

**DESCRIPTION OF THE IMMATURE STAGES OF
EURYTOMA SIVINSKII GATES AND GRISSSELL
(HYMENOPTERA: EURYTOMIDAE), AN
ECTOPARASITOID OF *ANASTREPHA* (DIPTERA:
TEPHRITIDAE) PUPAE IN MEXICO¹**

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ABSTRACT: We describe and illustrate for the first time the egg, larva, and pupa of *Eurytoma sivinskii* Gates and Grissell (Hymenoptera: Eurytomidae), a parasitoid that attacks *Anastrepha obliqua* (Macquart) pupae in Veracruz, Mexico. We obtained the immature stages of *E. sivinskii* using lab-reared *A. ludens* (Loew) pupae as hosts.

KEYWORDS: *Eurytoma*, Eurytomidae, ectoparasitoid, *Anastrepha*, Tephritidae

Eurytoma Illiger is remarkably diverse, both in number of species and the wide range of hosts utilized (Noyes 2003). Of approximately 700 nominal species worldwide, 204 are known from the New World, with 84 known from the Neotropical Region (Noyes 2003). Only 28 Neotropical species have documented hosts and only 3 of those species are known to attack Tephritidae (Gates and Grissell 2004). In North America, *Eurytoma gigantea* Walsh attacks *Eurosta solidaginis* (Fitch), a gall former in stems of *Solidago* spp. (Asteraceae), and at least seven other *Eurytoma* species are known from Tephritidae (Bugbee 1967, Noyes 2003). Most of these species that attack Tephritidae parasitize larvae in above-ground situations, often in flower heads or galls of Asteraceae (Bugbee 1967, 1975; Claridge 1961; Goeden 2001a,b; Peck 1963).

Eurytoma sivinskii Gates and Grissell (Hymenoptera: Eurytomidae) is a recently described parasitoid (Gates and Grissell 2004) discovered in *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) puparia whose larvae developed in tropical plum fruit, *Spondias mombin* L. (Anacardiaceae), in Tejería, Veracruz, Mexico.

METHODS

Specimens used in this study originated from a colony maintained at Unidad de Entomología Aplicada of the Instituto de Ecología A. C., Xalapa, Veracruz, Mexico. Environmental conditions were maintained at $27 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH, and 12:12 h

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photoperiod. The colony was established with specimens originally reared from *Anastrepha obliqua* (Macquart) pupae collected as larvae from tropical plum (*Spondias mombin*) in November 1997 at Tejería, Veracruz, Mexico.

To rear the parasitoids, we exposed 300 ml of two-day-old, laboratory-reared *A. ludens* pupae to three *E. sivinskii* cohorts kept in 30" x 30" x 30" Plexiglas cages (200 females and 100 males per cage; fed *ad libitum* with honey and water) for 6-8 days. Twenty-four hours after exposure to parasitoids, pupae were removed and placed in plastic vials (500 ml) with humidified vermiculite and covered with a lid. We made a randomized selection of pupae and dissected them one by one until obtaining a total of 26 eggs, 169 larvae (all stages represented), 40 prepupae, and 127 pupae. The specimens were placed in recently prepared Carnoy's fixing solution (60 ml of absolute alcohol, 30 ml of chloroform and 10 ml of acetic acid) for 24 hr. Subsequently, the specimens were washed and preserved in hermetic glass in 70% EtOH until needed. Another set of fly pupae was dissected systematically at 24-hr intervals for 23 days (start of *E. sivinskii* adult emergence) and preserved as above. Dissections were made in a physiological solution to minimize tissue contraction.

