

Pupal Orientation and Emergence of Some Aphelinid Parasitoids (Hymenoptera: Aphelinidae) of *Bemisia argentifolii* (Homoptera: Aleyrodidae)

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ABSTRACT Emergences of *Encarsia pergandiella* Howard and *E. formosa* Gahan were compared; and pupal orientation of several *Encarsia* and *Eretmocerus* species were examined. The species examined for pupal orientation were *E. pergandiella* from southwest Florida, *E. nr. pergandiella* from Brazil, *E. transvena* (Timberlake) from Florida, *E. formosa* from California (Davis), *E. formosa* from Georgia (Griffin), *E. formosa* from Nile Delta, Egypt, *Eretmocerus* sp. from Hong Kong, *Eretmocerus* sp. from Padappai, India, *E. mundus* (Mercet) from Murcia, Spain, *E. nr. californicus* Howard from Arizona (Tucson), parasitoids of *Bemisia argentifolii* Bellows & Perring [previously *B. tabaci* (Gennadius)]. The majority (98%) of *E. pergandiella* pupae from Florida and all other *Encarsia* species except *E. nr. pergandiella* from Brazil pupated facing the host venter, regardless of leaf orientation, so that rotation was necessary before emergence. *E. nr. pergandiella* from Brazil, 2% of *E. pergandiella* from Florida, and all species of *Eretmocerus* pupated facing the host dorsum, so that no rotation was necessary. All *E. pergandiella* pupae and adults from Florida were pigmented similarly regardless of pupal orientation. Less than 1% of *E. pergandiella* female pupae were oriented backwards, i.e., with the head toward the host posterior, but again, these were not distinguishable characteristics of color or form. We concluded that a low incidence of unusual pupal orientations represented natural variation in the population of *E. pergandiella* we studied.

KEY WORDS *Bemisia argentifolii*, *Bemisia tabaci*, adult emergence, pupal orientation, behavior

Encarsia pergandiella Howard, *E. transvena* (Timberlake), *E. formosa* Gahan, and *Eretmocerus* spp., are parasitoids of the whitefly, *Bemisia argentifolii* Bellows & Perring, the sweetpotato whitefly, *B. tabaci* (Gennadius), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). They can play a major role in suppression of whitefly populations in the field and greenhouse (Evans 1993, McAuslane et al. 1993, Stansly et al. 1994). Successful manipulation of parasitoid populations for enhanced biological control requires more detailed biological and taxonomic information than presently available. Furthermore, biology and taxonomy may be related in subtle ways. For example, Johnson (1996) found 2 color forms of *E. pergandiella* in Texas. Pupae of the more common form faced the host venter and had pigmented abdominal tergites both as pupae and adults. Abdominal tergites of the less common light form were entirely yellow and pupae were oriented facing the host dorsum. Johnson (1996) concluded that the 2 species were characterized by these and other distinctions, and demonstrated mating incompatibilities in reciprocal crosses.

Variations in pupal orientation occur in several families of hymenopteran parasitoids and have

been reported in the literature. Rosen and DeBach (1979) reported that pupae of *Aphytis* spp. (Aphelinidae) in armored scale insects (Homoptera: Diaspididae) always face the dorsum of the host although they may be oriented in different positions around the dorsoventral axis. Corrigan et al. (1990) reported that when egg masses of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), faced down, 98% of the egg parasitoid *Edovum puttleri* Grissell (Eulophidae) pupated face down in egg masses on the lower leaf surface, whereas 37% pupated face up in egg masses on the upper leaf surface. Lashomb et al. (1987) and Maini and Nicoli (1990) reported that the *E. puttleri* adults failed to emerge if the larvae pupated with their heads pointed toward the leaf surface. Ishay (1975) also found that 80% of *Vespa orientalis* F. pupae failing to emerge were oriented with the head toward the exterior of the comb.

