Comparative Morphology of the Immature Stages of Three Corn-Infesting Ulidiidae (Diptera)

GAURAV GOYAL,[1,2] GREGG S. NUESSLY,[1] GARY J. STECK,[3] JOHN L. CAPINERA,[4] AND DAKSHINA R. SEAL[5]

Ann. Entomol. Soc. Am. 104(3): 416–428 (2011); DOI: 10.1603/AN10180

ABSTRACT Several species of Ulidiidae (Diptera) are primary pests of maize (*Zea mays* L.). The ability to distinguish their immature stages would be useful for biological studies where their distributions overlap. Morphology of the immature stages was examined for three Ulidiidae that attack maíze in the southeastern United States: *Chaetopsis massyla* (Walker), *Euxesta eluta* Loew, and *Euxesta stigmatias* Loew. Egg, larval, and pupal characters were measured and described with the aid of light and scanning electron microscopy. Because of considerable overlap in character states, only a few traits in each stage could be used to separate these three species. *C. massyla* eggs had pores restricted to the posterior end, but the pores were evenly distributed in the two *Euxesta* species. Eggs of the two *Euxesta* species could not be differentiated. Larval mouth hooks of *C. massyla* had a distinct tooth on their ventral surface, whereas this tooth was lacking in *E. eluta and E. stigmatias*. Fewer oral ridges were observed on *C. massyla* larvae than on *E. eluta or E. stigmatias*. Posterior spiracular slits were apparent in *E. stigmatias* versus obscure in *E. eluta* larvae. The length and spinule arrangements on creeping welts could be used to separate *E. eluta* and *E. stigmatias* larvae. Posterior spiracular plates of *C. massyla* puparia were trapezoidal versus ovoid in the two *Euxesta* species. Puparium color varied significantly among the three species.

KEY WORDS *Euxesta eluta*, *Euxesta stigmatias*, *Chaetopsis massyla*, developmental stages, sweet corn

The picture-winged fly *Euxesta stigmatias* Loew (Diptera: Ulidiidae=Otitidae) feeds on a wide range of fruits, vegetables, and field crops (Seal and Jansson 1989) and is an economic pest of maíze (*Zea mays* L.), especially sweet corn (App 1938). This insect is highly injurious to maíze, with up to 100% damage to ears in untreated fields reported by many workers beginning in the 1940s (Bailey 1940, Seal and Jansson 1989, Nuessly and Hentz 2004). Several additional ulidiid species are known maíze pests in the Caribbean and in North, Central, and South America (Painter 1955, Arce de Hamity 1986, Barbosa et al. 1986, Evans and Zambrano 1991, Wyckhuys and O'Neil 2007). Four species in two genera are currently recognized pests of maíze in Florida: *Euxesta annonae* (F.), *Euxesta eluta* Loew, *Euxesta stigmatias* Loew, and *Chaetopsis massyla* (Walker) (Goyal et al. 2011). The genus *Chaetopsis* is restricted to the New World and represented by 10 species north of Mexico (Steyskal 1965) and 10 species south of the United States (Steyskal 1968), with four species common to both regions. The genus *Euxesta* occurs in both the Old and New worlds and is represented by 31 species north of Mexico (Steyskal 1965) and 69 species south of the United States (Steyskal 1968).

E. annonae, *E. eluta*, *E. stigmatias*, and *C. massyla* can frequently be observed on maíze in the same field in southern Florida; therefore, species identification is important. Although adults can be identified using wing and other body features (K. Ahlmark and G. J. Steck, unpublished key; Curran 1935; Goyal et al. 2010), no such distinguishing characteristics have been reported that can be used for the immature stages. General size and color characteristics have been reported for some immature stages of *E. annonae* (Frías-L 1981), *E. eluta* (Frías-L 1981, Arce de Hamity 1986), *E. stigmatias* (App 1938, Hayslip 1951, Seal et al. 1995), and *C. massyla* (Allen and Foote 1992), but they were all reared using different food sources under different environmental conditions. These descriptions provided inadequate details to separate the species. The current study was undertaken to describe in detail the immature stages of the three most common ulidiid species infesting corn in Florida (*C. massyla*, *E. eluta*, and *E. stigmatias*) and to search for characters

[1] Everglades Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 3200 E. Palm Beach Rd., Belle Glade, FL 33430.

[2] Corresponding author, e-mail: gaurav.pau@gmail.com.

[3] Division of Plant Industry, Florida Department of Agriculture and Consumer Services, P.O. Box 147100, Gainesville, FL 32614.

[4] Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, University of Florida, P.O. Box 110620, Gainesville, FL 32611.

[5] Tropical Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 18905 S.W. 280 St., Homestead, FL 33031.

Table 1. Range of body measurements (millimeters) on first- and second-instar larvae of three species of picture-winged fly pests of corn

Larval body measurement	Instar	Mean ± SEM (*n*; range)			*F*	df	*P*
		C. massyla	*E. eluta*	*E. stigmatias*			
Body length	First	1.83 ± 0.09A (12; 1.20–2.20)	1.78 ± 0.10A (13; 1.00–2.10)	1.4 ± 0.14B (9; 0.90–2.00)	4.30	2, 31	0.0225
	Second	4.25 ± 0.20 (15; 2.60–4.90)	3.72 ± 0.27 (10; 2.40–5.00)	3.52 ± 0.29 (12; 2.20–4.80)	2.58	2, 34	0.0905
Body width	First	0.19 ± 0.01 (12; 0.15–0.25)	0.20 ± 0.01 (13; 0.15–0.22)	0.18 ± 0.01 (9; 0.12–0.22)	1.43	2, 31	0.2555
	Second	0.40 ± 0.02 (15; 0.30–0.50)	0.36 ± 0.03 (10; 0.25–0.50)	0.39 ± 0.02 (12; 0.25–0.45)	1.27	2, 34	0.2926
Cephalopharyngeal skeleton length	First	0.26 ± 0.01 (12; 0.22–0.30)	0.28 ± 0.01 (13; 0.20–0.32)	0.26 ± 0.01 (9; 0.20–0.30)	1.49	2, 31	0.2407
	Second	0.56 ± 0.01 (15; 0.50–0.60)	0.55 ± 0.02 (10; 0.45–0.65)	0.52 ± 0.02 (12; 0.45–0.60)	2.60	2, 34	0.0893

ANOVA by PROC GLM (SAS Institute 2008). Means followed by different letters within a row are significantly different ($P < 0.05$; Tukey's test).

suitable for distinguishing these species without relying on the time- and space-consuming practice of rearing larvae to the adult stage.

Materials and Methods

Colony Maintenance. Separate colonies of *C. massyla*, *E. eluta*, and *E. stigmatias* were established using adults collected with sweep nets from sweet corn fields at the Everglades Research and Education Center, Belle Glade, FL. Flies were brought to the laboratory and provided with *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) artificial diet (product F9393B, Bio-Serv, Frenchtown, NJ) as an oviposition medium and larval food using the method of Hentz and Nuessly (2004). Mature larvae leaving the diet pupated in cotton balls used to plug the ends of diet tubes or in cotton sheets used to line trays where diet tubes containing developing larvae were maintained after removal from oviposition cages. Cotton balls and sheets with puparia were placed in the colony cages where adults emerged. All stages of all three species were maintained at 26.5 ± 1.0°C, 55–70% RH, and a photoperiod of 14:10 (L:D) h. All specimens used for this project were obtained from these laboratory colonies.

Eggs. Eggs were collected from the surface of the diet within test tubes attached to the inside top surface of adult cages. To obtain eggs of the same age for all three species, tubes with diet were placed in the cages of each of the species at the same time and then examined for eggs every 2 h. Newly deposited eggs were removed from the diet using a camel's-hair brush (size 0) and placed on filter paper within an airtight container. Older eggs were obtained for microscopic examination by subsampling eggs from the original cohort of 2-h-old eggs every 2 h for a total of 20 h. Eggs were stored at −20°C to kill the embryos in preparation for imaging.

Eggs ≤12 h old were placed on glass slides to measure their length and width by using light microscopy before preparing them for scanning electron microscopy (SEM). No differences in egg surface characteristics were observed among conspecific eggs aged 2–20 h old; therefore, eggs ≤12 h old were selected to standardize procedures for eggs of all three species. To prepare the various egg surfaces for examination, frozen eggs were thawed and air-dried at room temperature and then attached at different angles to sticky carbon paper on aluminum stubs and sputter-coated with gold-palladium (3 min) in a Vacuum Desk III (Denton Vacuum, Moorestown, NJ). Eggs were imaged using a JSM 5510 LV SEM (JEOL, Tokyo, Japan) with an accelerating voltage of 15 kV at working distances of 10 and 25 mm to look for additional surface features that were not apparent with light microscopy.

Larvae. Larvae in each of the three instars were initially examined for distinguishing characteristics. Larvae in diet tubes were examined daily from the day of eclosion to pupation and a subsample of larvae was collected daily to measure body length and widths. Larvae were then cleared in 10% NaOH for 12–15 h to allow measurements of the cephalopharyngeal skeleton (CPS). First instars could be separated from second instars within a species based on body length and width and nonoverlapping sizes of their CPS (Table 1). Third-instar larvae were used for the detailed morphological descriptions presented here, because their structures were larger and more easily manipulated and measured than those of earlier instars. Newly molted third instars were separated from other instars and then placed in new diet tubes to continue development for 3 d before being removed from the diet and prepared for examination and imaging.

Third-instar larvae were cleared in 10% NaOH for examination under dissecting and compound light microscopy. Different subsets of larvae were used for examining the structures on the posterior segment, the dorsal and lateral body surfaces, and the ventral body surface. The posterior larval segment was cut in cross section for viewing of the posterior spiracular plate and posterior spiracles. Other specimens were cut with iridectomy scissors longitudinally along the middorsal line to allow the cuticle to lie flat for careful examination of the ventral surface. Similarly, some other specimens were cut longitudinally on the mid-

ventral line for examination of the dorsal surface. Care was taken to avoid damage to the mouthparts. The cut larvae and posterior larval segments were then cleared for 12–15 h in 10% NaOH. Remaining body tissues were removed using a camel's-hair brush. After clearing, the sclerotized mouth parts were carefully removed from the cut larvae with the aid of very fine forceps and mounted laterally on a glass slide. Larval cuticles and posterior spiracular plates were mounted flat on glass slides, covered with glycerin and glass coverslips, and labeled.

