Description of Larvae of *Anastrepha* spp. (Diptera: Tephritidae) in the *fraterculus* Group

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ABSTRACT The second- and third-instar larvae of three species in the *fraterculus* group, *Anastrepha bahiensis* Lima, *Anastrepha coronilli* Carrejo & González, and *Anastrepha turpiniae* Stone are described and illustrated for the first time. Character states that can be used to distinguish among the three species and their overlap among other species in the *fraterculus* group are discussed.

KEY WORDS fruit fly, larvae, morphology, cephalopharyngeal skeleton

Anastrepha Schiner (Diptera: Tephritidae) is the most economically important genus of Tephritidae in the Neotropical region and also the most diverse with ≈250 species described thus far (Norrbom et al. 1999; Zucchi 2008; Norrbom and Korytkowski 2009, 2011). This genus ranges from the southern United States to northern Argentina and is restricted to tropical and subtropical environments (Aluja 1994).

Brazil harbors the largest known number of *Anastrepha* species, with reports for their occurrence in all of its 26 states. In total, 109 described species have been reported in the country and 33 occur exclusively there (Norrbom et al. 1999; Jesus et al. 2008; Uramoto et al. 2008; Zucchi 2007, 2008).

The *fraterculus* group consists of 29 species and is the most widespread species group, with 17 species in Brazil (Norrbom et al. 1999; Zucchi 2000, 2007). Some of the species in this group are the most polyphagous and economically important species within the genus (Aluja 1994, Norrbom et al. 1999, Zucchi 2007).

The larval stages of *Anastrepha* feed on living plant tissue and are responsible for fruit damage (White and Elson-Harris 1992). Larvae are the fruit fly stage commonly found in infested fruit intercepted in ports of entry, yet these are poorly known. There are published descriptions for third instars for only 13 species of *Anastrepha* so far and several are incomplete, which makes the identification of intercepted larvae extremely difficult (Carroll and Wharton 1988, Steck and Wharton 1989, Steck et al. 1990, Norrbom et al. 1999).

We describe the second and third instar of three species in the *fraterculus* group, *Anastrepha bahiensis* Lima, *Anastrepha coronilli* Carrejo & González, and *Anastrepha turpiniae* Stone. *Anastrepha bahiensis* has been reported from Mexico to Brazil (Norrbom et al. 1999, Norrbom and Korytkowski 2011). In Brazil, it has been reported in the states of Amapá, Amazonas, Bahia, Goiás, Espírito Santo, Minas Gerais, São Paulo, and Santa Catarina (Zucchi 2008). *Anastrepha coronilli* is found in Mexico, Guatemala, Costa Rica, Venezuela, Colômbia, Suriname, and Brazil. In Brazil, it has been reported only in the Brazilian Amazon (Norrbom 2004, Hernandez-Ortiz 2007, Morales and González 2007, Zucchi 2007). To date, *Anastrepha turpiniae* has been reported in Panamá and Brazil where it has been found in all regions, except for the southern region (Norrbom 2004, Zucchi 2007).

Materials and Methods

Larvae of *A. bahiensis*, *A. coronilli*, and *A. turpiniae* were dissected out of fruit collected in Manaus (03° 06′07″ S, 60° 01′30″ W), and Presidente Figueiredo (02° 02′04″ S, 60° 01′30″ W) in the state of Amazonas. To confirm species identity, some of the larvae were dissected out of each of these fruit and some were left in each fruit. The collected fruit were placed in 500-ml plastic containers with a layer of vermiculite at the bottom and covered with voile cloth until larvae emerged and pupated. All pupae obtained were placed in 30-ml plastic containers with a layer of vermiculite at the bottom and covered with voile cloth to allow for the emergence of adults. Larvae were killed and preserved in 100% ethanol. Of each plant species collected, only one species of *Anastrepha* emerged.

