Morphology and Development of Immature Stages of *Fidiobia dominica* (Hymenoptera: Platygastridae: Sceliotrachelinae)

JOSEP-ANTON JACAS,[1] JORGE E. PEÑA,[2] AND R. E. DUNCAN[2]

ABSTRACT   The external morphology and development of immature stages of *Fidiobia dominica* Evans & Peña (Hymenoptera: Platygastridae), a Neotropical solitary endoparasitoid of the eggs of *Diaprepes* spp. (Coleoptera: Curculionidae), are reported. This species behaves as a solitary idiobiont endoparasitoid. It has a stalked egg and two instars. The first instar is cyclopoid and seven-segmented, whereas the second instar is hymenopteriform and 11-segmented. Mandibulae are conspicuous in both instars. On completion of the larval development, the host egg turns amber transparent, making parasitized eggs easily recognizable. The pupa is exarate. *F. dominica* is a protandrous species and once emerged, males help females to emerge.

KEY WORDS   *Diaprepes abbreviatus*, biological control, egg parasitoid

Fidiobia dominica Evans & Peña (Hymenoptera: Platygastridae) is a solitary egg endoparasitoid that attacks at least two species of root weevils, *Diaprepes doublerii* Guérin and *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), indigenous to the Caribbean region (Evans and Peña 2005). *D. abbreviatus* was introduced into Florida in 1964 (Woodruff 1968) and since has become a serious pest of citrus throughout much of central and southern Florida, and it is currently spreading in the citrus-growing regions of Texas and California (Grafton-Cardwell et al. 2004). A lack of native egg parasitoids for this weevil in citrus orchards in Florida (Hall et al. 2001), and past failures of classical biological control (Beavers et al. 1980), have triggered renewed efforts to introduce, release, and evaluate candidate egg parasitoids from the Caribbean region into Florida (Peña et al. 1998, Peña and Amalin 2000, Hall et al. 2002, Jacas et al. 2005, Castillo et al. 2006). One of these candidate species, *F. dominica*, a platygastrid wasp collected in Dominica from *D. doublerii* egg masses, was subsequently introduced into Florida in April 2003, and it has been reared through ≈50 generations on *D. abbreviatus* eggs in the quarantine facility in Homestead, FL (Evans and Peña 2005). The release of this parasitoid was approved in June 2006, and releases are under way at various sites in Florida as part of the classical biological control program against this pest.

Little is known about the biology and the immature morphology of Platygastridae and even less so about that of species in the subfamily Sceliotrachelinae (Clausen 1940, Askew 1971, Masner 1995, Austin et al. 2005). The genera of this subfamily that parasitize coleopteran eggs are solitary idiobiont endoparasitoids and are thought to develop in a way similar to that of Scelionidae (Masner 1995). Although the first instar of some platygastrids is more or less hymenopteriform, first instars of many species are extraordinary in appearance and superficially resemble cyclopoid copepods (Clausen 1940, Askew 1971). When fully grown, these larvae are extremely bloated and ovoid. The number of instars seems to vary from one to three, depending on species (Clausen 1940, Masner 1995, Sampson et al. 2006). The incomplete knowledge on this important group of natural enemies compelled us to study and provide basic information on the external morphology and development of immature stages of *F. dominica.*

Materials and Methods

Specimens used in this study were obtained from a colony initiated from individuals of *F. dominica* imported from Dominica (Evans and Peña 2005). The colony was maintained on *D. abbreviatus* eggs in the insectary facilities of the University of Florida at the Tropical Research and Education Center (TREC) at 25.6 ± 1°C, ≈65% RH, and a photoperiod of 12:12 (L:D) h. Voucher specimens of *F. dominica* are deposited in the Florida Collection of Arthropods, Gainesville, FL, U.S. National Museum of Natural History (USNM), and in the Canadian National Collection, Ottawa, Canada.

Stock Colonies. Adult *D. abbreviatus* root weevils were collected from the foliage of ornamental trees in nurseries around TREC, Homestead, FL (25°30 N, 80°30, 1-m altitude). The weevils were placed in 30- by 30- by 30-cm Plexiglas cages with water and foliage of

[1] Corresponding author: Universitat Jaume I, Departament de Ciències Agràries i del Medi Natural, Campus del Riu Sec, E-12071-Castelló de la Plana, Spain (e-mail: jacas@exp.uji.es).

