MORPHOLOGY, HISTOLOGY, AND FINE STRUCTURE

Morphology and Development of the Immature Stages of Brachyufens osborni (Hymenoptera: Trichogrammatidae), an Egg Parasitoid of Broad-Nosed Weevil Species (Coleoptera: Curculionidae)

JOSEP-ANTON JACAS, JORGE E. PEÑA, AND RITA E. DUNCAN


ABSTRACT The external morphology and development of the immature stages of Brachyufens osborni (Dozier) (Hymenoptera: Trichogrammatidae), a Nearctic solitary endoparasitoid of the eggs of Pachnaeus spp. (Coleoptera: Curculionidae), are reported. Under our laboratory conditions (25°C and a photoperiod of 12:12 [L:D] h), B. osborni reared in eggs of Pachnaeus litus (Germar) (Entiminae) had a developmental time of 14.7 d. Egg hatching occurred within 15 h from oviposition and mandible measurements indicated the existence of two instars. First instars were mymariform, whereas second instars were saccoform. Pupation occurred 5–6 d after egg hatching and adults emerged 8 d later. On completion of the preimaginal development, the meconium was expelled and emergence took place by biting a hole in the chorion of the host egg. Both sexes emerged at the same time, and the sex ratio was female biased (3:1).

KEY WORDS Pachnaeus litus, Diaprepes abbreviatus, biological control, egg parasitoid

Brachyufens osborni (Dozier) (Hymenoptera: Trichogrammatidae: Trichogrammatinae) is an idiobiont endoparasitoid of weevil eggs restricted to the Nearctic region (Pitkin 2003, Owen et al. 2007). Dozier (1932) first described it from weevil eggs collected in Puerto Rico. The first record in the continental United States dates from 1959, when it was recovered from the indigenous root weevil Pachnaeus litus (Germar) (Coleoptera: Curculionidae: Entiminae) (Baranowski 1960). This endoparasitoid is common in Florida and is presumed to parasitize weevil egg masses of Diaprepes abbreviatus (L.), P. litus, and Pachnaeus opalus (Olivier) (Schauff 1987). Because parasitism on P. litus can be as high as 81% (Baranowski 1960), this parasitoid may be useful for the biological control of the exotic weevil D. abbreviatus. Currently, investigations are underway to ascertain its actual impact on field populations of this insect pest in Florida (J.A.J., J.E.P., and R.E.D., unpublished data).

A few genera of Trichogrammatidae are of interest for use in biological control, especially the genus Trichogramma Westwood. Although Trichogramma species are the most widely used natural enemy in inundative biological control programs (Heraty 2007), little is known about the preimaginal morphology and development of most of this and other genera, including B. osborni. The eggs of Trichogrammatidae described so far are somewhat elongate with a distinctly expanded middle and rounded ends (Flanders 1937, Clausen 1940, Pitkin 2003). The eggs can be pedunculated and when present, the form and length of the peduncle can be highly variable (Silvestri 1916, Bakkendorf 1933-34). The number of instars within this family is subject to controversy (Jarjees and Merritt 2002); only a single instar has been described for Chaetostrichia pulchra Kryger (Bakkendorf 1933–34), Oligosita utilis Kowalski (Taylor 1937), Prestuchiaca aquatica Lubbock (Saakian-Baranova 1991), and Trichogramma australicum Girault (Dahan and Gordh 1996), but five for Poropoera stollwecki Förster (Silvestri 1916). First instars can be either saccoform or mymariform (Clausen 1940). Additional instars can be of either type, but mature larvae are invariably similar in form, being robust, more or less distinctly segmented, and lacking spines or setae (Clausen 1940). Pupation takes place within the remains of the host egg and the adult parasitoid emerges by biting a hole in the chorion of this egg.

Aside from these brief descriptions of larval morphology and biology, little is known about the preimaginal stages of this important group of natural enemies. To fill this gap in our knowledge, we studied external morphology and development of the immature stages of B. osborni. Our goal in this study is to provide important biological information on B. osborni that could be useful in assessing the potential of this species as a manipulated natural enemy for the control of D. abbreviatus.