External measurements such as length, width, coloration, and number of segments were carried out using a Wild M3C stereomicroscope (Leica, Switzerland) at the Laboratório de Entomologia Agrícola, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil. Larvae were cleared in 10%

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potassium hydroxide at room temperature and placed in excavated slides with ethanol and glycerine (1:1). Rows of dorsal and ventral spinules were counted at the middorsal and midventral lines, respectively. The cephalopharyngeal skeleton (CPS) was removed, placed in glycerine, and measured at 80× magnification for third-instar larvae and 100× magnification for second-instar larvae. For both instars, measurements on posterior and anterior spiracles were taken using 200× and 400× magnification. These structures were photographed using a digital camera (Olympus DP72) attached to a microscope (Olympus BX51) and all drawings were made using a camera lucida (Leica DM 750) at the Laboratório de Citotaxonomia e Insetos Aquáticos (INPA). The cephalopharyngeal skeleton of third-instar larvae was placed in a petri dish with 80% ethanol, photographed, measured, and described using a digital camera (Leica DFC420) attached to a stereomicroscope (Leica M165C) at the Laboratório de Prospecção de Bioativos de Insetos (INPA).

The descriptions follow the terminology of Teskey (1981), Steck and Malavasi (1988), Steck and Wharton (1988), and Frías et al. (2009). We used the same endpoints for measurements, same abbreviations for thoracic segments, abdominal segments, posterior spiracular processes, and caudal tubercles as Steck and Malavasi (1988) and Steck and Wharton (1988).

In preparation for scanning electron microscopy (SEM), larvae were transferred to silicone capsules and dehydrated in an ethanol series, then critical point dried in CO_2 for 3 h, and sputter-coated with a gold layer. The larvae were examined in a LEO 1450VP scanning electron microscope at the Laboratório Institucional de Microscopia Eletrônica de Varredura do Museu Paraense Emílio Goeldi, Belém, Pará, Brazil. SEM was used to examine at least 10 larvae for each species.

Voucher specimens of larvae and associated females are deposited at the Coleção de Invertebrados of the Instituto Nacional de Pesquisas da Amazônia (INPA).

Results

The larvae of *A. bahiensis*, *A. coronilli*, and *A. turpiniae* are similar in their gross morphology. Diagnostic characters to differentiate among these three species include the presence or absence of dorsal spinules on segment T3, the shape of the anal lobe, the number of trunks and tips of the posterior spiracular hairs, and some characteristics of the cephalopharyngeal skeleton.

Anastrepha bahiensis Lima
(Fig. 1)

Material Examined. In total, 32 larvae were examined (25 third-instar larvae and seven second-instar larvae). Larvae were dissected out of fruit of *Helicostylis scabra* (Macbr.) C.C. Berg, Moraceae (locally known as "inharé"); and from fruit of *Pouroma cecropiaefolia* Moraceae (locally known as "mapati"), both native trees in the "terra firme" forest in the Amazon.

Features of Third-Instar Larvae. 5.99–10.65 mm long and 1.03–1.86 mm wide at the sixth abdominal segment. Elongate, cylindrical, tapered thoracic segments, and truncate caudal end. Color ranging from creamy white to creamy yellow.

On all segments, spinules in discontinuous rows of variable length dorsally and ventrally. Spinules conical, symmetrical to slightly curved. Spinule basal width 2.35–3.98 μm. Dorsal spinule pattern: T1 with 2–5 rows; T2 with 3–5 rows; T3, A1 to A8 lacking spinules. Ventral spinule pattern: T1 with 4–7 rows, T2 with 5–8 rows, T3 with 3–4 rows, A1 with 5–7 rows, A2 with 10–12 rows, A3 with 10–15 rows, A4 and A5 with 12–15 rows, A6 and A7 with 11–14 rows, A8 with 10–14 rows. Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with 3–4 irregular rows anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with 11.47–19.01 μm outside diameter, apical knob with 9.23–10.54 μm diameter, and combined height 5.63–8.72 μm. Maxillary sensory organ cylindrical to slightly tapered with 13.02–18.34 μm diameter and height 6.71–7.33 μm. Stomal sensory organ cylindrical with 4.15–7.05 μm height. Six to eight oral ridges with posterior margin irregularly serrated, accessory plates present (Fig. 1A).