An ocular micrometer was used to measure body parts large enough to be viewed with a compound light microscope. External larval characteristics observed included greatest length and width of third-instar larvae; total number of creeping welts; size, number of rows, and arrangement of spinules in individual creeping welts; shape, size, and position of the anal lobes with respect to the anal opening; and arrangement of spinules around the anal lobes. Several parameters of the CPS were examined including: total length and width; length of mandibles, hypopharyngeal sclerite, parastomal bar, dorsal arch, dorsal bridge, and dorsal and ventral cornua; and the presence or absence of a distinct tooth on the mandibles (see Fig. 3a–f). Larval mouthparts were photographed using an Automontage system (Syncroscopy, Frederick, MD) with a KY-F70B digital camera (JVC Headquarters and East Coast Sales, Wayne, NJ) mounted on a DMLB compound microscope (Leica Microsystems, Bannockburn, IL). Head and thoracic sections of larvae were examined for the size and shape of the antennae, number of oral ridges, and the size and shape of papillae on the anterior spiracles. The caudal segment was examined to measure the length of posts supporting the posterior spiracles; the distance between the posts; the number and arrangement of spiracular slits; the size and position of the spiracular scar; and the number, arrangement and branching pattern of spiracular hairs. The trunks and tips were counted for all the posterior spiracular hairs. Ratios of measurements on related structures also were calculated for use as characteristics to differentiate the species and to avoid the biases caused by unusually small or large specimens.

Some larvae were cross-sectioned and mounted vertically to obtain SEM views of the anterior and posterior surface structures. The methods of Grodowitz et al. (1982) were followed to prepare the larval specimens for examination under SEM. Live third-instar larvae were rinsed two times in deionized water followed by sonication in mild detergent for 30 s. The larvae were again rinsed two times in deionized water and then placed for 1 min in super skipper solution (kerosene, 17 parts; glacial acetic acid, 11 parts; 95% ethyl alcohol, 50 parts; iso-butyl alcohol, 17 parts; and dioxane, 5 parts) (Elzinga 1981) followed by two rinses in Carl's solution (95% ethyl alcohol, 28 parts; 40% formaldehyde, 11 parts; glacial acetic acid, 4 parts; water, 57 parts) (Barbosa 1974). The larvae were then placed in Carl's solution for 12–15 h followed by a dehydration series in increasing concentrations of ethyl alcohol (15 min in each of 30, 50, 70, 80, 90, and 95% and three times in 100%). The specimens were dried using a Samdri-780A critical point dryer (Tousimis Research Corporation, Rockville, MD). The specimens were attached at various angles to sticky carbon paper on aluminum stubs to allow SEM views of both dorsal and ventral sides. Specimens were sputter-coated with gold-palladium dorsally, ventrally, and laterally (3 min each).

Puparia. Four-day-old puparia were removed from the colony and stored in 70% ethyl alcohol for examination. Puparia were examined under light microscopy by using a dissecting microscope. The anterior and posterior surfaces were observed by placing puparia upright in small depressions in Styrofoam blocks. The greatest length and width of puparia, the number and position of papillae in anterior spiracles, the position of posterior spiracles, and the shape of posterior spiracular plates were observed and described (see Fig. 4a–d). The puparia were photographed with a JVC 3 charge-coupled device camera (JVC Headquarters and East Coast Sales) and Z16 APO lens (Leica Microsystems Inc.).

Voucher specimens were deposited in the Florida State Collection of Arthropods at the Division of Plant Industry (DPI), Florida Department of Agriculture and Consumer Services (FDACS), Gainesville, FL. Morphological terminology used in this manuscript follows Teskey (1981). Terminology of the maxillary palp and dorsolateral group follows Chu-Wang and Axtell (1972).

Statistical Analysis. One way analysis of variance (ANOVA) (PROC GLM, SAS Institute 2008) was used to determine whether the measurements and ratios of measurements were affected by species (fixed variable). The numbers of specimens for each characteristic were used as replicates in the model. The Tukey's honestly significant difference (HSD) test (SAS institute 2008) was used for means separation, with $P = 0.05$. Only characteristics with nonoverlapping ranges were used as diagnostic characteristics to separate the species.

Descriptions

Chaetopsis massyla

(Figs. 1a–c; 2a,d,e,i,l; 3a,d,g,j; 4a,d)

Eggs. White, elongate-ovoid to somewhat spindle shaped; posterior end smoothly rounded while tapering from micropylar end; widest in middle (Fig. 1a); length 0.68 ± 0.01 (mean ± SEM) (range, 0.60–0.75) mm; width 0.15 ± 0.003 (0.12–0.18) mm; length:width 4.61 ± 0.09 (3.55–5.71) (n = 36); diameter of collar surrounding micropyle 0.02 ± 0.0005 (0.02–0.03) mm (n = 36); hatching line (hl) starting from the micropylar end and spanning ≈20–25% of the egg length (Fig. 1b); micropyle situated in a shallow pit at the anterior end of the egg; chorion appearing to be smooth under a light microscope but reticulated with elevated ridges under SEM; entire egg surface covered

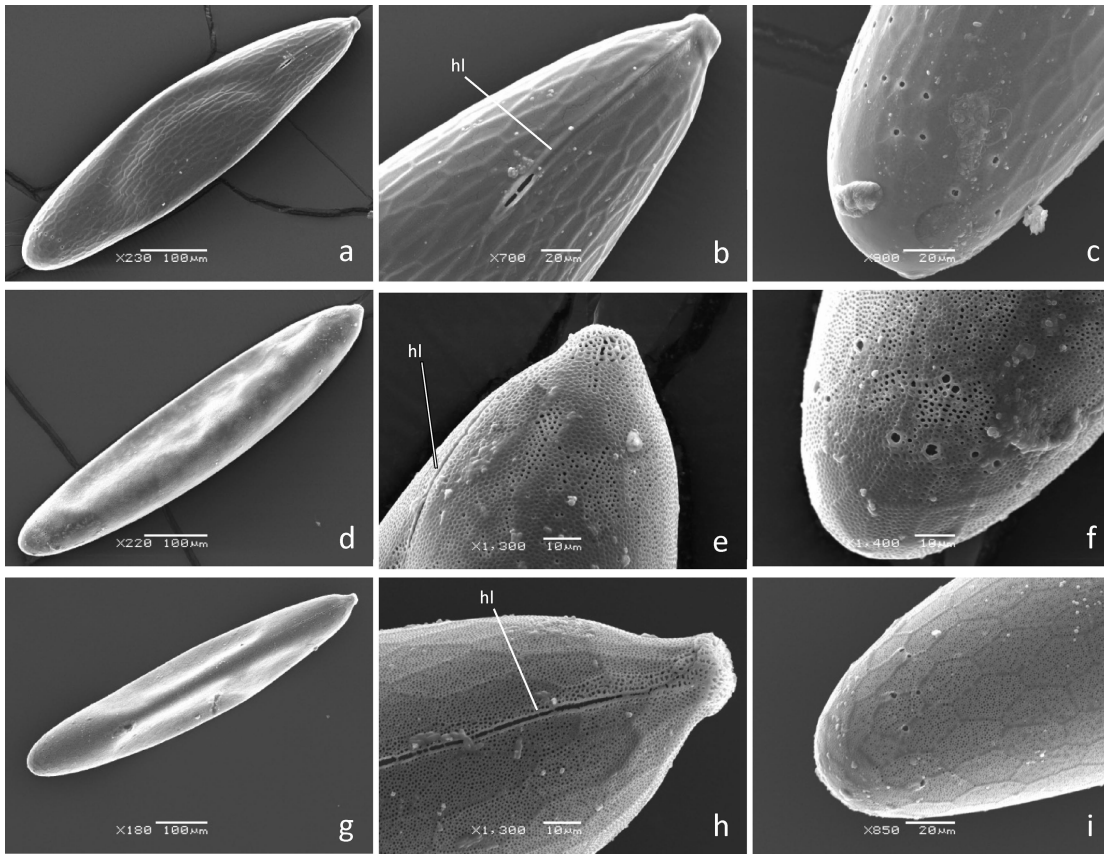

Fig. 1. Eggs of *C. massyla* (a–c), *E. eluta* (d–f), and *E. stigmatias* (g–i); habitus (a, d, g), anterior end (b, e, h) and posterior end (c, f, i); hl, hatching line.

mostly with elongate hexagons, occasionally irregular squares, pentagonal, and hexagonal cells; cell length reduces toward both ends of eggs and ranges 14–40 μm for anterior, 20–42 μm for middle and 16–34 μm for posterior cells (based on randomly selected cells from 100-μm-long sections in the anterior, middle, and posterior regions of eggs); distinct pores 1.95 ± 0.09 (1.05–2.92) μm in diameter (n = 36) were present at the vertices of polygons on the chorion surface at the posterior end of eggs (Fig. 1c).

Larvae. *General Structure.* Creamy white to yellow, elongate in shape with a pointed head, and broad, rounded posterior end; third-instar length 8.23 ± 0.09 (7.25–9.25) mm; width 1.17 ± 0.04 (0.45–1.50) mm; length:width 7.40 ± 0.50 (5.17–18.33) (n = 24) (Table 2).

Head. Antennomaxillary complex (Fig. 2a and d): cephalic segment bilobed with an antennal and maxillary sensory complex at the end of each lobe (Fig. 2a); antenna two-segmented, distal segment apically hemispherical; antennal length ≈1.5× greater than width (Table 2); maxillary complex with three papilla sensillae (p1, p2, p3) and two knob sensillae (k1, k2); dorsolateral group bearing two additional well-developed papilla sensillae (p, m) much closer to palp than to antennae (Fig. 2d).

