SYSTEMATICS

Descriptions of Immature Stages of *Trupanea nigricornis* and *T. bisetosa* (Diptera: Tephritidae) from Southern California

KHOUZAMA M. KNIO, RICHARD D. GOEDEN, AND DAVID H. HEADRICK

Department of Entomology, University of California, Riverside, CA 92521


ABSTRACT The immature stages of the sympatric, cryptic species *Trupanea nigricornis* (Coquillett), a flower head-infesting fruit fly that attacks a wide range of hosts in the Asteraceae in southern California, and *T. bisetosa* (Coquillett), an oligophagous congener that attacks only a few hosts in the tribe Heliantheae, are described and illustrated. Detailed examination with the aid of scanning electron microscopy of the eggs, all 3 instars, and puparia of this taxonomically closely allied generalist and specialist revealed many similarities and very few differences, for instance, most puparia of *T. nigricornis* were shorter and narrower than those of *T. bisetosa*. Therefore, major differences in the host-plant specificities of these cryptic, possibly sibling, species were not reflected by substantial morphological differences among their immature stages.

KEY WORDS *Trupanea*, cryptic species, immature stages, sympatry, taxonomy

THE FLOWER-HEAD INFESTING tephritids *Trupanea nigricornis* (Coquillett) and *T. bisetosa* (Coquillett) occur in sympathy in southern California. They are considered cryptic species because adults of both sexes show close morphological resemblance. The males are readily distinguished by the color of the 3rd antennal segments, brown in *T. nigricornis* and yellow in *T. bisetosa* (Foote 1960, Foote and Blanc 1963, Foote et al. 1993); however, females could not be distinguished until recently. Most (75%) females are now separated based on the shape of the basal stem of the apical Y-shaped mark in the wing. This stem is thin and distinct in *T. nigricornis* females, but broad and short in *T. bisetosa* females (Cavender and Goeden 1983, Foote et al. 1993). The phylogenetic relationships among *Trupanea* spp. have not been studied. However, based on wing morphology, the most useful diagnostic character in the genus *Trupanea nigricornis* and *T. bisetosa* are the closest relatives among the North American species and are probably sibling species (A. L. Norrbom 1993, personal communication). The immature stages of *T. nigricornis* have not been described. *T. bisetosa* immature stages were briefly described by Cavender and Goeden (1982). We provide detailed descriptions of all preimaginal stages for each of these cryptic species.

Materials and Methods

The immature stages of *T. nigricornis* were obtained from dissections of flower-head samples of *Encelia farinosa* Gray, *E. frutescens* Gray, and *E. virginensis* A. Nelson collected during 1989–1990 from 8 different interior valley locations in Riverside County, CA. The immature stages of *T. bisetosa* were obtained from dissections of flower heads of wild sunflower, *Helianthus annuus* L., collected from 2 interior valley locations near Riverside in Riverside County: Casa Blanca section of south Riverside in May and August 1989 and October 1990; and along Gilman Springs Road in San Jacinto Valley, 9 km east of Riverside in November 1989 and October-November 1990.

The ultrastructure of 6 each of eggs, each instar, and puparia of *T. nigricornis* and *T. bisetosa* was examined with a JEOL JSM-C-35 scanning electron microscope (SEM) in the Department of Nematology, University of California, Riverside. The specimens for SEM were fixed and preserved in 70% ethanol. Before scanning, they were rehydrated through decreasing serial dilutions of ethanol, washed in distilled water, and postfixed in 2% osmium tetroxide for 24 h. Afterward, the specimens were washed twice in distilled water, dehydrated in an increasing series of acidulated ethanol dilutions to 100%, and washed twice with 100% ethanol. Then, they were critical-point dried, mounted on stubs, and coated with a gold-palladium alloy before examination of 15 kV accelerating voltage. Micrographs were prepared using Polaroid 55 P/N film.

Egg and puparial measurements were obtained by direct measurement of the specimens, larval measurements were obtained by slide-mounting specimens according to the method described by
Phillips (1946). SEM measurements may show some discrepancies because of specimen shrinkage or nonplanar viewing.

Terminology used in the descriptions of the immature stages of *T. nigricornis* and *T. bisetosa* was based on the publications of Carroll and Wharton (1989), Headrick and Goeden (1990, 1991), Goeden and Headrick (1991a, b; 1992), and Goeden et al. (1993). Voucher specimens of *T. nigricornis* and *T. bisetosa* immatures are stored in the separate research collections of D.H.H. and Jeffrey A. Teerink, Department of Entomology, University of California, Riverside.