[2] Department of Entomology and Nematology, Tropical Research and Education Center, University of Florida, 18905 SW 280th St., Homestead, FL 33031.
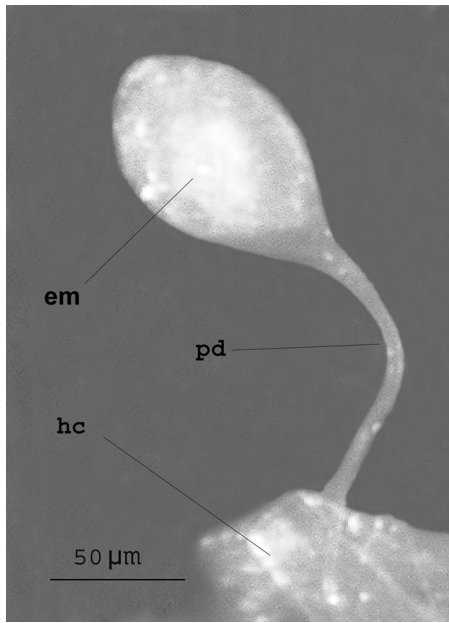
Fig. 1. Egg. em, embryo; pd, pedicel; hc, host chorion.

Conocarpus erectus L. (Myrtales: Combretaceae) that provided a food source and an oviposition substrate. Wax paper strips (3 by 10 cm) stapled together also were used as an oviposition substrate (Castillo et al. 2006). Both foliage and paper strips were replaced every 2–3 d. Paper strips containing *D. abbreviatus* eggs were removed and placed inside a similar cage. Adults of *F. dominica* were introduced into the cage and provided honey and water. Presumably, parasitized egg masses were removed from the cage 4–5 d later and placed in emergence cages (same dimensions as above) supplied with both water and a honey food source until adult emergence (≈12 d).

Experimental Specimens. Wax paper strips with <1-d-old eggs were carefully separated from each other and cut into one by 0.5-cm strips containing a single egg mass, introduced into a glass vial (Fisherbrand flint glass printed white 12- by 75-mm disposable culture tubes, Fisher, Pittsburgh, PA) with three naive *F. dominica* adults <1 d old. A smear of honey was provided on the inner surface of each test tube as

a food source, and the open end of the test tube was covered with two-ply Kimwipe (Kimwipes EX-L, Kimberly-Clarke, Neehah, WI) secured with rubber tubing to allow ventilation. Adult wasps were removed from the vials 6 h later. The latter were kept in a climatic chamber at 25°C and a photoperiod of 12:12 (L:D) h until dissected.

Eggs, presumably parasitized by *F. dominica*, were dissected at different times and checked under a stereomicroscope (Leica MZ6, Leica, Wetzlar, Germany) with a cold light source (Leica CLS100). Nonparasitized hosts were rejected and the inspection finished once 15–20 parasitized eggs had been obtained. These specimens were mounted on a glass microscope slide in PVA mounting medium (BioQuip Products Co., Rancho Dominguez, CA) for observation by using a light microscope. Immature stages were measured by use of the Eclipse Net software (Nikon 2003). The number of instars was determined using the width of the mandibles (Dyar 1890).

Results

Under laboratory conditions (25 ± 1°C and a photoperiod of 12:12 [L:D] h), *F. dominica* reared on eggs of *D. abbreviatus* has a developmental time of 18–22 d from oviposition to adult emergence. Parasitized eggs could be easily recognized by their transparent whitish appearance and unorganized structure when dissected as early as 24 h after exposure to the parasitoid. Conversely, unparasitized eggs have a more opaque white aspect and show some tissue/organ organization when examined. As time progresses, differences become more conspicuous, and the *D. abbreviatus* embryo can be easily observed in the unparasitized egg.

Eggs. *F. dominica* eggs are almost transparent, and the newly formed larvae become visible as hatching approaches (Fig. 1). Young eggs average $120 \pm 3 \ \mu m$ in length by $65 \pm 3 \ \mu m$ in width at the widest point ($n = 17$). The egg is lemon shaped, with a pedicel extending from the anterior end. The maximum length of the pedicel observed was 176 μm. However, this length was highly variable ($72 \pm 47 \ \mu m$; $n = 17$) in part because of pedicel breakage during dissection. The pedicel is internally attached to the host egg chorion, which prevents *F. dominica* egg from freely moving within the host.

