussets, Minnesota, Mississippi, Missouri, New York, North Carolina, South Carolina, Tennessee, Texas, Virginia and Washington D.C.

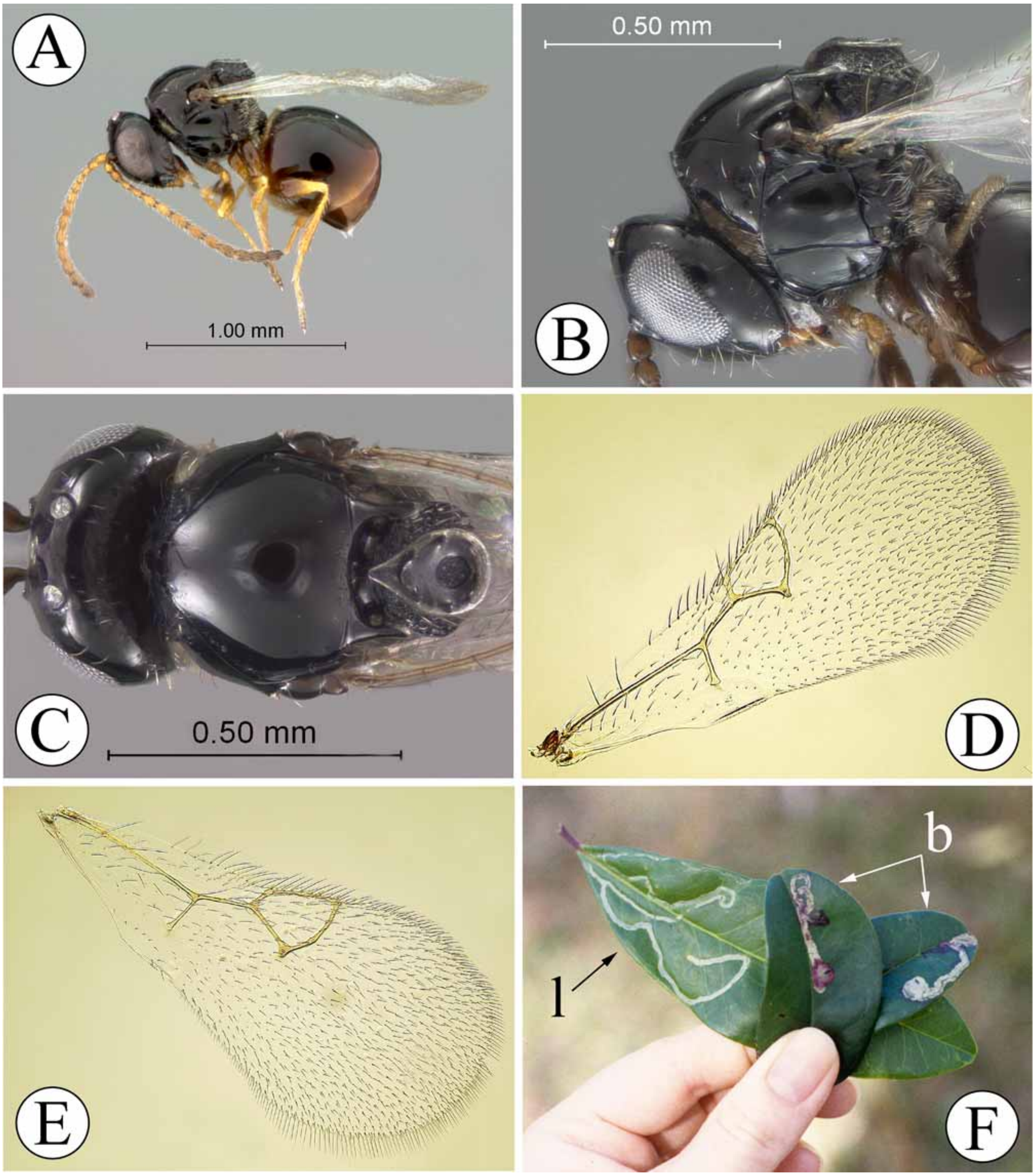

FIGURE 2. A–D, *Agrostocynips robusta* (Ashmead). A, habitus, female; B, close-up of head and mesosoma, female; C, female mesosoma, dorsal view; D, forewing of female, ventral view. E, *A. diastrophi* (Ashmead), forewing of female, ventral view. F, sympatric holly leafminers on *Ilex cassine*: linear mine (l) of *Phytomyza opacae* Kulp contrasted with two blotch mines (b) of *Phytomyza* n. sp. 1, a host of *A. robusta.*