1 Departament de Ciències Agràries i del Medi Natural, Campus del Riu Sec, Universitat Jaume I, E-12071-Castelló de la Plana, Spain.
2 Corresponding author, e-mail: jacas@canm.uji.es.
3 Department of Entomology and Nematology, Tropical Research and Education Center, University of Florida, 18905 SW 250th St., Homestead, FL 33031.

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Materials and Methods

Specimens used in this study were obtained from a colony initiated from individuals of *B. osborni* collected in different citrus orchards at the University of Florida Research and Education Center (TREC), Homestead, FL (25° 30’ N, 80° 30’ W, 1-m altitude).

The colony was maintained on *P. litus* eggs in the insectary facilities of TREC at 26 ± 1°C, a 12-h light photoperiod, and 65% RH. Voucher specimens of *B. osborni* were deposited in the Florida Collection of Arthropods, Gainesville, FL, and the U.S. National Museum of Natural History (USNM), Washington, DC.

**Stock Colonies.** Adult *P. litus* root weevils were collected from the foliage of citrus trees at TREC. The weevils were placed in 30 *×* 30 *×* 30 cm Plexiglas cages with distilled water and foliage of *Conocarpus erectus* L. (Myrtales: Combretaceae) that provided a food source and an oviposition substrate for the weevils. Foliage was replaced every 2–3 d. Foliage containing *P. litus* egg masses was removed and placed inside a similar cage. Adults of *B. osborni* were introduced into the cage and provided honey (streaked on paper placed on the inside wall of the cage) and distilled water (dispensed through a saturated cotton wick in a vial placed on the bottom of the cage). Egg masses that were exposed to adult wasps and assumed to be parasitized were removed from the cage 4–5 d later and placed in emergence cages (same dimensions as above) supplied with water and honey and maintained until adult parasitoid emergence.

**Experimental Specimens.** Foliage of *C. erectus* with <1-d-old *P. litus* eggs was placed into the *B. osborni* stock colony cage for 4 h. Subsequently, we removed the foliage supporting the egg masses, cut the foliage into 1–2-cm² pieces containing eggs, and placed the pieces individually into glass vials (Flint Glass Printed White 12- by 75-mm Disposable Culture Tubes, Fisherbrand, Thermo Fisher Scientific, Waltham, MA). The open end of the test tube was covered with two ply of Kimwipe (Kimwipes EX-L, Kimberly-Clarke Corp., Roswell, GA) and secured with rubber tubing to allow ventilation. Vials were kept in a climatic chamber at 25°C and 85% RH under a 12-h light photoperiod until larval dissections were performed. Parasitized eggs were collected as often as necessary to obtain enough specimens for dissection at various time intervals.

Eggs, presumably parasitized by *B. osborni*, were dissected at different time intervals until egg hatching (1, 6, 10, 13, and 18 h after oviposition), as indicated in Table 1. Eggs were checked under a stereomicroscope (MZ6, Leica Microsystems GmbH, Wetzlar, Germany) with a cold light source (CLS100, Leica). Nonparasitized hosts were rejected, and the inspections were finished once 10–15 specimens of *B. osborni* had been obtained. These immature stages were mounted on a glass microscope slide in PVA mounting medium (BioQuip Products, Rancho Dominguez, CA) for observation using a light microscope. Immature stages were measured by use of the Eclipse Net software (Nikon, Tokyo, Japan). The number of instars was determined based on the length of the mandibles (Dyar 1890). Analysis of variance was used to analyze the data. Developmental times were established for each stage.