Motivated by the discovery in Texas (Johnson 1996), we examined our colony of *E. pergandiella* in southwest Florida and also found a few of female pupae facing the dorsum of the puparium of the host. We proceeded to study the development and emergence process of *E. pergandiella* in more detail in comparison to related aphelinids. Avail-

Table 1. Parasitoid species studied

Parasitoid species	Host insects	Host plants	Original localities	Collected by	Determined by
<i>E. pergandiella</i>	<i>B. argentifolii</i>	Tomato, eggplant, cucumber, collard, salvia, watermelon, sweet potato, hibiscus	Florida (Immokalee)	T.-X.L.	G. Evans
<i>E. nr. pergandiella</i> ^a	<i>B. argentifolii</i>	Poinsettia, soybean, hibiscus	Sete Lagoas, Brazil	M. Rose	M. Rose, J. Wooley
<i>E. formosa</i>	<i>B. argentifolii</i>	Poinsettia, sweet potato	California (Davis)	J. Nelson	T.-X.L.
<i>E. formosa</i>	<i>T. vaporariorum</i>	Poinsettia, salvia, tomato	Georgia (Griffin)	R. Oetting	T.-X.L.
	<i>B. argentifolii</i>	Sweet potato, salvia, tomato	Florida	T.-X.L.	T.-X.L.
<i>E. formosa</i> ^a	<i>B. argentifolii</i>	Hibiscus, lantana	Nile Delta, Egypt	Kirk and Lacey	M. Schauff
<i>E. transvena</i>	<i>B. argentifolii</i>	Hibiscus, sweet potato, salvia, eggplant, collard	Florida	T.-X.L., Nguyen	G. Evans
<i>Eretmocerus</i> sp.	<i>B. argentifolii</i>	Hibiscus	Hong Kong	H. McAuslane	M. Rose
<i>Eretmocerus</i> sp. A ^a	<i>B. argentifolii</i>	Hibiscus, eggplant	Padappai, India	Kirk and Lacey	M. Schauff
<i>E. mundus</i> ^a	<i>B. argentifolii</i>	Hibiscus, cotton	Murcia, Spain	Kirk and Lacey	M. Schauff, M. Rose
<i>E. nr. californicus</i>	<i>T. vaporariorum</i>	Tobacco	Arizona (Tucson)	O. Minkenber	M. Rose
	<i>B. argentifolii</i>	Sweet potato	Florida	T.-X.L.	T.-X.L.

^aSpecimens were supplied by USDA-APHIS-PPQ, Mission Biological Control Center, Mission, TX.

able strains parasitizing *B. argentifolii* included *E. nr. pergandiella* from Brazil, *E. transvena* from Florida, *E. formosa* from California, Georgia, and Egypt, *Eretmocerus* sp. from Hong Kong, *Eretmocerus* sp. A. from India, *E. mundus* and *E. nr. californicus* from Arizona, and parasitizing *T. vaporariorum*, *E. formosa* from Georgia, and *E. nr. californicus* from Arizona.

Materials and Methods

Emergence. Native *E. pergandiella* from southwest Florida that naturally had parasitized a greenhouse colony of *B. argentifolii* in 1993 were collected from several host plant species, including collard, *Brassica oleracea* L. variety *acephala*, 'Georgia LS'; hibiscus, *Hibiscus rosa-sinensis* L., 'Brilliant Red'; tomato, *Lycopersicon esculentum* Miller, 'Lanai'; and sweet potato, *Ipomoea batatas* L., 'Carolina Bunch'. *E. formosa* was collected from a colony of *B. argentifolii* on poinsettias, *Euphorbia pulcherrima* Wild., in California (provided by J. Nelson, University of California at Davis). Adults were allowed to emerge and then introduced into a wood frame cage (60 by 60 by 60 cm) enclosed with 52-mesh polyethylene screen (Lumite, Chiopee, Gainesville, GA) containing potted eggplants. Parasitized pupae were collected, and parasitoid development and emergence recorded using a Sony EV-C100 video cassette recorder (Sony, Tokyo), and viewed on a Sony Monitor connected with a camera (Javelin, Chiromachip, JE-3662RGB, Los Angeles, CA) mounted on a stereo microscope (Leica, Wild M32, Heerbrugg, Switzerland). The major sequences were described and illustrated.

Pupal Orientation. Host species, collection and identification data for parasitoids observed are given in Table 1. All were from recent field collections

maintained subsequently in greenhouse or screen house culture on their respective whitefly hosts. Observations were made directly on live specimens under a stereo microscope, or by detaching whitefly puparia from the leaf surface and fixing to a microscopic slide (75 by 25 mm) with double-coated cellophane tape (3M Scotch, St. Paul, MN). Dissections were made using two 000 insect pins mounted on applicator sticks. Pupal orientation of *E. pergandiella* and *E. formosa* pupae in their *B. argentifolii* hosts on abaxial and adaxial leaf surface were compared.