Oral ridges (Fig. 2a) 23 ± 0.4 (19–27) oral ridges (or) (n = 29) below the antennomaxillary lobes; number varies between the two sides of the same larva; finely dentate with individual processes pointed and curved inward toward the mouth cavity; a few of the dentate processes in broken groups near the ends of the oral ridges close to the antennomaxillary complex; two medial oral lobes (mol) on the inside of the mandibles; labial lobe (lal) at posterior of mouth cavity.

Cephalopharyngeal skeleton (CPS) (Fig. 3a and d): sclerotized structure consisting of three major parts: mandibles (m), hypopharyngeal sclerite (hps), and tentoropharyngeal sclerite (tps) (Fig. 3a; Table 2); two mandibles (Fig. 3d) with elongate, heavily sclerotized basal half and lightly sclerotized distal half comprising sharp sickle-shaped mouth hooks that are distally grooved ventrally, a distinct tooth (at) ventrally at about the midpoint of the mouth hook just posterior to the end of the groove (Fig. 3d); hps lying between the mandibles and tps with posterior portion of hps overlapping the anterior margin of tps, two longitudinal bars articulated broadly with the mandibles anteriorly and with the tps posteriorly (Fig. 3a); posterior portion of the hps lies interior to the anterior ventral cornua; two bars of the hps joined on the

Table. 2. Comparison of third instar larval measurements among three species of picture-winged fly pests of corn

Larval measurement[a]	Mean ± SEM (*n*; range)			*F*; df; *P*
	C. massyla	*E. eluta*	*E. stigmatias*	
Larval				
Length (mm)	8.23 ± 0.0A (24; 7.25–9.25)	7.52 ± 0.09B (23; 6.39–8.63)	6.24 ± 0.14C (24; 5.25–7.75)	82.14; 2, 68; <0.0001
Width (mm)	1.17 ± 0.04A (24; 0.45–1.50)	1.18 ± 0.01A (23; 1.10–1.38)	0.95 ± 0.03B (24; 0.60–1.15)	20.91; 2, 68; <0.0001
Antenna				
Length (al)	15.29 ± 1.20A (12; 11.00–22.50)	14.69 ± 1.99A (10; 8.80–22.50)	15.29 ± 1.34A (12; 11.00–22.50)	0.05; 2, 31; 0.9509
Width (aw)	10.67 ± 0.33A (12; 9.00–12.50)	12.19 ± 1.13A (10; 8.80–20.00)	12.38 ± 0.49A (12; 11.00–15.00)	1.98; 2, 31; 0.1552
Anterior spiracle				
Length (asl)	115.88 ± 3.15A (15; 92.40–132.00)	105.93 ± 2.02B (23; 88.00–125.00)	103.57 ± 2.84B (15; 81.40–122.50)	5.66; 2, 50; 0.0061
Width (asw)	120.36 ± 3.42B (15; 99.00–145.00)	130.36 ± 3.35AB (22; 110.00–175.00)	135.66 ± 4.98A (16; 99.00–185.00)	3.44; 2, 50; 0.0399
Basal width (tw)	53.11 ± 1.93A (16; 33.00–66.00)	50.27 ± 1.46A (24; 39.60–62.50)	50.94 ± 1.88A (17; 32.50–67.50)	0.71; 2, 54; 0.4953
Papilla length (pa)	21.19 ± 0.62AB (14; 17.50–25.00)	19.57 ± 0.66B (19; 15.00–25.00)	24.03 ± 1.46A (12; 17.50–32.50)	6.14; 2, 42; 0.0046
Papilla width (pw)	13.24 ± 0.58A (14; 10.00–17.60)	14.13 ± 0.72A (19; 10.00–20.00)	12.82 ± 0.32A (14; 11.00–15.40)	1.27; 2, 44; 0.2899
Posterior spiracle				
Post diam	140.86 ± 2.88A (24; 121.00–175.00)	140.71 ± 3.22A (23; 106.80–162.50)	148.33 ± 4.91A (21; 120.00–187.50)	1.34; 2, 65; 0.2678
Post length	119.53 ± 3.42A (16; 87.50–137.50)	104.17 ± 3.69B (21; 62.50–125.00)	98.21 ± 3.14B (21; 75.00–137.50)	9.26; 2, 55; 0.0003
Distance between posts	115.47 ± 4.11B (23; 87.50–160.00)	144.39 ± 4.08A (23; 112.50–187.50)	106.67 ± 3.82B (21; 75.00–130.00)	24.08; 2, 64; <0.0001
Ecdysial scar width (es)	20.66 ± 0.67A (26; 15.40–28.00)	23.12 ± 1.63A (17; 15.50–35.00)	21.39 ± 1.08A (21; 15.40–27.50)	1.28; 2, 61; 0.2864
Rima thickness (rm)	4.63 ± 0.07A (18; 4.40–5.00)	—[b]	2.34 ± 0.03B (20; 2.20–2.50)	907.36; 1, 36; <0.0001
Opening length (sol)	31.12 ± 1.45A (12; 25.00–42.00)	32.10 ± 0.72A (17; 28.00–40.00)	30.35 ± 0.59A (17; 27.00–37.00)	1.10; 2, 43; 0.3434
Opening width (sow)	22.19 ± 0.73A (18; 17.50–28.60)	—[b]	18.36 ± 0.62A (14; 15.00–22.00)	14.72; 1, 30; 0.0006
Cephalopharyngeal skeleton (CPS)				
CPS length (mm)	0.99 ± 0.01A (19; 0.88–1.09)	0.87 ± 0.01B (21; 0.75–0.97)	0.90 ± 0.01B (21; 0.73–0.99)	21.70; 2, 58; <0.0001
Mandible segmensts				
a	58.61 ± 1.54B (16; 49.50–70.00)	53.75 ± 1.19B (18; 49.50–62.50)	65.32 ± 1.70A (17; 57.50–85.00)	15.81; 2, 48; <0.0001
b	52.94 ± 1.58A (16; 35.00–63.00)	48.43 ± 0.95AB (23; 37.50–57.50)	44.74 ± 1.65B (18; 30.00–55.00)	8.17; 2, 54; 0.0008
c	100.35 ± 1.91A (16; 80.00–110.00)	95.76 ± 2.68A (23; 66.00–117.50)	104.04 ± 3.51A (18; 80.00–149.60)	2.29; 2, 54; 0.1111
d	20.88 ± 1.57A (16; 10.00–30.00)	15.53 ± 0.95B (23; 7.50–25.00)	15.43 ± 1.69B (18; 6.60–30.00)	4.72; 2, 54; 0.0129
e	84.52 ± 1.82A (16; 67.50–92.40)	88.43 ± 1.75A (23; 75.00–107.50)	89.19 ± 1.28A (17; 77.50–105.00)	2.17; 2, 53; 0.1242
Dental sclerite length (ds)	54.27 ± 1.66A (23; 39.60–66.00)	44.74 ± 1.30B (28; 31.50–60.00)	45.29 ± 1.12B (25; 37.40–55.00)	14.72; 2, 73; <0.0001
hypopharyngeal sclerite (hps) length	176.29 ± 4.61A (20; 151.30–250.00)	146.98 ± 3.69B (22; 90.00–175.00)	156.51 ± 3.16B (23; 117.00–187.50)	14.82; 2, 62; <0.0001
Parastomal bar length (pb)	184.58 ± 3.56A (19; 162.00–225.00)	154.43 ± 2.95C (21; 126.00–187.50)	171.20 ± 3.34B (23; 150.00–212.50)	20.17; 2, 60; <0.0001
Overlap of tps over hps	58.61 ± 1.54A (16; 49.50–70.00)	53.75 ± 1.19B (18; 49.50–62.50)	65.32 ± 1.70B (17; 57.50–85.00)	15.81; 2, 48; <0.0001
Dorsal arch length (da)	129.85 ± 3.90A (17; 99.00–170.00)	128.12 ± 3.08A (19; 97.90–150.00)	118.13 ± 4.61A (16; 70.00–135.00)	2.60; 2, 49; 0.0847
Dorsal bridge length (db)	77.82 ± 2.24B (17; 63.00–99.00)	89.69 ± 2.25A (21; 60.00–106.80)	87.89 ± 1.39A (22; 70.00–100.00)	9.72; 2, 57; 0.0002
Dorsal cornu length (dc) (mm)	0.61 ± 0.01A (29; 0.51–0.74)	0.54 ± 0.01B (35; 0.45–0.65)	0.51 ± 0.01B (33; 0.45–0.56)	26.28; 2, 94; <0.0001
Notch length (*n*) (mm)	0.28 ± 0.01A (17; 0.25–0.34)	0.24 ± 0.01B (17; 0.20–0.30)	0.25 ± 0.01B (18; 0.12–0.28)	8.25; 2, 49; 0.0008
Ventral cornu length (vc) (mm)	0.70 ± 0.01A (31; 0.58–0.77)	0.57 ± 0.01C (28; 0.49–0.63)	0.61 ± 0.001B (34; 0.35–0.69)	52.55; 2, 90; <0.0001
Anal complex				
Anal organ width (aow)	0.45 ± 0.01C (18; 0.39–0.51)	0.60 ± 0.02A (21; 0.48–0.69)	0.52 ± 0.02B (24; 0.39–0.63)	27.29; 2, 60; <0.0001
Anal organ length (aol)	0.28 ± 0.01A (18; 0.24–0.32)	0.30 ± 0.01A (21; 0.26–0.35)	0.29 ± 0.01A (24; 0.23–0.39)	1.48; 2, 60; 0.2349
Anus length	0.16 ± 0.00A (18; 0.13–0.18)	0.15 ± 0.00AB (21; 0.13–0.17)	0.14 ± 0.00B (24; 0.10–0.17)	5.63; 2, 60; 0.0057
Anal hook	31.24 ± 1.16B (15; 23.20–38.40)	39.60 ± 0.85A (12; 36.40–45.00)	35.51 ± 2.38AB (21; 21.00–56.00)	3.83; 2, 45; 0.0290

Means followed by different letters within a row are significantly different ($P < 0.05$; Tukey's test).