Table 1. Measurements (mean ± SE in micrometers) of *F. dominica* immature stages at specified times (in days) after parasitization at 25°C

Time	Instar	n	Body length	n	Body width	n	Tail length	n	Mandible length	n	Mandible width
1	First	17	297 ± 10a	17	107 ± 2a[a]	17	61 ± 2a	15	30 ± 1a	15	15 ± 5a
2	First	17	694 ± 11b	17	241 ± 8b	15	67 ± 4a	13	31 ± 1a	13	11 ± 1a
3	First	17	884 ± 18c	17	288 ± 17c	17	63 ± 5a	13	31 ± 1a	13	12 ± 1a
4	Second	21	1,064 ± 27d	21	437 ± 13d				14	47 ± 1b	14	10 ± 1a
ANOVA			$F = 398.10$		$F = 346.15$		$F = 0.55$		$F = 17.25$		$F = 1.34$
			df = 3, 71		df = 3, 71		df = 2, 48		df = 3, 51		df = 3, 51
			$P < 0.00001$		$P < 0.00001$		$P = 0.5789$		$P < 0.00001$		$P = 0.2710$

Within columns, values followed by the same letter are not significantly different ($P < 0.05$).
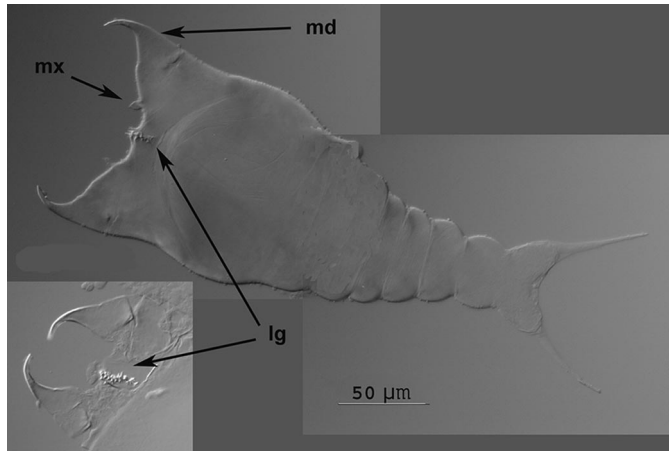
[a] Cephalothorax width.

Fig. 2. Neonate first instar. Dorsal view. md, mandibles; mx, maxillae; lg, spiked ligula.

Approximately 1 d after oviposition, the embryo increases to 158 ± 6 μm in length by 88 ± 4 μm in width at the widest point ($n = 15$) as it completes its development, at which time the neonate larva breaks through the egg. Only in two eggs of >400 host eggs examined were two parasitoid eggs observed in the same host egg, and only once were two larvae found within the same host egg. Correspondingly, no more than one adult emerged from each of these hosts.

Larvae. Mandible measurements (Table 1) indicate two instars in *F. dominica.* The neonate, or the first instar, is whitish, almost transparent, and cyclopoid (Clausen 1940, Askew 1971). The cephalothorax is enlarged ($\approx$107 μm in width) and bears relatively enormous, sickle-shaped mandibles (30 ± 1 and 13 ± 1 μm in length and width at the base, respectively; $n = 41$) and a spiked ligula with short, rasping teeth (Figs. 2 and 3). The abdomen tapers posteriorly and is reduced to seven segments. The caudal segment is bilobed with each lobe bearing one long spine-like process that is armed with a few smaller spines. When the larva is dislodged from the host egg, it often moves vigorously by ventral or backward thrusts of the caudal processes and the posterior portion of the abdomen. It also moves its mandibles vigorously when disturbed. The ligula and maxillae are clearly visible on the anterior margin of the cephalothorax at this stage of development (Fig. 2). At the end of the first instar, which takes 2–3 d, the larvae average 884 μm in length and 288 μm in width (Fig. 3).

The second instar is hymenopteriform (Fig. 4a), with an elongated oval body (1064 μm in length and 437 μm in width), and it has a small head bearing relatively large mandibles and a suctorial mouth (Fig. 4b). Ten body segments, in addition to the head, are clearly visible, and the cauda tapers posteriorly to a single point. By the end of its development, the parasitoid larva consumes the contents of its host, leaving only the egg chorion, which gradually takes an amber-like appearance that makes parasitized eggs clearly recognizable. Just before pupation, the larva, which occupies the entire host egg, shrinks, and a free space forms in the interior of the host (Fig. 4c); this process is completed in 2–3 d.