**Results**

**Trupanea bisetosa** (Coquillett)

(Figs. 1–5)

_Egg_. Smooth, shiny-white, elongate-ellipsoidal (Fig. 1A); 0.73 ± 0.01 mm long (n = 20; range, 0.71–0.80 mm), 0.18 ± 0.001 mm wide (range, 0.17–0.19 mm); anterior end with short, distinct pedicel, 0.03 ± 0.001 mm long (range, 0.03–0.04 mm), central micropyle (Fig. 1B-1), and 1 or 2 rows of aeropyles (Fig. 1B-2) connected by fine pores (Fig. 1C).

_First Instar_. White, slightly translucent, cylindrical, truncate at both ends (Fig. 2A); 0.55 ± 0.01 mm long (n = 20; range, 0.48–0.60 mm), 0.21 ± 0.003 mm wide (range, 0.20–0.23 mm); cephalopharyngeal skeleton averaged 0.12 ± 0.003 (n = 6; range, 0.11–0.13) mm long; mouth hooks bidentate (Fig. 2B-1); median oral lobe between mouth hooks (Fig. 2B-2), attached basally to labial lobe (Fig. 2B-3); labial lobe with 2 pores anteriorly; mouth hooks ventral to paired, flattened integumental petals (Fig. 2B-4); anterior sensory lobes dorsal to petals (Fig. 2B-5, 2C), each lobe with 4 sensory organs: terminal sensory organ (Fig. 2C-1), pit sensory organ (Fig. 2C-2), lateral sensory organ (Fig. 2C-3), and supralateral sensory organ (Fig. 2C-4); dorsal sensory organs (Fig. 2C-5) and subdorsal sensilla (Fig. 2C-6) dorsal to anterior sensory lobes; stomal sense organs flat with a single, central pore, lateral of mouth hooks, ventral of anterior sensory lobes; mesothorax, metathorax, and all abdominal segments each with lateral spiracular complex composed of single spiracle (Fig. 2D-1) and 2 dome-shaped sensilla, each with central papilla (Fig. 2D-2); caudal segment circumscribed by stelex sensory receptors (Fig. 2E-1); intermediate sensory complex ventral to spiracular plates, each composed of single stelex and medusoid sensillum (Fig. 2E-2); posterior spiracular plates bearing 2 rimae (Fig. 2F-1), 4 interspiracular processes (Fig. 2F-2) with 1–4 branches each, these varying in shape (blade-like narrow apically pointed or broad with dentate margin).

_Second Instar_. White, cylindrical; cephalopharyngeal skeleton moderately pigmented, 0.34 ± 0.02 mm long (n = 6; range, 0.24–0.4 mm); mouth hooks tridentate; median oral lobe laterally flattened (Fig. 3A-1), ventral lobe with ventral margin papillate (not shown); gnathocephalon bearing rugose pads with smooth or dentate edges (Fig. 3A-2); anterior sensory lobes as in 1st instar (Fig. 3A-3); stomal sense organ with several sensory papillae (Fig. 3A-4); prothorax with rugose pads on anterior margin, anterior prothoracic spiracles near posterior margin with 4 rounded papillae, each bearing longitudinal slit (Fig. 3B); posterior spiracular plates with 3 spiracular rimae (Fig. 3C-1), 4 interspiracular processes with 6–8
branches (Fig. 3C-2) and a median ecdysial scar (Fig. 3C-3); posterior spiracles surrounded by stelex sensilla, paired intermediate sensory complexes composed of single stelex and medusoid sensillum (Fig. 3C-4).

**Third Instar.** White, barrel-shaped (Fig. 4A); 2.78 ± 0.05 mm long (n = 50; range, 2.0–3.5 mm), 1.34 ± 0.02 mm wide (range, 1.0–1.5 mm); cephalopharyngeal skeleton darkly pigmented, 0.39 ± 0.01 mm long, (n = 6; range, 0.36–0.42); mouth
Fig. 3. Second instar of T. bisetosa. (A) Gnathocephalon, (1) median oral lobe, (2) rugose pads, (3) anterior sensory lobe, (4) stomal sense organ; (B) anterior thoracic spiracle; (C) posterior spiracular plate, (1) rima, (2) interspiracular process, (3) median ecdysial scar, (4) intermediate sensory complex.