Cephalopharyngeal skeleton with shape and sclerotization as in Fig. 1D. Total length from tip of mandible to end of ventral cornu 0.84–1.28 mm. Mandible length a 0.15–0.24 mm, length b 0.14–0.17 mm, height c 0.12–0.18 mm, ratio a to c 1.2–1.3. Tooth short and sharp, sometimes blunt. Hypopharyngeal sclerite 0.17–0.20 mm long, 0.16–0.20 mm wide at ventral bridge. Epipharyngeal sclerite visible only in dorsal view, with medial lobe directed anteriorly. Labial sclerite short, robust, fairly sclerotized, and horseshoe-shaped in dorsal view. Parastomal bar extending for almost the entire length of hypopharyngeal sclerite and arched slightly. Ventral sclerite present below pharyngeal sclerite. Dorsal cornu usually with well-defined sclerotized area 0.32–0.48 mm long, length including hyaline area 0.51–0.72 mm. Dorsal bridge prominently projecting anteriorly from dorsal cornu and slightly sclerotized. Dorsal arch 0.31–0.38 mm high. Anterior sclerite irregularly shaped and sclerotized. Cornu notch index (ratio N/DC) 0.6–0.7. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 8–9 ridges forming a series of grooves along length of ventral cornu. Ventral cornu 0.59–0.81 mm long from pharyngeal bar to posterior end of grooves. Ventral cornu 1.5 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 11–14 tubules (occasionally 15), in a single row (Fig. 1B, E). Tubule length 21.06–27.55 μm, distal width 16.45–19.87 μm, basal width 11.66–16.93 μm. The anterior spiracle height at midline 108.20–154.82 μm, distal width 200.63–225.86 μm, and basal width 90.49–129.58 μm at junction with trachea.