We used two different microscopy techniques to study corporal structures: a scanning electron microscope (SEM) and a stereomicroscope with attached Nikon Eclipse 50i camera. The stereomicroscope (1.6X main objective) set up along with Image Pro-plus® software was used to obtain images of specimens fixed with Carnoy's and preserved in 70% EtOH. Specimens were imaged and measured in 70% EtOH. Some specimens preserved in 70% EtOH were dyed with chlorazol black, using lactofenol as a support medium to enhance visualization of some structures. Specimens neither fixed nor dyed but preserved in ethanol were dehydrated through 100% ethanol and HMDS (Heraty and Hawks 1998) before point, SEM stub or card mounting. A Nikon SMZ1500 stereomicroscope with 10X (Nikon C-W10X/22) and Chiu Technical Corp. Lumina 1 FO-150 fiber optic light source were used for card- and point-mounted specimen observation. Mylar film was placed over the ends of the light source to reduce glare from the specimen. Scanning electron microscope (SEM) images were taken with an Amray 1810 (LaB₆ source). Specimens were cleaned of external debris with bleach and distilled water after Bolte (1996), dehydrated with HMDS, and affixed to 12.7 X 3.2 mm Leica/Cambridge aluminum SEM stubs with carbon adhesive tabs (Electron Microscopy Sciences, #77825-12). Stub-mounted specimens were sputter coated using a Cressington Scientific 108 Auto with a gold-palladium mixture from at least three different angles to ensure complete coverage (~20-30nm coating).

Measurements recorded for each include body length and width plus cephalic width. Descriptions for 1st-4th instars report basic measurements and qualitative observations while that of the 5th instar also includes chaetotaxy. This is done in accordance with previously published larval descriptions that focus primarily on the morphology of final instars, especially when comparing to conspecific taxa or differentiating genera (Phillips 1927, Plaut 1972, Roskam 1982, Henneicke et al., 1992, Dawah and Rohfritz 1996, Tormos et al., 2004). Larval chaetotaxy follows Roskam (1982) and Henneicke et al. (1992). The mandible is referred to as "Type

2" (Henneicke et al., 1992), overall elongate and slender, with a main apical tooth subtended by a smaller tooth approximately one-third the size of the former. The following abbreviations are used in the descriptions: A1-9 = abdominal segments; an = antennae; AS = anal segment; CI = clypeal seta; Di = inferior dorsal setae; Ds = superior dorsal setae; DT = dorsal terminal seta; Fi = inferior frontal setae; Fs = superior frontal setae; Ge = genal setae; Hy = hypostomal setae; L = lateral seta; La = labral setae; P = pleural setae; prls = lateral prelabial setae; prms = middle prelabial setae; TH1-3 = thoracic segments; ulc = under lip complex; V = ventral setae; VT = ventral terminal setae.

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Egg (n = 26). White, oval (0.402 ± 0.08 mm long; range: 0.371-0.416 mm), with two pedicels, one at each pole, one pedicel longer (0.180 ± 0.03 mm; range: 0.163-0.204 mm) than other (0.042 ± 0.02 mm; range: 0.028-0.056 mm). Chorion spinose (Fig. 7), the spines darkening over time.

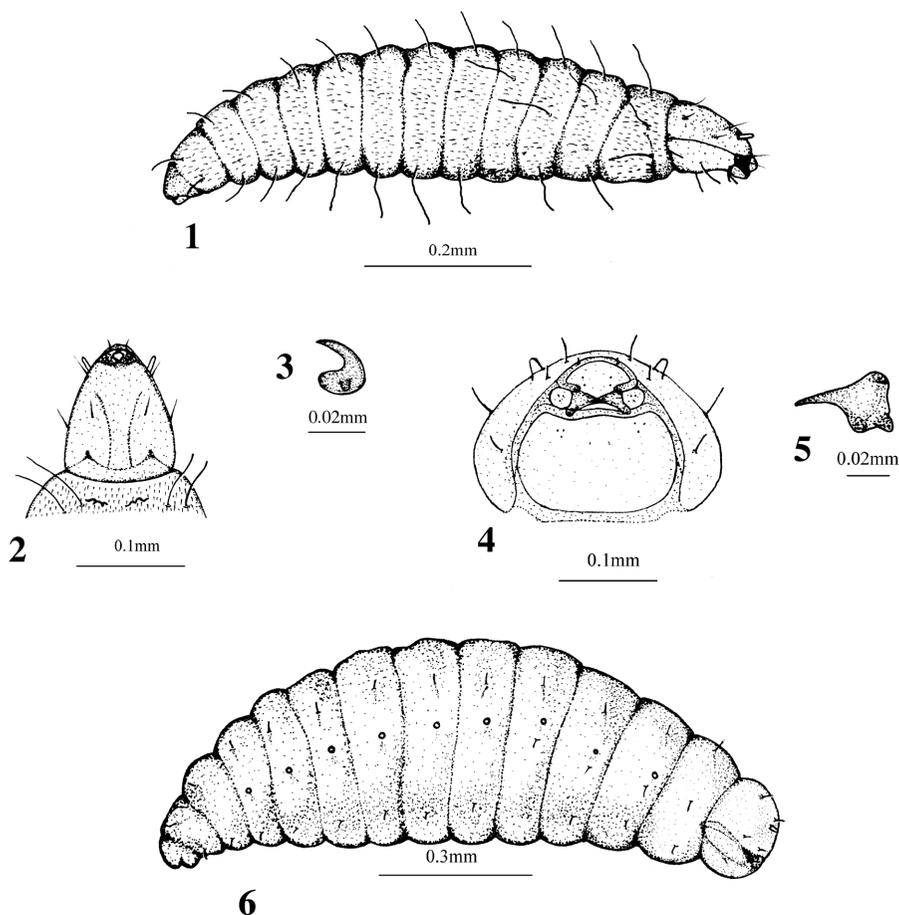
First instar (n = 29). White, 13-segmented plus the head (0.669 ± 0.10 mm long; range: 0.532-0.725 mm; 0.175 ± 0.04 mm wide; range: 0.137-0.201 mm). Head hypognathous, triangular in dorsal view (0.100 ± 0.02 mm wide; range: 0.094-0.104 mm) (Fig. 2); mandible falcate (Fig. 3).