hooks with 1 apical tooth, 1 subapical tooth, and 1 small median tooth; anterior sensory lobes with 4 sensory organs as described for previous instars (Fig. 4B-1); prothorax circumscribed by rugose pads on anterior margin (Fig. 4B-2), and with dome-shaped sensilla medially (Fig. 4B-3), anterior prothoracic spiracles with 4 rounded papillae, each bearing longitudinal slit (Fig. 4C); mesothorax, metathorax, and abdominal segments I–VI with irregular rows of posteriorly directed conical acanthes (Fig. 4D); last 2 segments with several sinuate rows of long conical acanthes directed anteriorly; lateral spiracular complex on all but gnathocephalon and caudal segments, each with open spiracle (Fig. 4E-1), 2 single dome-shaped sensilla with central papilla (Fig. 4E-2), and medial placoid-type sensilla (Fig. 4E-3); posterior spiracular plates bearing 3 oval rimae (Fig. 4F-1), 4 interspiracular processes (Fig. 4F-2) with 6–8 branches each, and median ecdysial scar (Fig. 4F-3); intermediate sensory complex each composed of single stellex and medusoid sensillum.

Puparium. Black, smooth, barrel-shaped, with truncate ends (Fig. 5A); 3.15 ± 0.02 mm long (n = 135; range, 2.75–3.8 mm), 1.4 ± 0.01 mm wide (range, 1.2–1.6 mm); cephalic end with open thoracic spiracles (Fig. 5B-1); spiracular plates slightly protruding and bearing 3 oval rimae, 4 interspiracular processes with 6–8 branches each, and ecdysial scar (Fig. 5C).

Trupanea nigricornis (Coquillett) (Figs. 6–10)

Egg. Shiny-white, smooth, elongate-ellipsoidal, (Fig. 6A); 0.72 ± 0.004 mm long (n = 72; range, 0.60–0.80 mm), 0.20 ± 0.001 mm wide (range, 0.18–0.23 mm); anterior end tapered, bearing short, poorly defined pedicel, 0.03 ± 0.001 mm long (range, 0.03–0.04 mm) with anterior, central micropyle (Fig. 6B-1), and 1 row of aeropyles (Fig. 6B-2); posterior end tapered acutely, smooth.

First Instar. Whitish, slightly translucent, elongate, cylindrical, bluntly rounded at both ends (Fig. 7A); 0.48 ± 0.11 mm long (n = 22; range, 0.30–0.70 mm), 0.18 ± 0.08 mm wide (range, 0.12–0.25 mm); cephalopharyngeal skeleton weakly pigmented, 0.12 ± 0.003 mm long (n = 6; range 0.11–0.13 mm); mouth hooks bidentate, with curved apical tooth and subapical tooth (Fig. 7B-1); median oral lobe laterally flattened lying between mouth hooks (Fig. 7B-2), attached (observed by dissections) basally to labial lobe; mouth hooks ventrad of paired, flattened integumental petals (Fig. 7B-3); anterior sensory lobes (Fig. 7B-4) each with 4 sensory organs: terminal sensory organ (Fig. 7C-1), pit sensory organ (Fig. 7C-2), lateral sensory organ (Fig. 7C-3), and supralateral sensory organ (Fig. 7C-4); terminal sensory organ with 5 rounded papillae in papillae in shallow depression; dorsal sensory organs dorsad of anterior sensory lobes (Fig. 7B-5) accompanied laterally by subdorsal sensillum, this consisting of rounded papilla surrounded by slightly raised cuticular ring (Fig. 7B-6); stomal sense organs flat, laterad of mouth hooks and ventrad of anterior sensory lobes, with pore- and cone-shaped sensilla (Fig. 7B-7); caudal segment with 2 conspicuous spiracular plates, each bearing 2 oval rimae (Fig.
Fig. 4. Third instar of *T. bisetosa*. (A) Habitus, anterior to left, dorsal to top; (B) gnathoccephalon, (1) anterior sensory lobes, (2) rugose pads, (3) prothoracic sensillum; (C) anterior thoracic spiracle; (D) surface acanthae, detail; (E) lateral spiracular complex, (1) spiracle, (2) sensilla, (3) placoid sensillum; (F) posterior spiracular plate, (1) rima, (2) interspiracular process, (3) median ecdysial scar.

7D-1), each with longitudinal slit, and 4 fan-shaped interspiracular processes each with 4 broad dentate branches (Fig. 7D-2); paired intermediate sensory complex ventral to spiracular plates, each composed of single stelex and medusoid sensillum (not shown).