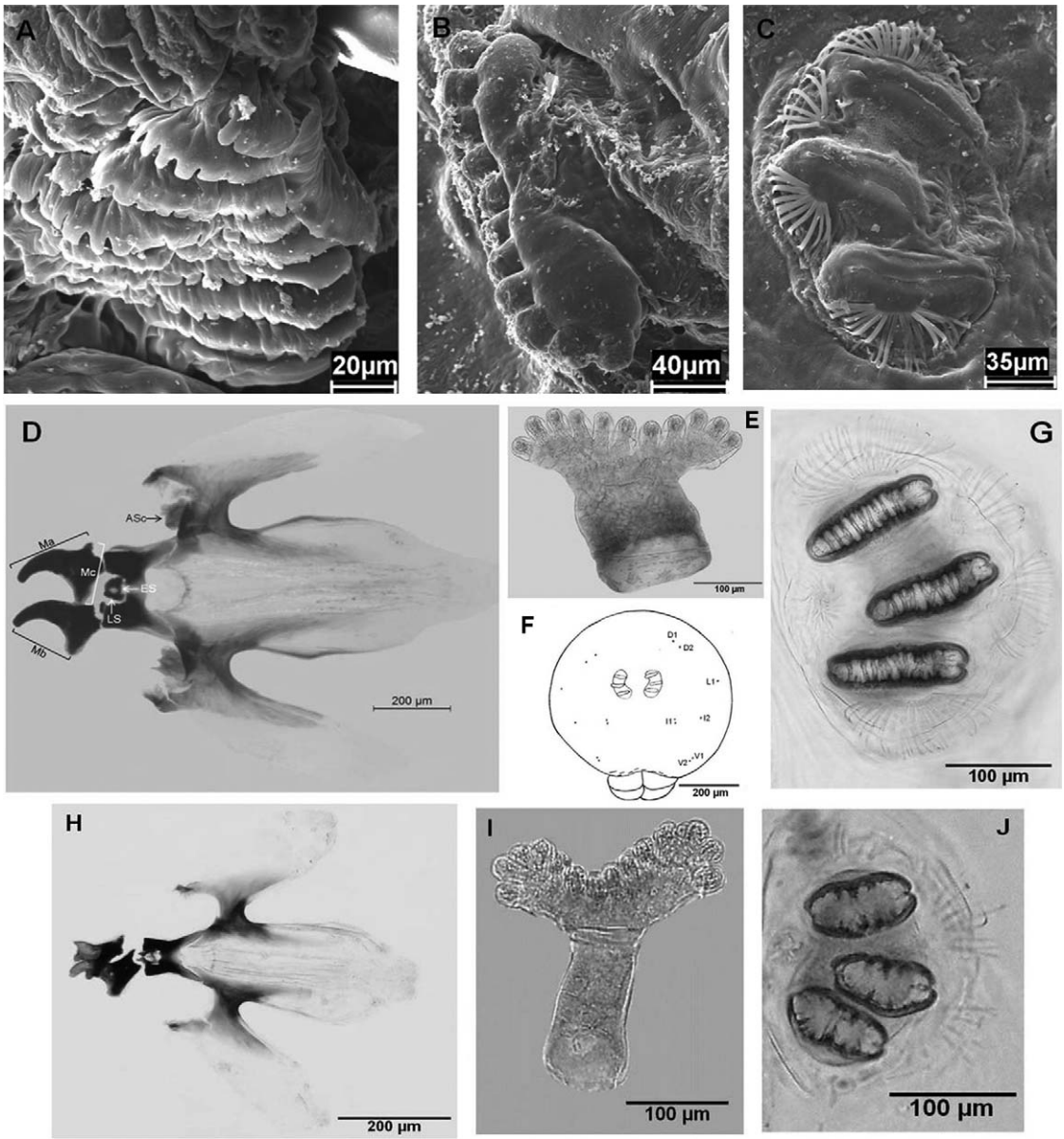

Fig. 1. Scanning electron microscopy (A to C) and optical microscopy views of third-instar (D to G) and second-instar (H to J) larvae of *A. bahiensis*. Third-instar larvae (A) Oral ridges. (B and E) Anterior spiracle. (C and G) Posterior spiracle. (D) Cephalopharyngeal skeleton. (F) Caudal view of posterior segment. Second-instar larvae (H) Cephalopharyngeal skeleton. (I) Anterior spiracle. (J) Posterior spiracle. Abbreviations: Ma, mandible length a; Mb, mandible length b; Mc, mandible height c; ASc, anterior sclerite; ES, epipharyngeal sclerite; LS, labial sclerite; D1 and D2, dorsal tubercles; L1, lateral tubercle; I1 and I2, intermediate tubercles; V1 and V2, ventral tubercles.

Fig. 1F illustrates the caudal segment with dorsal (D1 and D2), intermediate (I1 and I2), lateral (L1), and ventral (V1 and V2) tubercles and sensilla all well developed. D1 tubercle distinctly anterior to D2. Intermediate tubercles I1 and I2 more strongly developed, but associated sensilla weakly developed; I2 lateral and sometimes slightly ventral to I1. L1 and V all very weakly developed. D1 and I1 are associated with weakly developed sensillae. Some specimens have three lateral tubercles (V1, V2, and V3). Posterior spiracles located above horizontal midline. Anal lobe bifid.

Posterior spiracle with openings with thick rimae and numerous trabeculae (Fig. 1C, G); 72.24–98.63 μm long, 16.08–19.74 μm width, ratio length to width range 4.5–5.0. Ecdysial scar apparent. Felt chamber oval, 138.41–168.09 μm in diameter at junction with trachea. Spiracular process SP-I comprising 14–20

trunks and 20–40 tips, ratio tips to trunks 1.4–2.0, basal width 20.06–35.66 μm, ratio basal width to length of spiracular opening ≈0.2. SP-II comprising 4–9 trunks and 11–22 tips. SP-III comprising 5–12 trunks and 14–27 tips. SP-IV comprising 12–21 trunks and 23–41 tips, ratio tips to trunks 1.9, basal width 19.56–38.19 μm, ratio basal width to length of spiracular opening ≈0.2. Average bristle length ≈0.4 times length of spiracular opening.

Features of Second-Instar Larvae. 2.13–3.78 mm long and 0.37–0.52 mm wide at the sixth abdominal segment. Shape and color as in third instar.

Spinules shaped and in discontinuous rows as in third instar. Spinule basal width 2.16–3.11 μm. Dorsal spinule pattern: T1and T2 with 3–4 rows, the rows not completely traversing dorsum, usually with slight hiatus at middorsal line; T3, A1 to A8 lacking spinules. Ventral spinule pattern: T1 with 3–5 rows, T2 with four rows (occasionally two or three), T3 with 2–3 rows, A1 with five rows, A2 with 8–9 rows, A3 with 10 rows, A4 and A5 with 8–9 rows, A6 with 8–9 rows (occasionally 11), A7 with 9–11 rows, A8 with 8–9 rows (occasionally 10). Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with three (occasionally four) irregular rows anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with 6.89–12.05 μm outside diameter; and apical knob with 7.20–7.62 μm in diameter; combined height 4.97–5.57 μm. Maxillary sensory organ weakly sclerotized, cylindrical, with 11.26–17.31 μm diameter, and height 5.30–5.47 μm. Stomal sensory organ cylindrical with 2.82–3.89 μm height. 6–8 oral ridges with the same shape as in third-instar larvae and accessory plates present.