Pupa. The pupa of *F. dominica*, as in all Hymenoptera, is exarate. Initially, the pupa is whitish and does not occupy the whole inner volume of the host (Fig. 5a). Later, it becomes darker with red eyes, and the meconium is expelled. Subsequently, the pupa elongates and occupies almost all available space within the host (Fig. 5b). Eventually, the eyes darken and the thorax turns uniformly black. Mature pupae average 997 ± 100 μm in length ($n = 11$) (Fig. 5c), and adult characteristics can be easily observed through the amber-colored egg.

Upon completion of pupal development, the adult chews an emergence hole in the host egg chorion by using a dotted rim existing at the anterior end of *D. abbreviatus* eggs. Emerged adults (mainly males) were observed helping later emerging adults (females) to chew the exit hole (Fig. 6).
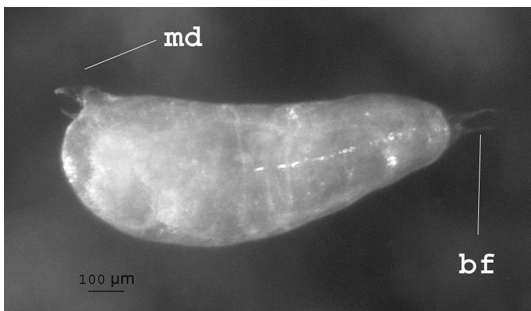
Discussion

As is typical of idiobiont parasitoids (Pennacchio and Strand 2006), female *F. dominica* probably inject some kind of venom at oviposition that prevents fur-
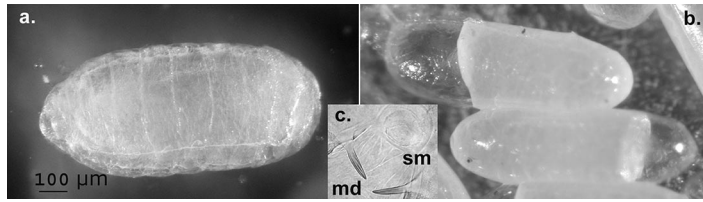

Fig. 3. Late first instar. md, mandibles; bc, bifurcated cauda.

Fig. 4. Second instar. (a) Prepupa. (b) Shrinking prepupa within egg. (c) Mandibulate suctorial mouth. md, mandibles; sm, suctorial mouth.

ther host development. This behavior could account for the distinctive features observed in parasitized eggs as early as 24 h after oviposition. Females also might be injecting host-marking substances during oviposition. Our results indicate that both multiparasitism and superparasitism are rare in this solitary species. Many platygastrid wasps exploit hosts that lay clumps of eggs, and strategies to prevent superparasitism are common. These strategies fall mostly into two categories: the first involves marking host eggs either externally or internally, and the second involves female wasps defending egg batches from competitors (Austin et al. 2005). No defending behavior has been observed in either our stock colony or the assays; therefore, host marking may be the strategy used by *F. dominica.*

Stalked eggs are not rare among platygastrids, and, as in other species, the pedicel of *F. dominica* egg is longer than the egg body (Clausen 1940, Sampson et al. 2006). The location of the egg in the host egg content for platygastrid egg parasitoids is fairly constant for any given species (Clausen 1940, Masner 1995). However, we could not determine a precise location of a *F. dominica* egg in the host embryo.

F. dominica has two instars, and this number fits within the range given by Masner (1995) for platygastrids. The first instar larva is cyclopoid and the second instar is hymenopteriform (Clausen 1940, Masner 1995, Sampson et al. 2006). *Allotropa burrelli* Muesebeck, another Sceliotrachelinae described by Clancy (1944), has a single instar, which is hymenopteriform and has fine pointed mandibles. However, *A. burelli* parasitizes nymphs of *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae), and its biology is completely different from that of *F. dominica.* Freeliving first instars of platygastrids usually feed directly upon the body fluids of the host, and their mandibles seem to be designed for fighting, rather than for feeding (Clausen 1940). Whether this is true for *F. dominica* deserves further investigation.