[a] All parameters are in μm unless specified.

[b] rm and sow not measured for *E. eluta* due to poor visibility of posterior spiracle under compound microscope.

ventral side by a lightly sclerotized bar forming an H shape when viewed ventrally; a pair of slender parastomal bars fused to the hps for half their length posteriorly and then tapering and diverging over the anterior half; the labial sclerite and the epipharyngeal sclerite lie below hps toward its anterior end; tps comprised mainly of dorsal cornu and ventral cornu, upper and lower arms of U-shaped sclerites on either side of the pharynx; ventral cornua fused on each side with the pharynx; dorsal cornua at their anterior end joined by a dorsal bridge.

Thorax. Three thoracic segments clearly visible; no creeping welts on any of the thoracic segments; 25–30 discontinuous rows of spinules (sr) encircle the anterior one third of the first thoracic segment (Fig. 2a).

Anterior spiracles: structure projects laterally on prothorax like a fan from middle part of segment (Figs. 2e and 3g); spiracular openings (so) in depressions at the ends of almost equal length finger-like papillae (pa) projecting from a common base; papillae in a single row in the shape of a fan, vary in number (eight to 10) between sides of the same larva; papillae increase in width toward their distal end; only the papillae and a small portion of the main body of the spiracle normally seen outside the larval body under SEM, whereas the remainder hidden within the body; length of spiracle (asl) about equal to its width (asw) (Table 2); papillae length (pl) ≈1.5–2× greater than their width (pw).

Abdomen. Eight abdominal segments clearly visible.

Locomotory structures: creeping welts (Fig. 2i–k; Table 3) present on the ventral surface of anterior margins of all eight abdominal segments (i.e., CW1 to CW8); welts comprise transverse swollen ridges bearing multiple rows (R) of spinules that were all pointed, curved, and symmetrical to asymmetrical; width of CW8 narrower than other welts; CW3, CW4, and CW6 wider than CW7 and CW8; CW7 wider than CW8; CW4 and CW5 longer than CW1 to CW3; welt width-to-length ratio greatest for CW1 followed by CW2 that was followed by CW4 to CW8. CW1 and CW8 with five rows of spinules (i.e., R1 to R5); CW2 usually with six rows but occasionally with five rows; CW3 to CW7 with six rows of spinules. Spinules in R1 of each welt anteriorly oriented while posteriorly oriented in following rows; CW1 with all the spinules of almost same

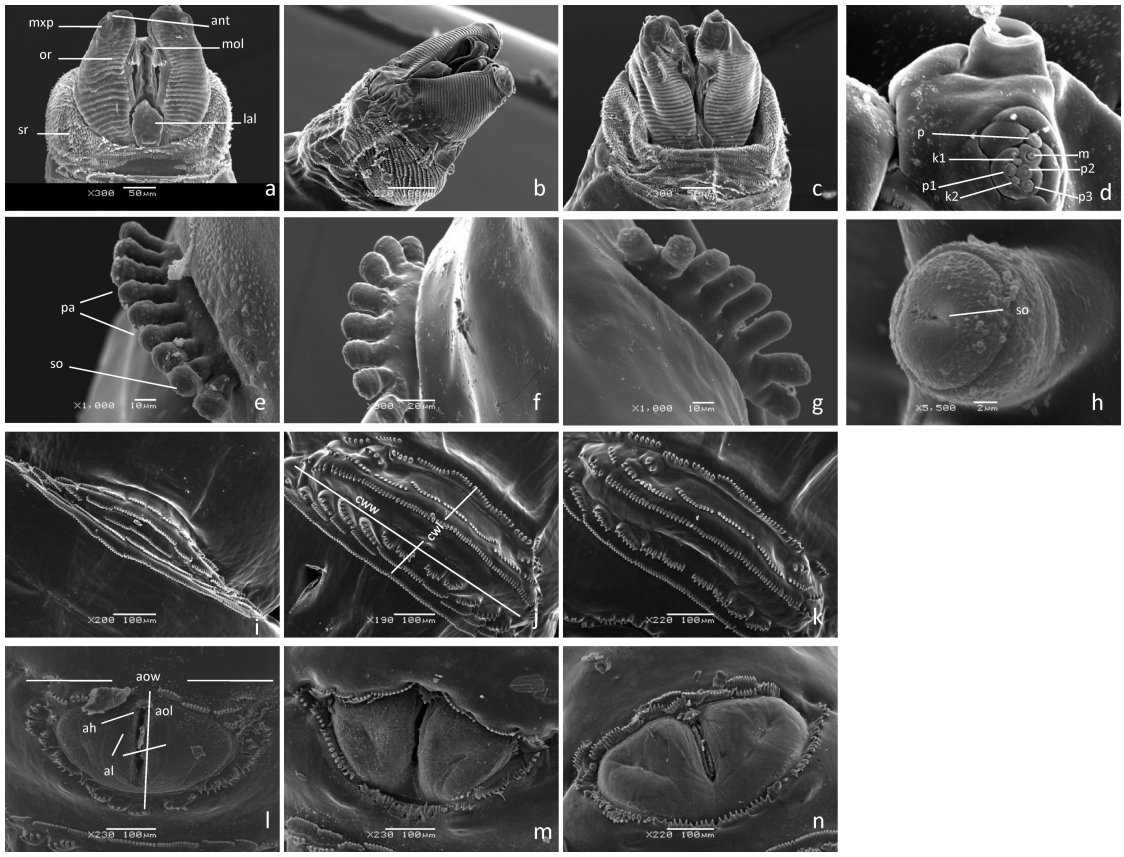

Fig. 2. SEM views of larvae of *C. massyla* (a, d, e, i, l), *E. eluta* (b, f, j, m), and *E. stigmatias* (c, g, h, k, n); face (a, b, c, d), anterior spiracle (e, f, g, h), creeping welts second (i), seventh (j), eighth (k), anal complex (l, m, n); ah, anal hook, al, anal lobe, aol, anal complex length, ant, antenna, aow, anal complex width, cwl, length of creeping welt, cww, width of creeping welt, k1, knob sensilla 1, k2, knob sensilla 2, lal, labial lobe, m, modified papilla sensilla one of dorsolateral group, mol, median oral lobe, mxp, maxillary palp, or, oral ridges, p, modified papilla sensilla two of dorsolateral group, pa, papillae, p1, papilla sensilla 1, p2, papilla sensilla 2, p3, papilla sensilla 3, so, spiracular opening, sr spinule row.

size; spinule groups in R4 of all welts except CW1 are angled to body midline and thus seem to radiate from the body with angle increasing laterally. CW1: R1 to R4 discontinuous, R5 continuous. CW2 to CW8: R1, R2, R4, and R5 discontinuous; R3 and R6 continuous, rarely discontinuous; spinules in R4 broader and in distinct groups on raised ridges with four to nine spinules in each group; spinule size largest in R4 reducing in size in rows anterior and posterior to R4. CW2 to CW7: spinules of R4 and R5 overlap spinule groups of the same row and join spinules between different rows at various points. CW8: spinules in R4 merge with those of R5 when the latter are discontinuous.

Posterior spiracles (Fig. 3j; Table 2): present on elevated sclerotized cylindrical posts on the last abdominal segment and facing posteriorly; spiracular plate bearing a circular ecdysial scar and three elongate-oval shaped spiracular openings arranged at right angles; ecdysial scar situated at the medial margin of the spiracular plate; moderately developed rimae (rm) and numerous trabeculae (tr) on the spiracular openings; four groups of spiracular hairs arising from the spiracular plate between the ecdysial scar and the spiracular openings; spiracular hairs more than half the length of the spiracular posts; SP-1 (first on left from ecdysial scar) comprising three to four trunks and 21–28 tips, basal width 6–16 μm (n = 23 specimens); SP-2 (second on left from ecdysial scar) comprising two to four trunks and 14–35 tips, basal width 8–18 μm; SP-3 (third on left from ecdysial scar) comprising two to four trunks and 14–28 tips, basal width 4–14 μm; SP-4 (fourth on left from ecdysial scar) comprising three to four trunks and 20–28 tips, basal width 6–19 μm.