### Table 1. Mandible measurements of larvae of *B. osborni* at different time intervals after oviposition (age)

<table>
<thead>
<tr>
<th><em>B. osborni</em> age</th>
<th>Mandible length (μm)</th>
<th>n</th>
<th>Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 h</td>
<td>5.7 ± 0.2 b</td>
<td>14</td>
<td>First</td>
</tr>
<tr>
<td>18 h</td>
<td>5.6 ± 0.1 b</td>
<td>10</td>
<td>First</td>
</tr>
<tr>
<td>36 h</td>
<td>5.7 ± 0.1 b</td>
<td>10</td>
<td>First</td>
</tr>
<tr>
<td>48 h</td>
<td>Mandibles not found</td>
<td>10</td>
<td>Second</td>
</tr>
<tr>
<td>55 h</td>
<td>Mandibles not found</td>
<td>10</td>
<td>Second</td>
</tr>
<tr>
<td>60 h</td>
<td>Mandibles not found</td>
<td>10</td>
<td>Second</td>
</tr>
<tr>
<td>69 h</td>
<td>51.0 ± 1.0 a</td>
<td>4</td>
<td>Second</td>
</tr>
<tr>
<td>72 h</td>
<td>48.3 ± 0.3 a</td>
<td>10</td>
<td>Second</td>
</tr>
<tr>
<td>84 h</td>
<td>47.4 ± 2.0 a</td>
<td>10</td>
<td>Second</td>
</tr>
<tr>
<td>96 h</td>
<td>50.1 ± 1.0 a</td>
<td>10</td>
<td>Second</td>
</tr>
<tr>
<td>120 h</td>
<td>47.8 ± 1.1 a</td>
<td>11</td>
<td>Second</td>
</tr>
<tr>
<td>144 h</td>
<td>50.5 ± 0.5 a</td>
<td>10</td>
<td>Second</td>
</tr>
</tbody>
</table>

ANOVA results: K-W: 68.97, P = 7.9 × 10⁻⁶

* Mandibles visible in only four larvae of 10 inspected.

**Results**

Under our laboratory conditions (25 ± 1°C and a photoperiod of 12:12 [L:D] h), *B. osborni* reared on eggs of *P. litus* had a developmental time of 14.7 ± 0.1 d (*n* = 32) from oviposition to adult emergence. Parasitized eggs could be recognized as early as 6 h after parasitism by the presence of small scars (Fig. 1a), presumably corresponding to either oviposition or feeding stings inflicted by female parasitoids. After parasitoid oviposition, further development of the host ceased. When dissected, parasitized eggs had a whitish appearance and an unorganized structure internally, whereas unparasitized eggs had a more opaque white aspect and showed some internal tissue organization. As time progressed, differences became more conspicuous and the *P. litus* embryo could be observed in unparasitized eggs, whereas *B. osborni* first instars could be seen in parasitized eggs.

**Egg.** The egg stage under our laboratory conditions lasted 12.5 ± 0.3 h (*n* = 31). Recently laid eggs (1 h old) of *B. osborni* averaged 193 ± 4 μm in length by 41 ± 2 μm in width at the widest point (*n* = 11) and were almost transparent (Fig. 1b, c). The egg was spindle-shaped and had a short pedicel. Further measurements as hatching approached (6 and 10 h old) indicated no increase in egg length (*F* = 0.20; df = 2, 36; *P* = 0.8191) or width (*F* = 2.33; df = 2, 36; *P* = 0.1125). The majority of hosts (99%; *n* = 185 host eggs) supported only a single parasitoid egg, but two hosts were found with three eggs. Similarly, only in one case did we find both an egg and a neonate larva in the same host egg (13-h old). Coincidence of further developed immature stages within the same host was never
found. Correspondingly, no more than one adult emerged from parasitized hosts.

Larva. Mandible and body measurements (Table 1; Fig. 2) indicated that *B. osborni* has two instars. The neonate, or first instar, was mymarid (Clausen 1940) (Fig. 3a). Early first instars, found in 13 h old eggs, had an enlarged head and thorax (69 ± 3 μm in width; 139 ± 6 μm in length; *n* = 9). The anterior part of the larval head consisted of a conical process which bore sickle-shaped mandibles (5.7 ± 0.1 μm in length; *n* = 35) (Fig. 3b). The abdomen was much slenderer than the thorax, tapered posteriorly and was reduced to five segments (Fig. 3c). The caudal segment was long and thin with one long spine-like process. Another similar spine was found dorsally on the fourth abdominal segment, and many long setae were present on the abdomen. The young larvae moved vigorously within the host egg by ventral and backward thrusts of the abdomen. As time passed, larvae became bloated (Fig. 3d and e). At the end of the instar, which took 29.7 ± 0.7 h (*n* = 32), larvae had almost doubled in size and measured 219 ± 6 μm in length and 128 ± 6 μm in width (*n* = 10).