As larval development progresses, *F. dominica* parasitized eggs turn amber. Such a change in color is
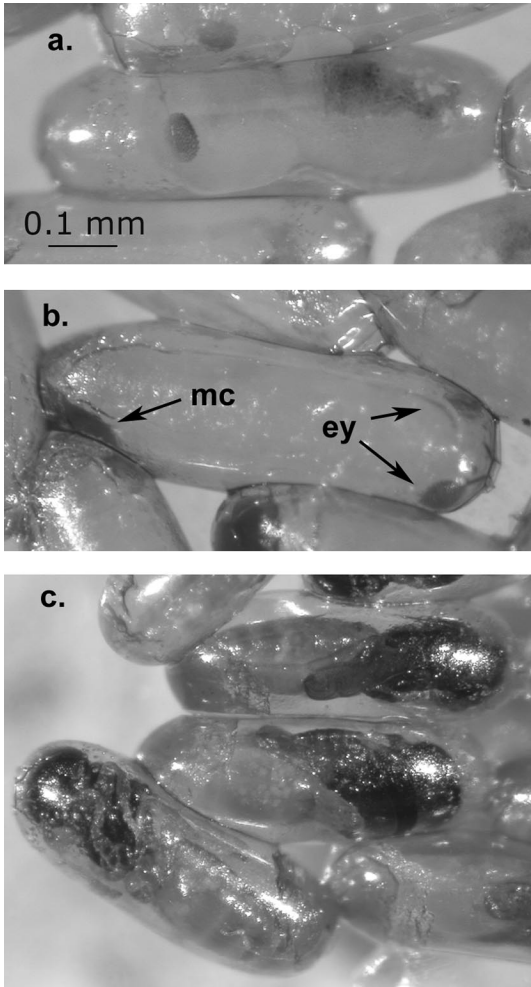

Fig. 5. Exarate pupae. (a) Young white pupa. (b) Elongated white pupa. (c) Mature pupa. ey, eye; mc, meconium.
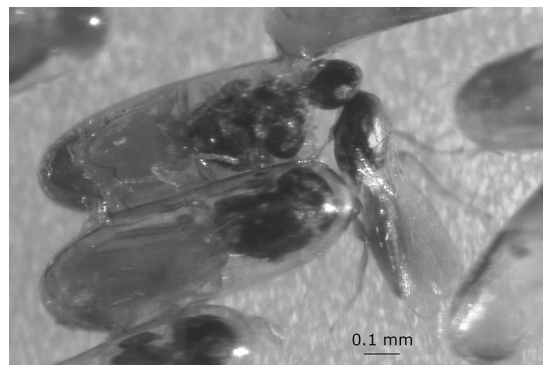

Fig. 6. Recently emerged adult male chewing exit hole for a sister.

common in eggs parasitized by platygastrids (Austin et al. 2005). Other species of egg parasitoids of *D. abbreviatus* released in Florida do not cause this color change. Therefore, this trait may provide a tool to quickly estimate the level of parasitism in the field when this species is released from quarantine, provided that this trait does not change under field conditions.

Mating at the emergence site is very common among species that attack gregarious hosts or hosts living in discrete, well-defined patches (Godfray 1994). In such cases, it is not uncommon that males emerge first and wait for females to emerge. Males of *F. dominica* not only waited but also helped females exit the weevil egg. Such altruistic behavior also has been observed in other systems. Suzuki and Hiehata (1985) and Kurosu (1985) reported males of another egg parasitoid, *Trichogramma papilionis* Nagarkatti (Hymenoptera: Trichogrammatidae), helping females emerge by pulling their bodies through a hole chewed in the chorion.

Acknowledgments

References Cited

Askew, R. R. 1971. Parasitic insects. Elsevier, New York.

Austin, A. D., N. F. Johnson, and M. Dowton. 2005. Systematics, evolution, and biology of scelionid and platygastrid wasps. Annu. Rev. Entomol. 50: 553–582.

Beavers, J. B., S. A. Lovestrand, and A. G. Selhime. 1980. Establishment of the exotic parasite *Tetrastichus haitiensis* (Hym: Eulophidae) and recovery of a new *Trichogramma* (Hym: Trichogrammatidae) from root weevil egg masses in Florida. Entomophaga 25: 91–94.