Anal complex: paired wrinkled and grooved anal lobes on either side of the anus (Fig. 2l; Table 2) present ventrally on anterior portion of last abdominal segment; anal lobes surrounded by one or occasionally two rows of spinules overlapping each other at several places and a few groups of spinules scattered around the anal lobes more laterally; spinules pointed and curved and almost always posteriorly oriented, except a few of the spinules anterior to anal lobes oriented anteriorly in few specimens; a

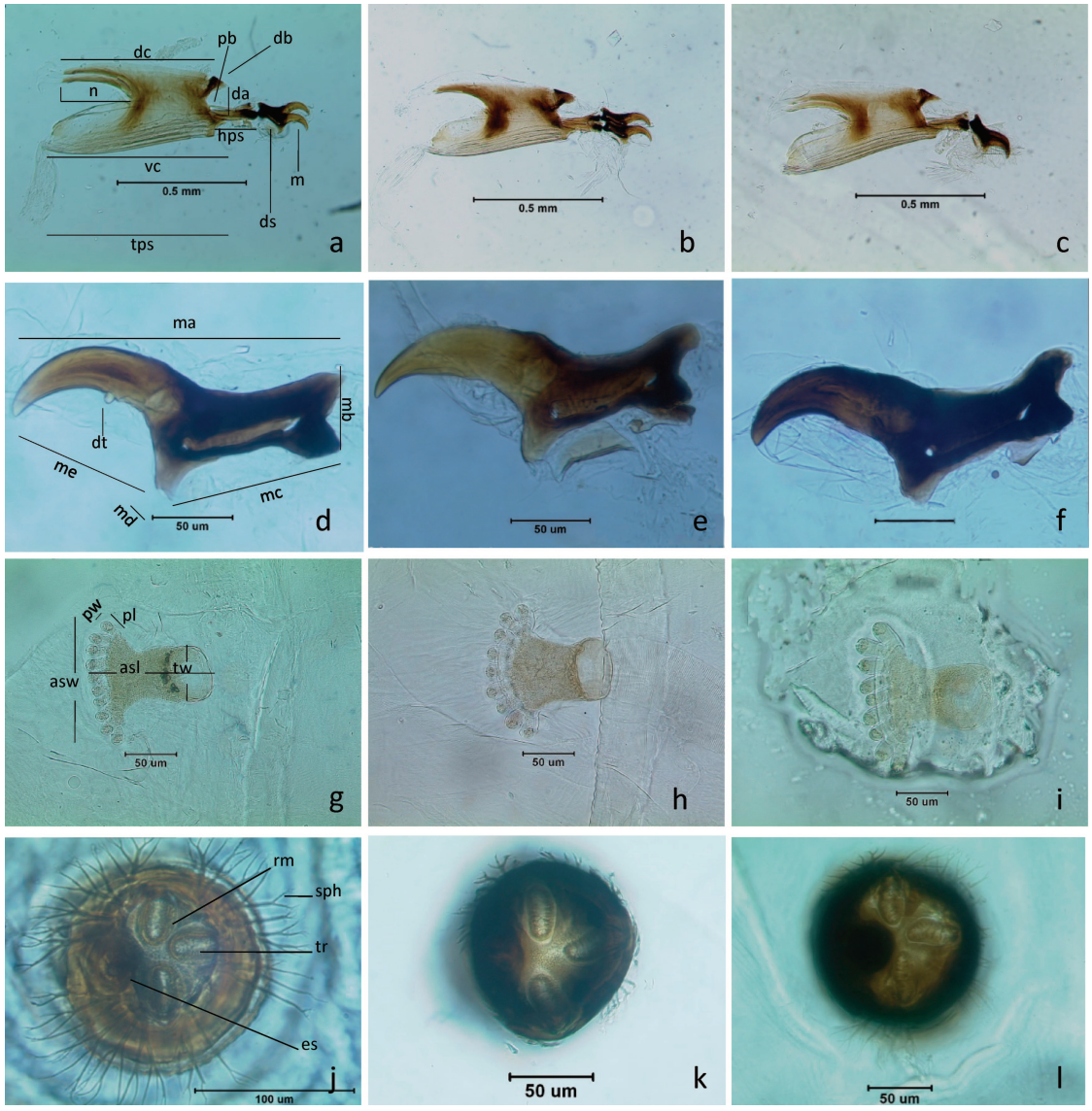

Fig. 3. Light microscope views of larvae *C. massyla* (a, d, g, j), *E. eluta* (b, e, h, k), and *E. stigmatias* (c, f, i, l); Cephalopharyngeal skeleton (a, b, c), mandibles (d, e, f), anterior spiracle (g, h, i), posterior spiracle (j, k, l), asl, anterior spiracle length, asw, anterior spiracle apical width, da, dorsal arch, db, dorsal bridge, dc, dorsal cornu, ds, dental sclerite, dt, distinct tooth es, ecdysial scar, hps, hypopharyngeal sclerite, m, mandible, ma, total mandible length a, mb, mandible length b, mc, mandible base length c, md, mandible length d, me, mouth hook length e, n, notch, pb, parastomal bar, pl, papilla length, pw, papilla width, rm, rima, sph, spiracular hair, tps, tentoropharyngeal sclerite, tr, trabeculae, tw, anterior spiracle basal width, vc, ventral cornu. (Online figure in color.)

robust anal hook with six to nine sharp points at the posterior end of the anus.

Puparia. Length 4.95 ± 0.06 (4.38–5.75) mm; maximum width at third abdominal segment 1.58 ± 0.01 (1.35–1.75) mm ($n = 39$) (Fig. 4d); reddish to reddish brown; barrel shaped, narrowing sharply toward the anterior end but broadly toward the posterior end; notable cuticle shrinkage at the anterior end; anterior spiracle visible with only papillae and small portion of stalk protruding as in third instar; all creeping welts clearly visible similar to third instar; anal lobes reddish brown to black; spinules around anal lobes clearly visible; posterior spiracular plate surrounded by grooves forming trapezoidal shape (Fig. 4a); the posts supporting posterior spiracles brown; posterior spiracles dark black with spiracular hairs apparent.

Euxesta eluta

(Figs. 1d–f; 2b,f,m; 3b,e,h,k; 4b,d)

Eggs. Color and shape of eggs similar to those of *C. massyla;* length 0.72 ± 0.01 (0.62–0.80) mm; width

Table 3. Comparison of creeping welt measurements (millimeters) among larvae of three species of picture-winged fly pests of corn

Welt no.	Parameter[a]	Mean ± SEM (*n*; range)			*F*; df; *P*
		C. massyla	*E. eluta*	*E. stigmatias*	
1	Width (W)	0.80 ± 0.03[a]Abc (18; 0.58–0.98)	0.75 ± 0.02Ac (24; 0.53–0.94)	0.65 ± 0.02Bb (18; 0.52–0.80)	10.29; 2, 57; 0.0002
	Length (L)	0.14 ± 0.01Ad (18; 0.09–0.28)	0.13 ± 0.01ABd (21; 0.09–0.22)	0.10 ± 0.01Bc (18; 0.07–0.14)	4.48; 2, 54; 0.0158
2	W	0.86 ± 0.03Aabc (18; 0.62–1.02)	0.83 ± 0.02Abc (24; 0.67–1.02)	0.71 ± 0.03Bab (15; 0.53–0.90)	9.55; 2, 54; 0.0003
	L	0.18 ± 0.01Bcd (18; 0.12–0.23)	0.20 ± 0.00Ac (21; 0.17–0.23)	0.14 ± 0.01Cb (15; 0.12–0.16)	32.89; 2, 51; <0.0001
3	W	0.93 ± 0.03Aab (15; 0.76–1.20)	0.97 ± 0.02Aa (24; 0.77–1.16)	0.74 ± 0.02Bab (18; 0.61–0.89)	26.85; 2, 54; <0.0001
	L	0.22 ± 0.01ABbc (15; 0.17–0.30)	0.23 ± 0.01Ab (21; 0.17–0.29)	0.19 ± 0.00Ba (18; 0.17–0.22)	7.03; 2, 51; 0.0020
4	W	0.96 ± 0.03Aa (12; 0.80–1.16)	0.98 ± 0.02Aa (24; 0.76–1.20)	0.77 ± 0.02Ba (18; 0.60–0.95)	19.68; 2, 51; <0.0001
	L	0.28 ± 0.02Aa (12; 0.21–0.37)	0.26 ± 0.00Aa (21; 0.23–0.29)	0.21 ± 0.01Ba (18; 0.18–0.24)	22.64; 2, 48; <0.0001
5	W	0.91 ± 0.03Aabc (12; 0.71–1.11)	0.97 ± 0.02Aa (24; 0.76–1.17)	0.73 ± 0.02Bab (18; 0.59–0.89)	26.27; 2, 51; <0.0001
	L	0.28 ± 0.02Aa (12; 0.19–0.37)	0.26 ± 0.01Aa (21; 0.19–0.29)	0.21 ± 0.01Ba (18; 0.19–0.26)	15.42; 2, 48; <0.0001
6	W	0.94 ± 0.03Aa (12; 0.73–1.13)	0.91 ± 0.03Aab (24; 0.71–1.16)	0.72 ± 0.02Bab (18; 0.59–0.92)	19.38; 2, 51; <0.0001
	L	0.25 ± 0.01Aab (12; 0.19–0.33)	0.27 ± 0.00Aa (24; 0.26–0.29)	0.20 ± 0.01Ba (18; 0.15–0.24)	27.23; 2, 48; <0.0001
7	W	0.79 ± 0.02Ac (12; 0.67–0.93)	0.83 ± 0.02Abc (24; 0.67–1.05)	0.65 ± 0.02Bb (18; 0.52–0.83)	17.62; 2, 51; <0.0001
	L	0.24 ± 0.02Aabc (12; 0.15–0.32)	0.25 ± 0.00Aab (24; 0.21–0.28)	0.19 ± 0.01Ba (18; 0.10–0.26)	10.07; 2, 48; 0.0002
8	W	0.65 ± 0.03Bd (12; 0.49–0.82)	0.78 ± 0.02Ac (18; 0.62–0.94)	0.55 ± 0.03Cc (15; 0.37–0.76)	22.36; 2, 42; <0.0001
	L	0.22 ± 0.01Aabc (12; 0.14–0.28)	0.23 ± 0.01Ab (18; 0.17–0.28)	0.15 ± 0.01Bb (15; 0.08–0.21)	16.98; 2, 39; <0.0001

Means within a row comparing species followed by different uppercase letters, and within a column comparing parameters between welts of the same species followed by different lowercase letters are significantly different ($P < 0.05$; Tukey' test).

0.14 ± 0.001 (0.12–0.14) mm; length:width 5.27 ± 0.06 (4.56–6.11) ($n = 33$); diameter of collar surrounding the micropyle 0.02 ± 0.0003 (0.02–0.03) mm ($n = 33$) (Fig. 1d and e). The remainder of the description is the same as for eggs of *C. massyla*, except for the following: length of polygonal cells on chorion surface range 12–36 μm for anterior, 24–35 μm for middle, and 20–37 μm for posterior cells; chorion covered with two sizes of pores over entire surface when observed under SEM; minute pores 0.89 ± 0.04 (0.72–1.27) μm in diameter ($n = 20$) widely distributed across the surface but do not cover the seam of the hatching line (hl); distinct pores 2.56 ± 0.07 (1.91–3.00) μm diameter ($n = 33$) present at the vertices of polygons at posterior end of eggs (Fig. 1f).