Parasitoids used for observation were maintained in an air conditioned insectary at $23 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h. Voucher specimens of parasitoids and whiteflies were deposited in the Insect Collection at Southwest Florida Research and Education Center, University of Florida at Immokalee.

Results

Coloration of *E. pergandiella*. Female pupae from Florida were yellowish with dark markings on the pronotum and the dorsal surface of the abdomen which varied through a range of intensity to none at all. Male pupae and pharate adult males inside the whitefly puparium were uniformly yellow with an inverted dark triangle on the dorsal surface of the mesoscutum and with a dark spot on the dorsal surface of the abdomen. A layer of black pigment attached to the inner surface of the whitefly puparium gave an appearance of a large dark area on the dorsal surface of the abdomen of some pupae. Dark pigmentation on abdominal tergites of all adult female *E. pergandiella* from Florida was clearly visible on either fresh or slide-mounted specimens, in contrast to *E. nr. pergandiella* which is entirely yellow.

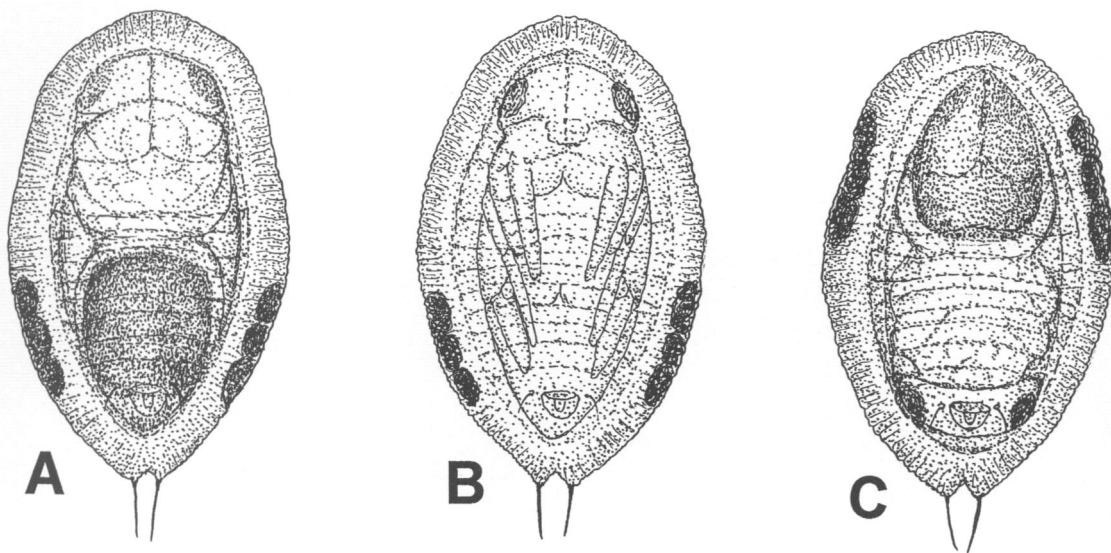


Fig. 1. Observed orientations of *E. pergandiella* pupae inside host puparium. (A) Facing host venter, head anterior. (B) Facing host dorsum, head anterior. (C) Facing host venter, head posterior.

Pupal Orientation. Few morphological characters were discernable when viewing parasitoid pupae facing the host venter (Fig. 1 A and C). The head, thorax, and abdomen were recognizable, but not distinctly defined, particularly the thoracic and abdominal segments. In contrast, the geniculate antennae, mouthparts, and legs and wing tips were distinct on pupae facing the dorsum (Fig. 1B). Sternal plates were also evident but less distinct.

Ninety-eight percent of all female *E. pergandiella* pupae from Florida, and all *E. formosa* from California, Georgia, and Egypt, and *E. transvena*

from Florida observed on abaxial (lower) leaf surfaces developed facing the venter of the host puparium (Table 2). *E. nr. pergandiella* from Brazil all developed facing the dorsum of the host (Fig. 1B; Table 2). All species of *Eretmocerus* faced the dorsum of the host. Male *E. pergandiella* pupae ($n = 113$) all faced the dorsum of the host puparium. Of 254 *E. pergandiella* pupae from Florida on adaxial (upper) leaf surfaces of 19 eggplant and tomato leaves, only 5 (2.0%) faced the dorsum as did 100% ($n = 52$) of all *E. formosa* from California in this position. Thus, we could find no evidence for any effect of leaf orientation on pupal orientation within the host.