The second instar was sacciform (Fig. 4a and b), lacked apparent segmentation, and measured 318 ± 15 μm in length and 169 ± 4 μm in width when young (48-h-old parasitized eggs; *n* = 11). Second instars had relatively large mandibles (49.2 ± 0.5 μm in length; *n* = 61) (Fig. 4c) that were difficult to distinguish before they were completely sclerotized at ≈24 h after the onset of this instar (Table 1; Fig. 4d). During this 24-h postmolt period, larvae increased enormously in size (1062 ± 26 μm in length at 72 h) (Fig. 2). Later growth leveled off and remained fairly constant (Fig. 2). By the end of the second instar, which lasted for 97.3 ± 1.0 h (*n* = 30), the larva had consumed the entire contents of its host egg, leaving only the egg chorion, which gradually assumed an amber color. Just before pupation, the larva, which occupied the entire host egg, shrunk (1198 ± 22 μm in length and 388 ± 10 μm in width; *n* = 9), and a free space occurred in the interior of the egg chorion (Fig. 4e).

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**Fig. 1.** (a) Eggs of *P. litus* showing scars (arrows), typical symptoms of parasitism by *B. osborni*. (b and c) Less than 2-h-old eggs of *B. osborni*.  
**Fig. 2.** Preimaginal developmental times of *B. osborni* at 25°C. Larval length at different time intervals after oviposition.
Pupa. After sclerotization, the exarate pupa of *B. osborni* was dark with red eyes (Fig. 5a). Pupae were first observed at 168 h postparasitization and occupied almost all available space within the egg chorion (Fig. 5b). Eventually, the typical black stripes on the abdomen were revealed. Mature pupae averaged 1085 ± 13 μm in length and 341 ± 7 μm in width (n = 10) (Fig. 5c), and adult characteristics could be easily recognized through the amber colored egg shell. Upon com-

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**Fig. 3.** First instars of *B. osborni*. (a) Neonate mymariform larva with enlarged cephalothorax showing the head produced in front into a conical process and slender abdomen with cauda. (b) Sickle-shaped mandibles. (c) Abdominal segments I to V. (d) Twelve-hour-old larva. (e) Twenty-four-hour-old larva.

**Fig. 4.** Second instars of *B. osborni*. (a) Larva within the host egg (arrow). (b) Sacciform larva. (c) Mouth showing mandibles. (d) Sixty-hour-old larva within the egg chorion showing mandibles (arrow). (e) Shrinking late second instar larvae within the host egg.
pletion of pupal development the adult chewed an emergence hole in the host egg chorion. Both sexes emerged simultaneously and sex ratio was strongly female biased (3:1). The meconium was expelled just before emergence and could be observed through the empty host egg chorion. The pupal stage lasted 192.0 ± 0.7 h (n = 30).

Discussion

Trichogrammatinae are almost exclusively egg parasitoids (Pennacchio and Strand 2006, Pitkin 2003). This relatively short-lasting host stage provides a narrow window of opportunity for the parasitoid development (Strand 1986). Egg hatching of *B. osborni* occurred between 11 and 15 h after oviposition, and larvae consumed the host egg contents in approximately five more days. This is a comparatively short time considering that *P. litus* embryonic development takes ≈9 d (J.A.J., J.E.P., and R.E.D., unpublished data) but rather long compared with different *Trichogramma* species that take <3 d to reach the pupal stage (Flanders 1937, Manweiler 1986). Endoparasitoids frequently modify their host environment to suit their nutritional needs of the developing immature stages (Vinson and Iwantsch 1980). Some species maintain the host in a living state, whereas others, such as *B. osborni*, behave as idobiонт parasitoids, arresting host development upon parasitism. It is likely that female *B. osborni*, like other idobiонт parasitoids (Pennacchio and Strand 2006), probably inject a venom at oviposition that prevents further development of *P. litus* eggs. This behavior has been described previously for the genus *Trichogramma* (Strand 1986, Jarjees and Merritt 2003). As with another weevil egg parasitoid, *Fidiobia dominica* Evans & Peña (Hymenoptera: Platygasteridae) (Jacas et al. 2007), such a behavior could account for the distinctive absence of internal organization found in parasitized eggs soon after parasitism. Similarly, because superparasitism was not commonly observed in our assays, we think that females might inject host-marking substances during oviposition that would prevent subsequent parasitization.