Cephalopharyngeal skeleton with total length from tip of mandible to end of ventral cornu 524.22–616.91 μm (Fig. 1H). Mandible length a 102.17–122.38 μm, length b 67.44–78.43 μm, height c 68.05–88.59 μm, ratio a to c 1.4–1.5, apical half well sclerotized, basal half heavily sclerotized, some mandibles show two openings at base (Fig. 1E), teeth sharply pointed, and well-developed secondary tooth. Hypopharyngeal sclerite 90.93–114.61 μm long, anterior and apical half heavily sclerotized, posterior and basal half well sclerotized, and 48.07–77.14 μm wide at ventral bridge. Epipharyngeal sclerite, labial sclerite, and parastomal bar as in third instar. Dorsal cornu usually with well-defined sclerotized area 114.58–202.43 μm long, length including hyaline area 221.54–340.55 μm. Dorsal arch 146.83–174.70 μm high. Anterior sclerite apparently absent. Cornu notch index 0.5. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 6–7 ridges forming a series of grooves along length of ventral cornu. Ventral cornu 270.85–367.44 μm long from pharyngeal bar to posterior end of grooves. Ventral cornu 1.2 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, and bearing 11–14 tubules (occasionally 9–10), in a single row (Fig. 1I). Tubule length 8.85–13.19 μm, distal width 9.12–11.22 μm, and basal width 5.88–8.97 μm. The anterior spiracle height at midline 73.74–96.77 μm, distal width 82.13–94.87 μm, and basal width 24.35–44.28 μm at junction with trachea.

Caudal segment with sensilla-bearing tubercles arranged as in third instar. Posterior spiracle and anal lobe located as in third instar. Anal lobe bifid.

Posterior spiracle with openings with thinner rimae and fewer trabeculae than in third instar (Fig. 1J), 22.38–29.91 μm long, 11.27–15.12 μm width, ratio length to width 1.9. Ecdysial scar apparent. Felt chamber round, 56.27–63.54 μm diameter at junction with trachea. Spiracular process SP-I comprising 7–12 trunks and 14–18 tips, ratio tips to trunks 1.5–2.0, basal width 6.93–15.46 μm, ratio basal width to length of spiracular opening ≈0.4–0.5. SP-II comprising 4–6 trunks and 6–8 tips. SP-III comprising 7–9 trunks and 10–13 tips. SP-IV comprising 7–11 trunks and 14–16 tips, ratio tips to trunks 1.4–2.0, basal width 7.75–12.33 μm, ratio basal width to length of spiracular opening ≈0.4. Average bristle length ≈0.5 times length of spiracular opening.

Anastrepha coronilli Carrejo and González (Fig. 2)

Material Examined. In total, 36 larvae were examined (25 third-instar larvae and 11 second-instar larvae). Larvae were dissected out of fruit of *Bellucia grossularioides* L. Melastomataceae (locally know as "goiaba-de-anta") a native tree in the "terra firme" forest in the Amazon.

Features of Third-Instar Larvae. 9.51–10.96 mm long and 1.55–2.37 mm wide at the sixth abdominal segment. Elongate, cylindrical, tapered thoracic segments, and truncate caudal end. Color creamy yellow.

On all segments spinules in discontinuous rows of variable length dorsally and ventrally. Spinules conical, symmetrical to slightly curved. Spinule basal width 2.19–5.29 μm. Dorsal spinule pattern: T1 with 2–3 rows (occasionally 4); T2 with 3–4 rows (occasionally 1–2); T3, A1 to A8 lacking spinules. Ventral spinule pattern: T1 with 3–5 rows, T2 with 3–4 rows, T3 with 1–2 rows, A1 with 3–7 rows, A2 with 9–11 rows, A3 with 10–14 rows, A4 and A5 with 13–15 rows, A6 with 10–14 rows, A7 with 12–14 rows, A8 with 10–14 rows. Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with four irregular rows anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with 17.48–25.06 μm outside diameter, apical knob with 10.79–13.09 μm diameter, and combined height 8.04–13.18 μm. Maxillary sensory organ cylindrical to slightly tapered with 20.18–43.47 μm diameter, and height 10.23–14.60 μm. Stomal sensory organ cylindrical with 4.90–8.67 μm height. 9–10 oral ridges (occasionally 7–8) with posterior margin quite irregularly serrated, accessory plates present (Fig. 2A).