Larvae. *General Structure.* Third-instar larvae of *E. eluta* are the same as in *C. massyla* larvae, except for the following: length 7.52 ± 0.09 (6.39–8.63) mm; width 1.18 ± 0.01 (1.10–1.38) mm; length:width 6.38 ± 0.10 (5.17–18.33) ($n = 23$) (Table 2).

Head. Antennomaxillary complex (Fig. 2b): same as in *C. massyla*, except that the antennal length is ≈1.18× greater than the width (Table 2).

Oral ridges: 34 ± 0.6 (30–42) oral ridges (or) ($n = 25$) present below the antennomaxillary lobes (Fig. 2b); remainder of description is the same as for *C. massyla.*

CPS: same as that of *C. massyla*, except that no distinct tooth as seen on mandibles of *C. massyla* (Fig. 3b and e; Tables 2 and 4).

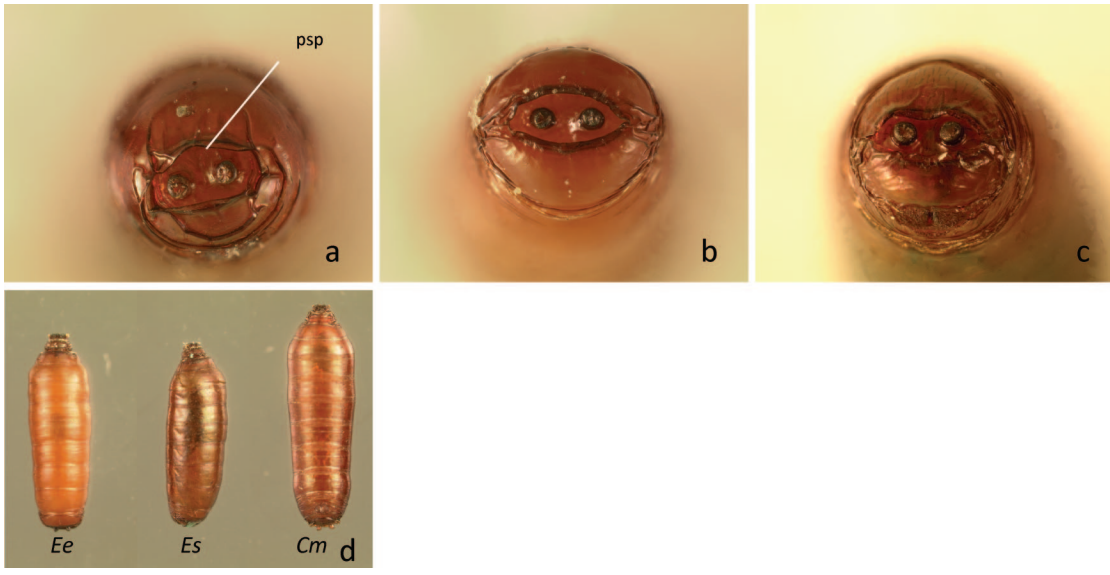

Fig. 4. Light microscope views of puparia. Posterior spiracular plate of *C. massyla* (a), *E. eluta* (b), *E. stigmatias* (c); habitus view of *E. eluta* (*Ee*), *E. stigmatias* (*Es*, and *C. massyla* (*Cm*); psp, posterior spiracular plate. (Online figure in color.)

Table 4. Diagnostic characteristics separating the immature stages of three species of picture-winged fly pests of corn

Stage	Characteristic	*C. massyla*	*E. eluta*	*E. stigmatias*
Egg	Surface structure	Pores present at vertices of polygons only (Fig. 1c)	Pores present across entire surface (Fig. 1e and f)	Pores present across entire surface (Fig. 1h and i)
Larva	Posterior spiracle	Light brown (Fig. 3j)	Black (Fig. 3k)	Dark brown (Fig. 3l)
	Distinct tooth on mandible	Present (Fig. 3d)	Absent (Fig. 3e)	Absent (Fig. 3f)
	No. oral ridges	23 ± 0.4 (range, 19–27)	34 ± 0.6 (range, 30–42)	35 ± 0.6 (range, 31–40)
	Creeping welt length		Mean and range for CW2 and CW6 longer than *E. stigmatias*	Mean and range for CW2 and CW6 shorter than *E. eluta*
	Spinule arrangement	Spinules in row 1 discontinuous in CW2 to CW8	Spinules in row 1 continuous in CW4 to CW8	Spinules in row 1 continuous in CW3 to CW8
Puparium	Color	Reddish to reddish brown	Light brown to black	Light brown black
	Shape of posterior spiracular plate	Trapezoidal (Fig. 4a)	Oval (Fig. 4b)	Oval (Fig. 4c)

Thorax. Three thoracic segments visible; no creeping welts on any of the thoracic segments; 27–30 discontinuous rows of spinules (sr) encircle the anterior third of the first thoracic segment (Fig. 2b).

Anterior spiracles (Figs. 2e, 3 h): length (asi) ≈1.23× greater than width (asw) (Table 2); seven to 10 finger-like papillae in a single row; papillae length (pl) ≈1.4× greater than width (pw) (Table 2); remainder of the description is the same as that of *C. massyla.*

Abdomen. Eight abdominal segments are clearly visible.

Locomotory structures: creeping welts on the ventral side of the anterior margins of all eight abdominal segments (Table 3); welts comprise transverse swollen ridges bearing rows of spinules that are all pointed, curved and symmetrical to asymmetrical. CW3 to CW5 wider than CW1, CW2, CW7 and CW8; CW4 to CW6 longer than CW1–CW3 and CW8; CW1 shorter than other welts; welt width to length ratio greatest for CW1 followed by CW2 and CW3 and then by CW6 to CW8. Spinule groups in R4 of all welts except CW1 angled to body midline and thus seem to radiate from the body with the angle increasing laterally. CW1 and CW8 have five rows of spinules; CW2 to CW7 have six rows of spinules; CW1, majority of spinules were of equal size; CW2 to CW8, spinule size largest on R4 and in distinct groups on raised ridges with three to eight spinules in each group and reducing in size in rows anterior and posterior to R4. R1 spinules on CW1 and CW2 with anterior orientation (occasionally with posterior orientation); R1 spinules on CW3 to CW7 with anterior orientation, but R2 to R6 with posterior orientation, R4 spinules angled to body midline and thus pointing opposite to each other. CW1 to CW3: R1 and R2 usually continuous (rarely discontinuous), R3 and R4 discontinuous. CW2 and CW3: R5 and R6 continuous. CW4 to CW8: R2, R4, R5 discontinuous but R1, R3, and R6 continuous. CW2 to CW7: spinules of R4 and R5 overlap spinule groups of the same row and join spinules between different rows at various points. CW8: spinules in R4 merged with those of R5 when the latter were discontinuous.

Posterior spiracles (Fig. 3k; Table 2): SP-1 comprising two to three trunks and 19–25 tips. basal width 5–15 μm (14 specimens); SP-2 comprising two to three trunks and 14–26 tips, basal width 6–17 μm; SP-3 comprising two to four trunks and 12–21 tips, basal width 6–18 μm: SP-4 comprising two to four trunks and 18–29 tips; basal width 6–21 μm. Remainder of description is the same as in *C. massyla.*

Anal complex: the description is the same as that of *C. massyla* (Fig. 2k; Table 2).

Puparia. Length 4.25 ± 0.06 (3.75–4.75) mm; maximum width at third abdominal segment 1.42 ± 0.03 (1.25–1.70) mm (n = 34) (Fig. 4b and d). Light brown (but not reddish) to black. The remainder of description is the same as in puparia of *C. massyla* except for the following: posterior spiracular plate surrounded by grooves forming ovoid shape; posts supporting posterior spiracles black.

Euxesta stigmatias

(Figs. 1g–i; 2c,g,h,n; 3c,f,i,l; 4c,d)

Eggs. Color and shape of eggs similar to those of *C. massyla;* length 0.76 ± 0.01 (0.69–0.83) mm; width 0.15 ± 0.004 (0.12–0.19) mm; length:width 5.20 ± 0.12 (4.05–6.69) (n = 39); (Fig. 1g–i); diameter of collar surrounding the micropyle is 0.02 ± 0.0002 mm (n = 39). The remainder of the description is the same as for eggs of *C. massyla* and *E. eluta,* except for the following: length of polygonal cells on chorion surface range 12–44 μm for anterior, 24–38 μm for middle, and 15–37 μm for posterior cells; chorion covered with two sizes of pores over entire surface when observed under SEM; minute pores 0.63 ± 0.06 (0.16–0.81) μm in diameter (n = 14) widely distributed across the surface, but do not cover the seam of hatching line (hl); distinct pores 2.84 ± 0.07 (2.35–3.52) μm diameter (n = 39) present at vertices of polygons at posterior end of eggs (Fig. 1i).

Larvae. *General Structure.* Third-instar larvae the same as in *C. massyla,* except the following: length, 6.24 ± 0.14 (5.25–7.75) mm, width 0.95 ± 0.03 (0.60–1.15) mm, and length:width 6.64 ± 0.24 (5.83–11.67) (n = 24) (Table 2).

Antennomaxillary complex: same as in *C. massyla* (Fig. 2c; Table 2).

Oral ridges: 35 ± 0.6 (31–40) oral ridges (or) (n = 22) present below the antennomaxillary lobes (Fig. 2c). Remainder of the description is the same as for *C. massyla.*

CPS: same as that of *C. massyla,* except no distinct tooth as seen on mandibles of *C. massyla* (Fig. 3c and f; Table 2).

Thorax. Three thoracic segments clearly visible; no creeping welts on any of the thoracic segments; 27–29 discontinuous rows of spinules (sr) encircle the anterior third of the first thoracic segment (Fig. 2c).