Castillo, J., J. A. Jacas, J. E. Peña, B. J. Ulmer, and D. G. Hall. 2006. Effect of temperature on life history of *Quadrastichus haitiensis* (Hymenoptera: Eulophidae), an endoparasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biol. Control 36: 189–196.

Clancy, D. W. 1944. Biology of *Allotropa burrelli,* a gregarious parasite of *Pseudococcus comstocki.* J. Agric. Res. 69: 159–167.

Clausen, C. C. 1940. Entomophagous insects. McGraw-Hill, New York.

Dyar, H. G. 1890. The number of moults of lepidopterous larvae. Psyche 5: 420–422.

Evans, G. A., and J. E. Peña. 2005. A new *Fidiobia* species (Hymenoptera: Platygastridae) reared from eggs of *Diaprepes doubleirii* (Coleoptera: Curculionidae) from Dominica. Fla. Entomol. 88: 61–66.

Godfray, H.C.J. 1994. Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ.

Grafton-Cardwell, E., K. Godfrey, J. E. Peña, C. McCoy, and R. Luck. 2004. Diaprepes root weevil. University of California, Agricultural and Natural Resources Publ. 8131.

Hall, D., J. E. Peña, R. Franqui, R. Nguyen, P. Stansly, C. Mccoy, S. Lapointe, R. Adair, and B. Bullock. 2001. Status of biological control by egg parasitoids of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in citrus in Florida and Puerto Rico. BioControl 46: 61–70.

Hall, D. G., J. Eger, J. E. Peña, R. Duncan, C. O'Brien, G. Evans, and C. McCoy. 2002. Exploration in Belize for parasitoids attacking eggs of citrus weevils and an evaluation of *Pediobious irregularis* and *Horismenus bennetti* (Hymenoptera: Eulophidae) as potential biological control agents of *Diaprepes abbreviatus* and *Pachnaeus litus* (Coleoptera: Curculionidae). Fla. Entomol. 85: 663–666.

Jacas, J. A., J. E. Peña, and R. E. Duncan. 2005. Successful oviposition and reproductive biology of *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae): a predator of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) Biol. Control 33: 352–359.

Kurosu, U. 1985. Male altruism and wing polymorphism in a parasitic wasp. J. Ethol. 3: 11–19.

Masner, L. 1995. The proctotrupoid families, pp. 209–265. *In* P. E. Hanson and I. D. Gauld [eds.], The Hymenoptera of Costa Rica. Oxford University Press, Oxford, United Kingdom.

Pennacchio, F., and M. R. Strand. 2006. Evolution of developmental strategies in parasitic Hymenoptera. Annu. Rev. Entomol. 51: 233–258.

Peña, J. E., J. Étienne, R. Duncan, and J. Pinto. 1998. Introduction of *Ceratogramma etiennei* (Hymenoptera: Trichogrammatidae) for biological control of *Diaprepes abbreviatus* in Florida, USA, pp. 145–148. *In* S. Hassan [ed.], Egg parasitoids, 5th International Symposium, 4–7 April 1998, IIBC, Cali, Colombia. Parey Buchverlag, Berlin, Germany.

Peña, J. E., and D. M. Amalin. 2000. Biological control of *Diaprepes abbreviatus* by parasitoids, pp. 66–76. *In* S. H. Futch [ed.], *Diaprepes* short course, 22 March 2000, Cooperative Extension Service Florida Agricultural Experiment Station. Citrus Research & Education Center, Lake Alfred, FL.

Sampson, B. J., T. A. Rinehart, O. E. Liburd, S. J. Stringer, and J. M. Spiers. 2006. Biology of parasitoids (Hymenoptera) attacking *Dasineura oxycoccana* and *Prodiplosis vaccinii* (Diptera: Cecidomyiidae) in cultivated blueberries. Ann. Entomol. Soc. Am. 99: 113–120.

Suzuki, Y., and K. Hiehata. 1985. Mating systems and sex ratios in the egg parasitoids, *Trichogramma dendrolimi* and *T. papilionis* (Hymenoptera: Trichogrammatidae). Anim. Behav. 33: 1223–1227.

Woodruff, R. E. 1968. The present status of a West Indian weevil (*Diaprepes abbreviatus* (L.) in Florida. Fla. Dep. Agric. Div. Plant Ind. Entomol. No. 77.