Second instar (n = 33). White, 13-segmented plus the head, slightly dorsoventrally flattened (Fig. 1) (1.289 ± 0.42 mm long; range: 0.871-1.825; 0.430 ± 0.17 mm wide; range: 0.289-0.681) (Fig. 6). Head ovate to pentagonal (0.218 ± 0.07 mm wide; range: 0.139-0.299), mandible triangular (Figs. 4-5).

Third instar (n = 27). White, 13-segmented plus the head (1.768 ± 0.43 mm long; range: 1.186-2.102 mm), (0.605 ± 0.15 mm wide; range: 0.374-0.706 mm). Head broad, ovate (0.296 ± 0.06 mm wide; range: 0.186-0.315); mandible triangular.

Fourth instar (n = 25). Beige, 13-segmented plus the head (2.673 ± 0.48 mm long; range: 1.796-3.654 mm; 0.865 ± 0.14 mm wide; range: 0.641-1.087). The head is circular (0.384 ± 0.06 mm wide; range: 0.271-0.444 mm); mandible triangular, elongated.

Fifth instar (n = 55). Beige with white granules, 13-segmented plus the head (3.248 ± 0.44 mm long; range: 2.419-3.612 mm; 1.062 ± 0.13 mm wide; range: 0.847-1.202 mm) (Figs. 11-12). Body barrel shaped, broadest medially, tapering anteriorly and posteriorly (Figs. 11-12). Head with usual complement of setae (Henneicke et al., 1992): one pair of Fs, one pair of Fi, one pair of Ge, one pair of La, and one pair of Hy (Figs. 9-10); ulc typically collapsing but ms, prms, and prls present. Antenna $\sim 2.0x$ as long as broad (Figs. 9-10, an). Mandibles of type 2, moderately sclerotized. Setae moderately sclerotized, papilla borne, long, conspicuous (Figs. 9-12). TH1-2 with four dorsal setae (2 Ds and 2 Di), one pair of pleural setae (P), one pair of lateral setae (L), and one pair of ventral setae (V); TH3 with two dorsal setae (2 Ds), one pair of P, one pair of L, and one pair of V; A1-8 each with less conspicuous pair Ds setae, a single pair P, and a single pair V setae, A1 lacks V setae. AS with two pair inconspicuous DT, one pair of VT present.