Anterior spiracles: same as for *C. massyla* except the following (Figs. 2g and h and 3i); spiracle (asl) length ≈1.3× width (asw) (Table 2); seven to 10 finger-like papillae in a single row; papillae ≈2× longer than wide (Table 2).

Abdomen. Eight abdominal segments are clearly visible.

Locomotory structures: Creeping welts on the ventral side of anterior margins of all eight abdominal segments (Table 3); welts comprise transverse swollen ridges bearing rows of spinules which were all pointed, curved and symmetrical to asymmetrical. CW1, CW7, and CW8 narrower than CW4; CW3 to CW7 longer than CW1, CW2 and CW8; width to length ratio significantly greater for CW1 followed by CW2 followed by CW3 to CW8. The size, shape, number of rows, angle, grouping, and the orientation of spinules on the rows were the same as for *E. eluta.* CW1 and CW2: R1, R2, R4 and R5 discontinuous (the later two rarely continuous), R3 and R6 (CW2) continuous; CW3 to CW8: R2, R4, and R5 discontinuous but R1, R3, and R6 continuous (R1 rarely discontinuous).

Posterior spiracles (Fig. 3l): SP-1 comprising two to four trunks and 22–31 tips, basal width 5–14 μm (14 specimens); SP-2 comprising two to four trunks and 11–25 tips, basal width 5–15 μm; SP-3 comprising two to four trunks and 13–27 tips, basal width 5–15 μm; SP-4 comprising two to four trunks and 15–29 tips, basal width 6–18 μm. The remainder of the description is the same as in *C. massyla* (Table 2).

Anal complex (Fig. 2l): same as for *C. massyla* and *E. eluta* except that the anal hook has five to eight points (Table 2).

Puparia. Length 3.92 ± 0.05 (3.40–4.75) mm; maximum width at third abdominal segment of 1.36 ± 0.02 (1.05–1.60) mm (n = 33) (Fig. 4c and d). Light brown (but not reddish) to black. Description is the same as that of puparia of *C. massyla* except the following: posterior spiracular plate surrounded by groves forming an ovoid shape; posts supporting posterior spiracles black.

Diagnoses

Eggs. The only characteristic found to differentiate eggs of the two genera was the size and distribution of pores. All three species had pores at the vertices of polygons on the egg surface, but only *E. eluta* and *E. stigmatias* also had pores over the entire egg surface (Table 4). Means for several additional egg characteristics differed, but overlapping ranges prevent them from being diagnostic for species. The mean diameter of the pores at the vertices of polygons at the posterior end of eggs was greatest in *E. stigmatias* and smallest in *C. massyla* (F = 33.68; df = 2, 105; $P < 0.0001$). The mean length of *E. stigmatias* eggs was greater than that of *C. massyla* (F = 38.95; df = 2, 105; $P < 0.0001$), and the mean width of *E. eluta* eggs was less than the other two species (F = 7.78; df = 2, 105; P = 0.0007). Mean length-to-width ratio was smaller in *C. massyla* than the other two species (F = 14.58; df = 2, 105; $P <$ 0.0001). No consistent differences in egg shape or color were found. Diameter of the egg collar was similar (F = 1.68; df = 2, 105; P = 0.1906).

Larvae. First instars of *C. massyla* and *E. eluta* were on average longer than those of *E. stigmatias* (Table 1). Body width and CPS length were similar among species. Due to considerable overlap in ranges of body measurements, none were suitable for differentiating first- and second-instar larvae of the three species.

Several characteristics of third-instar larvae could be used to distinguish among the species including the presence or absence of a distinct tooth on the mandibles, number of oral ridges, differences in the continuity of the first row of spinules (R1) in creeping welts, difference in length of a creeping welt, and color of the posterior spiracles (Table 4). The mandibles have a distinct tooth in all *C. massyla* specimens but the tooth was absent on the mandibles of *E. eluta* and *E. stigmatias.* The mean number and range of oral ridges was less in *C. massyla* than in either of the *Euxesta* species (F = 166.97; df = 2, 73; $P < 0.0001$). The R1 in creeping welts was discontinuous in CW2 to CW8 of *C. massyla,* but continuous in CW4 to CW8 of *E. eluta* and CW3 to CW8 of *E. stigmatias.* The width, length, and width:length ratio of creeping welts varied among species (Table 3); however, CW2 was the only welt with nonoverlapping ranges suitable for distinguishing *E. eluta* from *E. stigmatias.* The posterior spiracles of *E. eluta* remained dark black after the 12–15 h 10% NaOH treatment, whereas those of *C. massyla* weakened to light brown and dark brown to black in *E. stigmatias* (Fig. 3j–l). The spiracular slits and spiracular scar of the posterior spiracular plate of *C. massyla* were distinct after clearing, but these structures were indistinguishable in *E. eluta* and difficult to differentiate in *E. stigmatias.*

Means of several other larval characteristics differed among the three species but could not be used to distinguish the species due to their overlapping ranges. The mean length and width of third instar *E. stigmatias* larvae were less than those of the two other species (Table 2). The mean length of *C. massyla* larvae was longer than *E. eluta,* but the mean widths of the two species were similar. No significant difference was found in mean larval width:length ratios among the three species. Mean antennal length, width, and their ratio were similar among the three species. The mean overall length of the CPS was longer in *C. massyla* than in the *Euxesta* species. Mandible segment *d* was longer in *C. massyla* than the other species, whereas mandible segment *a* was longer in *E. stigma-*

tias than the other species. Mandible *b* was longer in *C. massyla* than *E. stigmatias.* None of the ratios of various mandible lengths was consistently largest or smallest in any one species. The dental sclerite, hps, overlap of tps over hps, dorsal cornu and notch lengths were all significantly greater in *C. massyla* than in *E. eluta* and *E. stigmatias.* Mean ventral cornu and parastomal bar lengths were significantly greater in *C. massyla* than in *E. stigmatias*, which were greater than in *E. eluta.* The dorsal bridge length was significantly shorter in *C. massyla* than in the other species. Other CPS parameters were not found to be different among species. The mean length of the anterior spiracles was significantly greater in *C. massyla* than in the other two species. The length:width ratio of the anterior spiracles was greatest in *C. massyla* (0.97 ± 0.03) and least in *E. stigmatias* (0.77 ± 0.03) ($F = 14.08$; df = 2, 49; $P < 0.0001$), with *E. eluta* (0.82 ± 0.02) intermediate. The width of the anterior spiracles was greater in *E. stigmatias* than in *C. massyla.* The length of papillae on the anterior spiracles of *E. stigmatias* was significantly greater than on *E. eluta* ($F = 4.98$; df = 2, 42; $P = 0.0114$). The length:width of papillae on the anterior spiracles of *E. stigmatias* (1.85 ± 0.13) was significantly greater than on *E. eluta* (1.44 ± 0.08) ($F = 4.98$; df = 2, 42; $P = 0.0114$). The mean width of CW1 to CW7 was greater in *C. massyla* and *E. eluta* than in *E. stigmatias.* The majority of *E. stigmatias* welts were narrower than the other species. Significant differences in the width: length ratio were only observed in CW2 and CW4. The length:width ratio of CW2 was greater in *C. massyla* (4.88 ± 0.14) and *E. stigmatias* (5.04 ± 0.14) than *E. eluta* (4.28 ± 0.12) ($F = 9.29$; df = 2, 51; $P = 0.0004$). The length:width ratio of CW4 was greater in *E. eluta* (3.82 ± 0.08) and *E. stigmatias* (3.72 ± 0.08) than *C. massyla* (3.46 ± 0.13) ($F = 3.26$; df = 2, 48; $P = 0.04$). The length of creeping welts varied within welts of the same species for *C. massyla* ($F = 13.48$; df = 7, 103; $P < 0.0001$), *E. eluta* ($F = 69.11$; df = 7, 154; $P < 0.0001$), and *E. stigmatias* ($F = 34.82$; df = 7, 130; $P < 0.0001$). The width of creeping welts significantly varied within welts of the same species for *C. massyla* ($F = 11.24$; df = 7, 103; $P < 0.0001$), *E. eluta* ($F = 16.62$; df = 7, 178; $P < 0.0001$), and *E. stigmatias* ($F = 9.66$; df = 7, 130; $P < 0.0001$). Spinule size and orientation (anterior/posterior) did not vary in any welt among the three species. The mean length of the posts supporting the posterior spiracles was significantly greater in *C. massyla* than in the other species, whereas the distance between the two posterior spiracles was significantly greater in *E. eluta* than in the other species (Table 2). Rima thickness (rm) in *C. massyla* was nearly 2× as wide as in *E. stigmatias.* Rima thickness could not be accurately measured for *E. eluta* by using a light microscope due to the poor clearing of the posterior spiracles by using 10% NaOH. The mean width of the anal complex was significantly wider for *E. eluta* than *E. stigmatias* followed by *C. massyla.* The mean length: width ratio of anal complex was significantly greater for *E. eluta* (2.01 ± 0.05) than *E. stigmatias* (1.79 ± 0.04) followed by *C. massyla* (1.61 ± 0.00) ($F = 24.45$; df = 2, 60; $P < 0.0001$). The mean length of the anal hook was significantly shorter in *C. massyla* than in *E. eluta.* The mean length of the anal complex did not differ significantly among the three species; however, the mean length of the anus in *E. stigmatias* was significantly shorter than in *C. massyla.*

Puparia. Puparia of the three species could be differentiated by their color and the shape of the posterior spiracular plate. The puparia of *C. massyla* were reddish compared with the light brown to black puparia of *E. eluta* and *E. stigmatias* (Table 4). The posterior spiracular plate of *C. massyla* was trapezoidal, whereas those of *E. eluta* and *E. stigmatias* were ovoid, lacking the hard angles observed in *C. massyla* (Fig. 4a–c). Some pupal characteristics were found with significant differences among the three species, but they could not be used to distinguish the species due to their overlapping ranges. Mean puparium length ($F = 87.84$; df = 2, 103; $P < 0.0001$), width ($F = 28.96$; df = 2, 103; $P < 0.0001$), and their ratio ($F = 13.18$; df = 2, 103; $P < 0.0001$) were greater in *C. massyla* than *E. stigmatias.*