Table 2. Pupal orientation on abaxial and adaxial leaf surfaces of several aphelinid parasitoid species of *B. argentifolii*

Parasitoid species	Pupae	Pupal orientation, %	
		Pupa facing host dorsum	Head toward anterior
On abaxial leaf surface			
<i>E. pergandiella</i> (Florida)	5,794	2.0	99.6
<i>E. nr. pergandiella</i> (Brazil)	245	100.0	100.0
<i>E. formosa</i> (California)	824	0.0	100.0
<i>E. formosa</i> (Georgia) ^a	523	0.0	100.0
<i>E. formosa</i> (Egypt)	115	0.0	100.0
<i>E. transvena</i> (Florida)	267	0.0	100.0
<i>Eretmocerus</i> sp. (Hong Kong)	232	100.0	100.0
<i>Eretmocerus</i> sp. A (India)	212	100.0	100.0
<i>E. nr. californicus</i> (Arizona) ^a	223	100.0	100.0
<i>E. nr. californicus</i> (Arizona)	105	100.0	100.0
<i>E. mundus</i> (Spain)	223	100.0	100.0
On adaxial leaf surface			
<i>E. pergandiella</i> (Florida)	254	2.0	98.8
<i>E. formosa</i> (California)	52	0.0	100.0

^aHost whitefly was *T. vaporariorum*.

The preponderance of female *E. pergandiella* pupae observed were oriented parallel to the host with <1% headed backward toward the host posterior (Table 2). No pupae in the latter position happened to be facing the host dorsum. We could not discern any consistent color variation in *E. pergandiella* female adults corresponding to their orientation as pupae in either the rare dorsum-facing or backward positions, although dorsum-facing pupae were apparently light-colored because the dark sternum faced the host venter.

Pupation of *Encarsia* spp. After positioning themselves head forward in the host puparium, pre-pupae of *E. pergandiella*, *E. formosa*, and *E. transvena* commenced a series of undulating motions excreting meconium inside the lower, lateral margins of the host remains. Ten to 35 min was required for the completion of a defecation cycle ($n = 3$ per species) each resulting in deposition of a meconium pellet for a total of from 2 to 4 pellets on each side.

Emergence of Adult *Encarsia* spp. The exarate pupa excreted a fluid inside the pupal case just