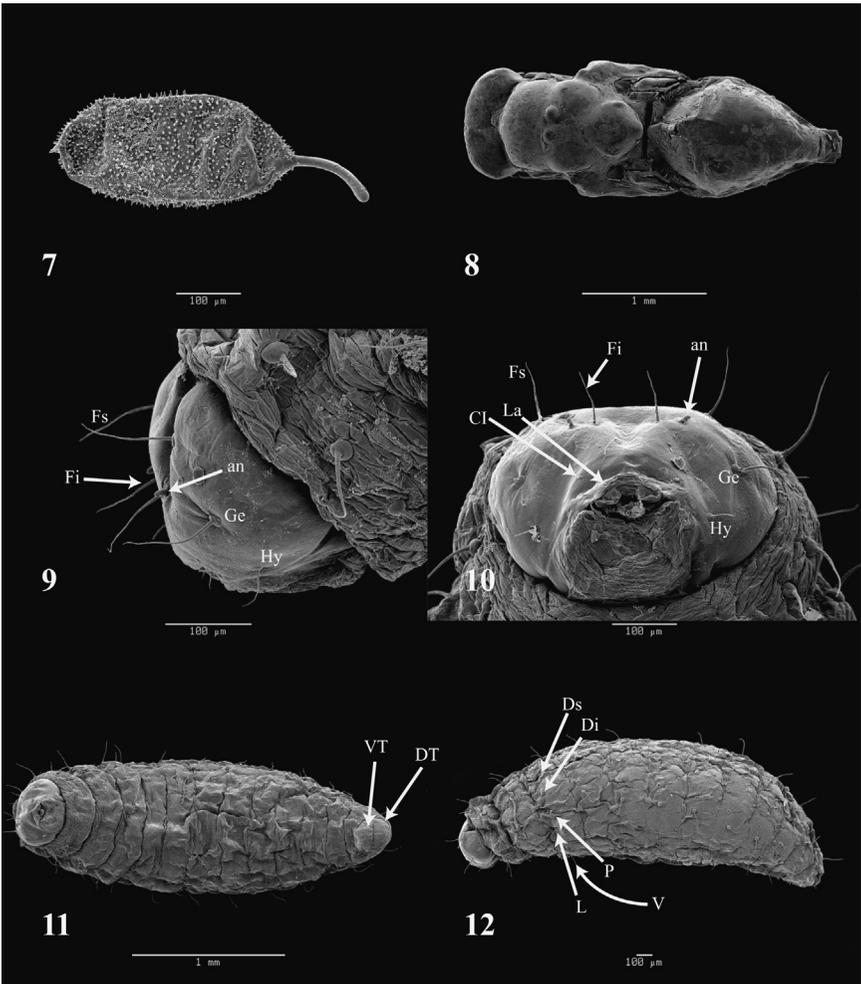
Figures 1-6. *Eurytoma sivinskii*: 1, first instar, lateral habitus. 2, first instar, dorsal head capsule. 3, first instar, falcate mandible. 4, second instar, ventral head capsule. 5, second instar, triangular mandible. 6, second instar, lateral habitus.

Prepupa (n = 40). White to beige without well-defined segmentation (0.3080 ± 0.37 mm long; range: 2.442-3.200 mm; 0.984 ± 0.13 mm wide; range: 0.777-1.074 mm). Spiracles covered on surface by an operculum and a reservoir sac. Cephalic width 0.429 ± 0.11 mm; range: 0.170-0.555 mm.

Pupa (n = 30). White to beige dorsally (2.826 ± 0.38 mm long; range: 1.672-3.124 mm; 0.859 ± 0.10 mm wide; range: 0.487-1.053) (Fig. 8). Cephalic width 0.714 ± 0.08 mm; range: 0.510-0.971 mm. Darkens significantly during course of development with adult characteristics increasingly visible.

DISCUSSION

The immature stages of *E. sivinskii* are similar to those described for other *Eurytoma* species such as *E. pini* Bugbee (Arthur 1961) and *E. amygdali* Enderlein (Plaut 1972). However, our results indicated that body length is a good indicator to distinguish immature stages of *E. sivinskii*. The structural description of the egg of *E. sivinskii* is similar to that of *E. parva* (Girault) (Phillips 1927), *E. pini* Bugbee (Arthur 1961) and *E. amygdali* (Plaut 1972) with oval shape, a spinose and bipedicellate chorion. Five instars are identified in *E. sivinskii* which is the same number reported for *E. pini* by Arthur (1961).



Figures 7-12. *Eurytoma sivinskii*: 7, egg. 8, pupa, dorsal. 9, final instar, lateral head capsule. 10, final instar, ventral head capsule. 11, final instar, ventral habitus. 12, final instar, lateral habitus. See Materials and Methods for explanation of abbreviations.

Significant differences were evident between lengths ($F=252.502$; 4 df; $P<0.001$), widths ($F=251.378$; 4 df; $P<0.001$) and cephalic widths ($F=186.353$; 4 df; $P<0.001$) of each instar.

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