**Second Instar.** White, cylindrical, tapered anteriorly, truncated posterior; moderately pigmented cephalopharyngeal skeleton, 0.29 ± 0.02 mm long (*n* = 6; range, 0.25–0.37 mm); mouth hooks tridentate (Fig. 8A-1), median oral lobe laterally flattened, ventral lobes with ventral margin papillate (Fig. 8A-2); fleshy, flattened projections dorsal to mouth hooks (Fig. 8A-3); stomal sense organ with ~5 papillate and pit sensoria (Fig. 8A-4); integument surrounding mouth hooks with flattened,
Fig. 5. Puparium of *T. bisetosa*. (A) Habitus, anterior to left, dorsal to top; (B) anterior end, detail, (1) anterior thoracic spiracle; (C) posterior spiracular plate.

subrectangular pads with dentate or smooth posterior edges (Fig. 8A-5); anterior sensory lobes with 4 sensilla (Fig. 8A-6) as described for 1st instar; dorsal sensory organ a single domed papilla (not shown); with anterior prothoracic spiracles dorsolateral on prothorax near posterior margin, each spiracle bearing 4 rounded papillae, each with single, longitudinal slit (Fig. 8B); posterior spiracular plate with 3 spiracular rims (Fig. 8C-1), median ec dysial scar (Fig. 8C-2), and 4 branched, fan-shaped interspiracular processes, with 5 or 6 branches each (Fig. 8C-3); paired intermediate sensory complexes each composed of single stellex and medusoid sensillum.

**Third Instar.** Creamy white, barrel-shaped (Fig. 9A), $2.66 \pm 0.40$ mm long ($n = 30$; range, 1.75–3.25 mm), $1.18 \pm 0.17$ mm wide range, 0.80–1.50 mm); cephalopharyngeal skeleton darkly pigmented, averaging $0.36 \pm 0.01$ mm in length ($n = 6$; range, 0.33–0.39 mm); mouth hooks with curved apical and subapical teeth, medial tooth at base of subapical tooth prominent (Fig. 9B-1); mouth hooks ventral to several flattened integumental petals (Fig. 9B-2); median oral lobe laterally flattened (Fig. 9B-3); anterior sensory lobes with 4 sensory organs (Fig. 9B-4); stomal sense organs with several sensoria (9B-5); integument of gnathocephalon with rugose, subrectangular pads with dentate margins (Fig. 9B-6); prothorax with rugose pads located on anterior margin (Fig. 9C-1); prothorax circumscribed medially by single row of domed sensilla (Fig. 9C-2); anterior prothoracic spiracles dorsolateral near posterior margin (Fig. 9C-3), with 4 rounded papillae, each with longitudinal slit (Fig. 9D); domed sensilla circumscribing anterior portion of mesothorax (Fig. 9C-4); all thoracic segments and abdominal segments I–VI with several straight rows of acanthae near anterior margins (Fig. 9C-5), acanthae conical, 5–6 $\mu$m, oriented posteriorly; lateral spi-

Fig. 7. First instar of T. nigricornis. (A) habitus, anterior to right; (B) gnathocephalon, dorsal to left, (1) mouth hook, (2) median oral lobe, (3) integumental petal, (4) anterior sensory lobe, (5) dorsal sensory organ, (6) subdorsal sensillum, (7) stomal sense organ; (C) anterior sensory lobe, detail, (1) terminal sensory organ, (2) pit sensory organ, (3) lateral sensory organ, (4) supralateral sensory organ; (D) posterior spiracular plate, (1) rimae, (2) interspiracular process.

Pedicel complex on meso- and metathoracic segments and abdominal segments I–VII, each complex with single spiracular opening (Fig. 9E-1) and 2 dome-shaped sensilla with central papilla (Fig. 9E-2); abdominal segments VII–VIII with curved rows of acanths on posterior margins, acanthes conical, =9 μm long and oriented anteriorly (Fig. 9F-1); caudal segment bearing 2 posterior spiracular plates each with 3 rimaes, 0.034 mm long (Fig. 9F-2), 4 branched interspiracular processes with 3–6 branches each (Fig. 9F-3), and a median ec dysial scar (Fig. 9F-4); plates surrounded by dorsal, ventral, and lateral sensoria (Fig. 9F-5); intermediate sensory complex each composed of single stelex and medusoid sensillum (Fig. 9F-6).

Comments. Sexually dimorphic adults of T. nigricornis and T. bisetosa are distinguishable (Cavender and Goeden 1983, Foote et al. 1993). Their immature stages, however, are very similar in their external anatomy with only minimal differences observed.

The pedicel of T. nigricornis eggs only slightly protrudes and in all specimens examined has a single row of aeropyles; T. bisetosa eggs often have 2 rows of aeropyles.

The lateral spiracular complex was observed on segments TII–AVII on T. bisetosa 1st instars. Lateral spiracles were not observed on T. nigricornis; however, T. nigricornis 1st instars were dissected from plants and were often covered with plant exudates. T. bisetosa 1st instars were hatched from eggs held in petri dishes with moistened filter paper, and thus remained clean. We presume that lateral spiracles in T. nigricornis are present.