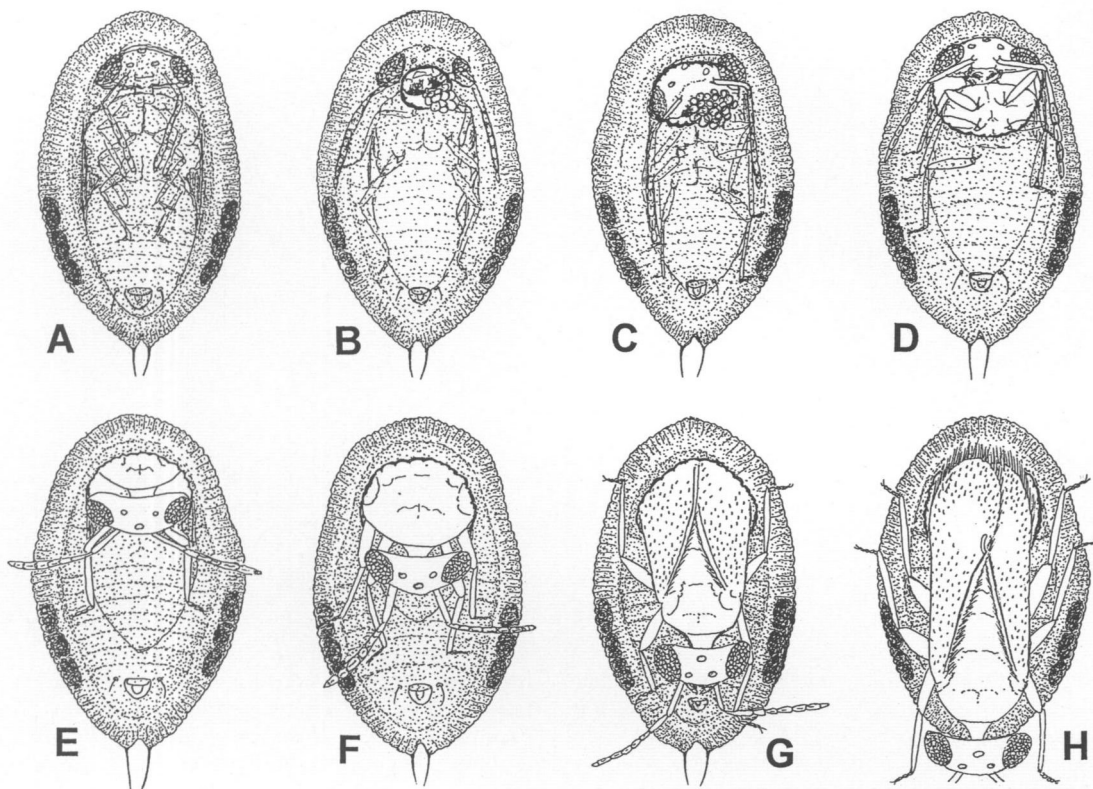


Fig. 2. Postrotation emergence of female *E. pergandiella*. (A) Pharate at rest. (B) Initiation of emergence hole. (C and D) Enlargement of emergence hole. (E-H) Escape from puparium.

before eclosion in situ. The fluid appeared to serve as a lubricant and a solvent helping the pharate adult struggle free from pupal exuvia.

Rotation. The pharate female *E. pergandiella* moved the head from side to side 10–60 min before commencing rotation. Head movements gradually became more vigorous. At the same time, the body moved from side to side, using the legs, first the front legs and later the middle and hind legs, pushing against the side wall of the host puparium to allow the thorax, and lastly, the abdomen free from the pupal case. Rotation was complete in 25–98 min ($n = 3$).

Emergence. A few minutes were spent following rotation adjusting position of the appendages before head movements recommenced, accompanied by movement of mouthparts and front legs (Fig. 2A). Head and front legs moved in synchrony to thrust the mandibles against the host puparium wall which was soon pierced (Fig. 2B). The female then began chewing along the edge of the resulting hole, accumulating debris around the edge. Enlargement of the opening allowed longer and more vigorous movement the head and front legs (Fig. 2 C and D). Between 61 and 92 min ($n = 4$) was required for completion of the emergence hole. The parasitoid appeared to test the dimensions of the hole by pushing the head along the edge be-

fore attempting to emerge, head first, followed by antennae, front legs, middle legs, hind legs, and finally entire body (Fig. 2 E–H). Pupae facing the dorsum of the host puparium emerged as above without the necessity of rotation.

The newly emerged wasp remained motionless on top of the whitefly puparium for 2–15 min allowing the cuticle to dry and harden. Grooming on or off the host pupal case commenced first with the hind legs sweeping alternatively over the upper and lower wing surfaces. Later antennae, mouthparts, and neck and middle legs were groomed by the front legs, whereas the middle legs were used to groom the hind legs. More than 40 min were spent grooming the wings, and ≈ 6 min each for the antennae and legs during the 1st h after emergence.

Discussion

Gerling (1966a) reported rotation of *E. pergandiella* and *E. californicus* (Gerling 1966b) inside their *T. vaporariorum* hosts, although he stated that it was the pupa that rotated. We observed the adult already free from the pupal exuvia before the onset of rotation and no rotation of *E. nr. californicus*, which developed facing the dorsum of both *B. argentifolii* and *T. vaporariorum*. Gerling and