Discussion

The above-mentioned descriptions of morphological characters for the immature stages of *C. massyla, E. eluta* and *E. stigmatias* add to the physical descriptions for these species in the literature. The descriptions and measurements published by previous workers generally fall within the ranges determined in the current study even though most of these studies were conducted on different diets, with a few notable exceptions. Allen and Foote (1992) described eggs of *C. massyla* reared on decayed cattail and lettuce leaves as 0.86–0.94 mm long compared with 0.60–0.75 mm long in our study. The measurements of *E. stigmatias* larval length reported by App (1938) were 9.38 mm, nearly 33% longer than those observed in our study. Similarities in the results from the current study and those of previous studies on different diets indicate that the differences in the immatures reared on artificial diet in the current study could be used to differentiate the immatures of these three species reared on other foods (e.g., corn ears). *E. eluta* and *E. stigmatias* eggs in our study were within the range of size described in other studies. Frías-L (1981) described *E. eluta* eggs found in sweet corn in Chile as white, 0.7 mm long, and 0.15 mm wide. Arce de Hamity (1986) reported that eggs of *E. eluta* collected in Jujuy, Argentina, reared on artificial diet were "cylindrical, dull white in color with a thin and sculpturous chorion," 0.73 ± 0.03 mm long, and 0.12 ± 0.002 mm wide. App (1938) found eggs of sweet corn-reared *E. stigmatias* to be "minute, elongate and whitish." Seal et al. (1995) found *E. stigmatias* eggs from field collected sweet corn ears to be "creamy white, slender," 0.85 ± 0.004 mm long (slightly longer than those in our study), and 0.16 ± 0.001 mm long. Hayslip (1951) reported *E. stigmatias* eggs in sweet corn to be "white, slender" and 0.78 mm long.

Our measurements of third-instar larval characters for these three species closely resemble those made by previous workers. Allen and Foote (1992) described

third-instar larvae of *C. massyla* as white with transparent integument, 4.50–9.90 mm long; 0.57–1.28 mm wide; CPS length 0.99–1.10 mm; anterior spiracles with 9–12 finger-like marginal papillae. Arce de Hamity (1986) reported third-instar larvae of *E. eluta* to be 7.55 ± 0.73 mm long, 1.18 ± 0.33 mm wide, with anterior spiracles 0.09 ± 0.008 mm long and 0.15 mm wide. They also found the ratio of the hypostomal (other name for hps) to pharyngeal sclerites (other name for tps) as 1:4, nearly identical to the value 1:3.87 [hps/ventral cornu (tps)] found in our study. Frías-L (1981) described *E. eluta* mandibles as black posteriorly and brown anteriorly. Seal et al. (1995) reported *E. stigmatias* larvae as 5.39 ± 0.04 mm long and white with a glossy cuticle that turns yellow as larvae mature.

Published observations of puparium characteristics were nearly identical to those found in our study. Allen and Foote (1992) described puparia of *C. massyla* as reddish brown, 3.06–5.50 mm long, and 0.96–1.63 mm wide. Arce de Hamity (1986) found the length and width of *E. eluta* puparia to be 4.25 ± 0.42 and 1.44 ± 0.15 mm, respectively. Seal et al. (1995) found puparia of *E. stigmatias* to be barrel shaped, reddish brown, 3.91 ± 0.02 mm long, and 1.37 ± 0.01 mm wide.

Only a few characteristics for each developmental stage were found in our study that could be used to separate the immature stages of *C. massyla*, *E. eluta*, and *E. stigmatias* without rearing them to the adult stage. Although the means of most of the characteristics were significantly different among species, their overlapping ranges made them unsuitable for separating the species. Moreover, some of the characteristics found to separate the species (egg surface, larval mandibles, larval creeping welts, and larval posterior spiracles) require the use of compound and scanning electron microscopes, which limits their usefulness to field scouts, growers, or others working in field conditions. However, puparium color and shape of the posterior spiracular plates in puparia can be observed using a hand lens in the field to separate members of the *Chaetopsis* and *Euxesta* genera compared in this study.

Acknowledgments

Assistance in fly colony maintenance and specimen preparation was provided by H. K. Gill (University of Florida). This work would not have been possible without access to the laboratory supplies and imaging equipment at the DPI, FDACS in Gainesville, FL, and the assistance of P. Skelley with SEM imaging and J. Wiley with critical point drying. Lyle Buss (University of Florida) provided instruction and assistance with a photo Automontage system at the Department of Entomology and Nematology, Gainesville, FL. This research was funded in part by a Hand Fellowship awarded by the Dolly and Homer Hand Group.

References Cited

Allen, E. J., and B. A. Foote. 1992. Biology and immature stages of *Chaetopsis massyla* (Diptera: Otitidae), a secondary invader of herbaceous stems of wetland monocots. Proc. Entomol. Soc. Wash. 94: 320–328.

App, B. A. 1938. *Euxesta stigmatias* Loew an otitid fly infesting ear corn in Puerto Rico. J. Agric. Univ. P R 22: 181–187.

Arce de Hamity, M. G. 1986. Biology of *Euxesta eluta* (Diptera: Ulidiidae): behavior in the attack and putrefaction of corn ears. Acta Zool. Lilloana 38: 119–128.

Bailey, W. K. 1940. Experiments in controlling corn ear pests in Puerto Rico. Puerto Rico Agricultural Experiment Station Circular 23.

Barbosa, P. 1974. Manual of basic techniques in insect histology. Autumn Publisher, Amherst, MA.

Barbosa, P., A. E. Segarra-Carmona, and W. Colon-Guasp. 1986. *Eumecosomyia nubila* (Wiedemann), a new otitid in Puerto Rico, with notes on the habits of the dipteran species complex of corn. J. Agric. Univ. P R 70: 155–156.

Chu-Wang, I. W., and R. C. Axtell. 1972. Fine structure of the terminal organ of the house fly larva *Musca domestica.* Z. Zellforsch. 127: 287–305.

Curran, C. H. 1935. New American Diptera. Am. Mus. Novit. 812: 1–24.

Elzinga, R. J. 1981. Fundamentals of entomology, 2nd ed. Prentice-Hall, Upper Saddle River, NJ.

Evans, D. C., and E. Zambrano. 1991. Insect damage in maize of highland Ecuador and its significance in small farm pest management. Trop. Pest Manage. 37: 409–414.

Frías-L, D. 1981. Diferencias de microhabitats entre *Euxesta eluta* y *Euxesta annonae* (Diptera, Otitidae). Agric. Técnica (Chile) 41: 89–94.

Goyal, G., G. S. Nuessly, D. R. Seal, J. L. Capinera, G. J. Steck, and K. J. Boote. 2011. Distribution of picture-winged flies (Diptera: Ulidiidae) infesting corn in Florida. Fla. Entomol. 94: 35–47.

Goyal, G., G. S. Nuessly, G. J. Steck, D. R. Seal, J. L. Capinera, and K. J. Boote. 2010. New report of *Chaetopsis massyla* (Diptera: Ulidiidae) as a primary pest of corn in Florida. Fla. Entomol. 93: 198–202.

Grodowitz, M. J., J. Krchma, and A. B. Broce. 1982. A method for preparing soft bodied larval Diptera for scanning electron microscopy. J. Kans. Entomol. Soc. 55: 751–753.

Hayslip, N. C. 1951. Corn silk fly control on sweet corn, pp. 1–6. University of Florida Agricultural Experiment Station Circular S-41.

Hentz, M. G., and G. S. Nuessly. 2004. A technique for rearing the sweet corn pest, *Euxesta stigmatias* (Diptera: Otitidae), on a *Helicoverpa* Diet. J. Entomol. Sci. 39: 140–143.

Nuessly, G. S., and M. G. Hentz. 2004. Contact and leaf residue activity of insecticides against the sweet corn pest *Euxesta stigmatias* Loew (Diptera: Otitidae). J. Econ. Entomol. 97: 496–502.

Painter, R. H. 1955. Insects on corn and teosinte in Guatemala. J. Econ. Entomol. 48: 36–42.

SAS Institute. 2008. PROC user's manual, version 9th ed. SAS Institute, Cary, NC.

Seal, D. R., and R. K. Jansson. 1989. Biology and management of corn silk fly, *Euxesta stigmatias* Loew (Diptera: Otitidae), on sweet corn in southern Florida. Proc. Fla. State Hortic. Soc. 102: 370–373.

Seal, D. R., R. K. Jansson, and K. Bondari. 1995. Bionomics of *Euxesta stigmatias* (Diptera: Otitidae) on Sweet corn. Environ. Entomol. 24: 917–922.

Steyskal, G. C. 1965. Family Otitidae, pp. 642–654. *In* A. Stone, C. W. Sabrosky, W. W. Wirth, R. H. Foote, and J. R. Coulson (eds.), A catalog of the Diptera of America North of Mexico, vol. 276. U.S. Department of Agriculture, Washington, DC.

Steyskal, G. C. 1968. 54. Family Otitidae, pp. 1–31. *In* P. E. Vanzolini and N. Papavero (eds.), A catalogue of the Diptera of the Americas south of the United States. Departamento de Zoologia, Secretaria da Agricultura, Sao Paulo, Brazil.

Teskey, H. J. 1981. Morphology and terminology—larvae, pp. 65–88. *In* J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth, and D. M. Wood (eds.), Manual of Nearctic Diptera, vol. 1. Agriculture Canada Monograph 27. Research Branch, Supply & Services Canada, Hull, QC, Canada.

Wyckhuys, K.A.G., and R. J. O'Neil. 2007. Local agro-ecological knowledge and its relationship to farmers' pest management decision making in rural Honduras. Agric. Hum. Values 24: 307–321.

Received 11 November 2010; accepted 6 March 2011.