Biology. Reared from *Phytomyza* n. sp. 1 (*sensu* Scheffer & Wiegmann, 2000) on *Ilex cassine* and *I. myrtifolia* (holly, Aquifoliaceae; SJS reared material, Fig. 2 F); from *Phytomyza* sp. on *Solidago* (goldenrod, Asteraceae); from *Liriomyza trifolii* (Burgess) on *Solanum americanum* P. Mill. (American black nightshades, Solanaceae) and *L. trifolii* on *Capsicum anuum* L. (jalapeno and Cuban hots peppers, Solanaceae). Also recorded from an undetermined leafminer on *Symphoricarpos* (snowberry, Caprifoliaceae); this species has also been observed host searching on *Celtis* (hackberry, Ulmaceae; MLB, pers. obsv.).

Distribution. Southwestern**,** Central and Eastern United States, South Eastern Canada and the Carribbean (see material examined). Specimens of this species have also been examined from Baja as well as central and Southern Mexico. The species is likely present throughout Central America.

Conclusion

Biological Notes–holly leafminer parasitoids

Although as many as 12 *Phytomyza* species can be found mining the leaves of various holly species in the eastern US, figitids have been reared regularly from only a single *Phytomyza* holly leafminer, an undescribed species currently referred to as *Phytomyza* n.sp. 1 (Scheffer and Wiegmann 2000). This leafminer routinely forms mines in two closely related (and sometimes lumped) evergreen holly species: *I. myrtifolia* Walter and *I. cassine* L. which are broadly sympatric from Texas/Louisiana into North Carolina (*I. cassine* is also reported from Mexico and Cuba; Galle 1997). The lifecycle of the fly appears to be univoltine (as in several other holly leafminers (Kulp 1968, Potter and Kimmerer 1986, Scheffer 2002)), with the adult flies and parasitoids emerging from pupae during February and early March. On *I. myrtifolia* populations seen in southern Alabama and northern Florida, the leafmines of first instar larvae are distinctly linear, but then become conspicuously blotch-like during the later two instars. Because the leaves in these populations are so small (mined leaves ranging only to 27mm x 5mm), fully developed mines may encompass nearly the entire leaf. In contrast, the *I. cassine* populations observed in central and southern Florida have very large leaves (mined leaves ranging to 110mm x 55mm), and on these plants *Phytomyza* n. sp 1 generally forms a type of blotch mine that conspicuously widens as the larva grows (Figure 2F: “b”). *Ilex cassine* in Florida is also host to *P. opacae* Kulp which makes a serpentine linear mine that can usually be distinguished from that of *Phytomyza* n. sp. 1 by its much greater length and very gradual increase in width (Figure 2F: “l”). Populations observed in North Carolina and South Carolina often have intermediate leaf sizes, making species identification difficult; in collections of leafminers and parasitoids by SJS, these host plants with intermediate sized leaves are often referred to as *Ilex cassine* “medium.”

Although figitids are not often reared from other holly leafminers, *A. robusta* is frequently reared from *Phytomyza* n. sp. 1; of 14 collections of this leafmining species made in 1997 and 1998, 10 resulted in *A. robusta* at levels ranging up to 40% (47/118) of pupal emergences. While most agromyzids, including the other holly leafminers, are regularly parasitized by braconids at high levels (SJS, pers. obs.), *Phytomyza* n. sp. 1 appears to be less commonly attacked by braconids. In fact, collections that result in high numbers of *A. robusta* tend to have very few braconids, possibly indicating ecological interference of some sort. Further studies of geographic and genetic variation in the host plants, the leafminer, and its parasitoids would undoubtedly prove interesting.

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