The eggs of Trichogrammatidae are very small and contain no yolk (Flanders 1937, Klomp and Teerink 1967, Pak and Oatman 1982). These features are adaptations to egg parasitism that are compensated after oviposition by increasing their volume by absorption of host egg material (Fisher 1971). This situation did not occur for *B. osborni* and the size of the egg did not significantly change from oviposition to hatching. Egg size was similar to that reported for fully grown eggs in other Trichogrammatinae (Flanders 1937, Pak and Oatman 1982, Saakian-Baranova 1991, Dahlan and Gordh 1996). This lack of increase in volume might be linked to the short duration of the embryonic development of *B. osborni* (12.5 h), about half of that reported for other Trichogrammatinae (Flanders 1937, Pak and Oatman 1982, Strand 1986, Volkoff et al. 1995, Dahlan and Gordh 1996).

*B. osborni* develops through two instars, and this number fits within the range given for Trichogrammatinae (Volkoff et al. 1995, Jarjees and Merritt 2002). The first instar is mymariform and the second instar is sacciform. However, because no mandibles could be observed from ages 48–60 h, we cannot discard the existence of an extra instar which would coincide with the exponentially growing phase observed during this period (Fig. 2). Mandibles are sometimes difficult to observe in *Trichogramma* larvae (Pak and Oatman 1982, Manweiler 1986, Dahlan and Gordh 1996). Furthermore, amandibulate larvae have been described for second instars of aphidiine braconids (O’Donnell 1987). Mymariform first instars have been observed in other Trichogrammatinae [*e.g., Poropoea stollwecki* (Silvestri 1916, Grassé 1951)] but have been more extensively detected in Mymaridae parasitoids (Pitkin 2004). The morphology of the mymariform larvae has been described several times in different terms (Clausen 1940, Grassé 1951, Hagen 1964). Consistent with the description of Clausen (1940), larvae of *B. osborni* exhibit externally visible segmentation of the body. However, neither Grassé (1951) nor Hagen (1964) mentioned this feature, and Grassé (1951) considered the absence of mandibles a characteristic of...
the mymariform larval form. In contrast, Hagen (1964) described mymariform larvae as having mandibles, which is consistent with our observations on B. osborni.

Results from studies on different species of the genus Anaphes (Hymenoptera: Mymaridae) have demonstrated that these species can regulate either super- or multiparasitism within their hosts by larval fights between their mymariform first-instar larvae (Baaren et al. 1997). Adaptations to physical combat with competitors take the form of strong mandibles, active cauda and dorsal spines that allow crawling motion. These morphological characteristics also were found in B. osborni and could aid in eliminating con-specific larvae rather than assist in feeding. Several authors refer to the integumental absorption of the nutrients, mostly yolk proteins from the young host egg, by first instars (Hesami et al. 2004). In contrast to the active first instars, saccoform second instars were never seen actively moving within the host egg. It has been suggested that this type of larvae take food material by diffusion and absorption (Sahad 1984, Moratorio and Chiappini 1995). As larval development progressed, B. osborni parasitized weevil eggs turned amber. Such a change in color also occurs with other egg parasitoids of D. abbreviatus, such as F. dominica, that were previously released in Florida (Jacas et al. 2007). Therefore, this trait may provide a tool for rapidly estimating parasitization levels (although unspecific in the field).

Immature development took almost 15 d irrespective of the sex of the developing parasitoid. Although prandyly is common in species attacking gregarious hosts that mate at the emergence site (Godfray 1994), this was not the case with B. osborni.

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