Interspiracular processes on the posterior spiracular plates of T. bisetosa 1st instars are variable
among individuals, having 1–4 branches, and each branch being narrow and pointed, or broad with a dentate margin. The processes on *T. nigricornis* all appear to be 4-branched, broad, and dentate.

Interspiracular processes of *T. bisetosa* 2nd instars have 6–8 branches each, whereas *T. nigricornis* 2nd instars have 5–6 branches each. Interspiracular processes of *T. bisetosa* and *T. nigricornis* 3rd instars have between 3 and 8, narrow, pointed branches each, the number of branches varying among individuals; however, the branches on *T. bisetosa* 3rd instars are slightly narrower.

Puparia of *T. nigricornis* are shorter and narrower on average than those of *T. bisetosa*, but their size ranges overlap. Moreover, the sizes of *T. nigricornis* puparia varied according to the size of the flower heads of their different hosts (K.M.K. and R.D.G., unpublished data).

**Discussion**

The eggs of *T. nigricornis* and *T. bisetosa* are similar in shape to the eggs of other *Trupanea* species, for example, *T. californica* Malloch (Headrick and Goeden 1991), *T. conjuncta* (Adams) (Goeden 1987), and *T. imperfecta* (Coquillett) (Goeden 1988). They also are similar in shape to a taxonomically related gall-former, *Tephritis baccharis* (Coquillett) (Goeden and Headrick 1991a), and to the facultative stem-mining/flower-head infesting species, *Tephritis arizonaensis* Quisenberry (Goeden et al. 1993).

Sensoria on the anterior sensory lobes are similar in *T. nigricornis* and *T. bisetosa*, as are the dorsal sensory organs. A subdorsal sensillum observed on *T. nigricornis* and *T. bisetosa* may be homologous to 1 of the 3 pit sensilla described by Carroll and Wharton (1989) near the antenna of the Mexican fruit fly, *Anastrepha ludens* (Loew). This particular sensillum has not been observed in other tephritid species examined by us to date, and its presence or absence should be confirmed in all future descriptions.

The lateral sensory complex was first described in 3rd instars of the tephritine, *Trupanea californica* (Headrick and Goeden 1991). Since then, all tephritine species examined have been reported as having the lateral spiracular complex. However, the lateral spiracular complex has not previously been observed in 1st instars, although its presence was predicted (Headrick and Goeden 1991). The difficulty in observing these small spiracles is compounded because most larvae examined were dissected from plant material, and thus plant exudates and debris often obscure thorough examination. Early instars also are prone to shrinkage during the process of fixation for SEM, thus making observations difficult. Confirmation of the presence of the lateral spiracular complex in all larval stages of *T. bisetosa* provides impetus for confirmation in other tephritid species. Tephritine species have been reported or observed to have lateral spiracles (*A. ludens*, Carroll and Wharton [1989]; *Bactrocera dorsalis* Hendel & *Ceratitis capitata* [Wiedemann], D.H.H., and R.D.G., unpublished data), but their presence in all instars, again, needs confirmation.
Trupanea bisetosa has a limited host range that does not overlap with the wide host range of *T. nigricornis*. This suggests that these 2 species either once shared a common ancestor and diverged in their host ranges or 1 species evolved from the other. The morphological similarity between *T. nigricornis* and *T. bisetosa* immature stages shows that the ability to feed and survive on new hosts does not present a major barrier to host-range expansion or host-shifting by either species. No major differences in mouthpart structures or sensory organs associated with feeding
Fig. 10. Puparium of *T. nigricornis*. (A) Habitus, anterior to right; (B) anterior end, (1) invagination scar, (2) anterior thoracic spiracle; (C) posterior spiracular plate, (1) median ecdysial scar, (2) rima, (3) interspiracular process.

have been reported among nonfrugivorous tephritid larvae examined to date having different food niches (galls, flower head, crowns, roots, or stems) (compare, Phillips 1946; Headrick and Goeden 1990; Goeden and Headrick 1991a, b; Goeden et al. 1993). Thus, the mouthparts and their associated sensory organs apparently are versatile in nonfrugivorous tephritids.

To further understand the basis of the well-documented differential host preferences of these 2 cryptic, sympatric, probably sibling species, and to build further hypotheses about their evolutionary history and host selection, additional, comparative studies on their biology, ecology, and behavior have been completed by us (unpublished data).

Acknowledgments

We sincerely thank F. L. Blanc, L. E. Carroll, J. A. Teerink, and 1 anonymous reviewer for their helpful reviews of earlier drafts of this article. We also thank Gordon Gordh for his assistance and material support for this study.

References Cited


Received for publication 19 July 1994; accepted 6 September 1995.