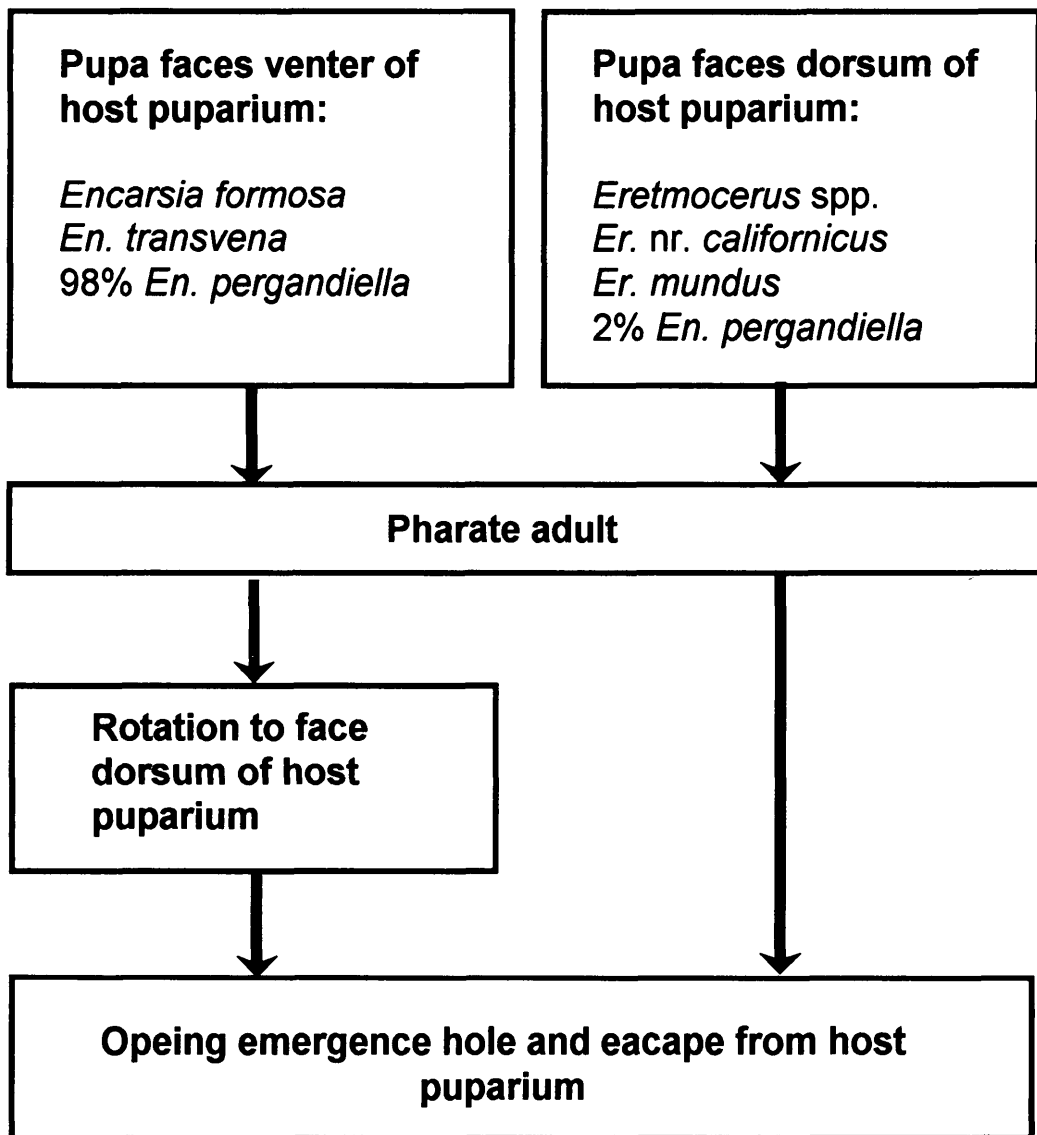


Fig. 3. Summary of pupal orientation and emergence in some species of *Encarsia* and *Eretmocerus*.

Foltyn (1987) and Gerling (1990) later reported rotation by pharate adults of *E. lutea* (Masi) and *E. mundus* within their *Bemisia* hosts.

We only observed a small percentage of *E. pergandiella* pupae facing the host dorsum in the population we studied. This orientation did not appear to be related to variation in form or color. Some variation in pupal orientation might be found in other aphelinid populations if enough individuals were observed. The backward facing orientation is another variation, also observed in some species of *Encarsia*, although as an exception rather than the rule (Viggiani 1984). However, sibling species exhibiting distinctive pupal orientations could occur elsewhere, just as they do with *E. pergandiella* from Florida and *E. nr. pergandiella* from Brazil.

Many if not most *Encarsia* parasitoids of *B. argentifolii* appear to pupate facing the host venter, necessitating the extra step of rotation during emergence (Fig. 3). There must be some risk involved in rotation, although we did not observe evidence of failure. What compensatory benefit face-down pupation has for *Encarsia* species, or why *Eretmocerus* species are different has yet to be determined.

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