Cephalopharyngeal skeleton with shape and sclerotization as in Fig. 2D. Total length from tip of man-

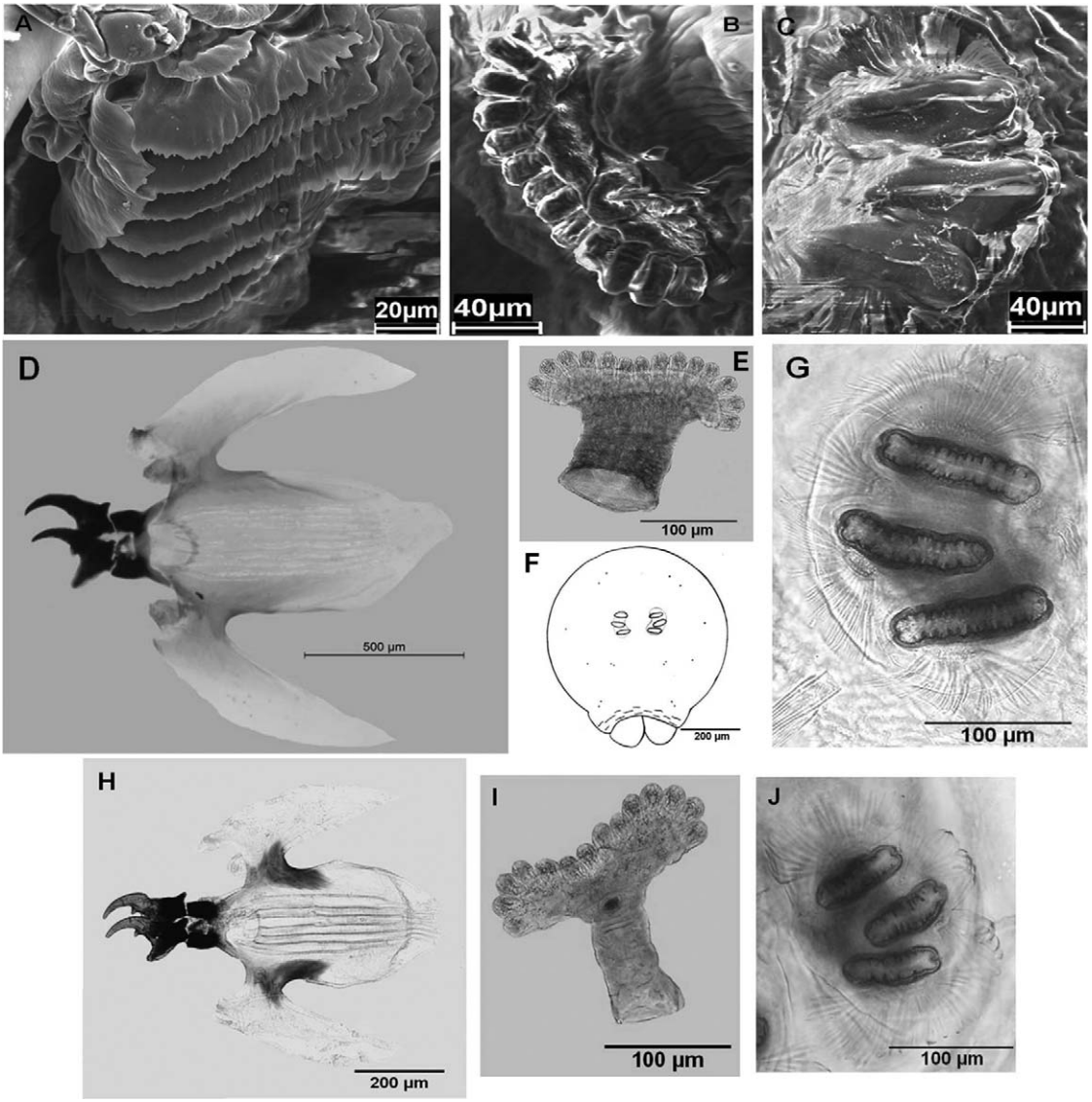

Fig. 2. Scanning electron microscopy (A to C) and optical microscopy views of third-instar (D to G) and second-instar (H to J) larvae of *A. coronilli*. Third-instar larvae (A) Oral ridges. (B and E) Anterior spiracle. (C and G) Posterior spiracle. (D) Cephalopharyngeal skeleton. (F) Caudal view of posterior segment. Second-instar larvae (H) Cephalopharyngeal skeleton. (I) Anterior spiracle. (J) Posterior spiracle.

dible to end of ventral cornu 1.15–1.47 mm. Mandible length a 0.25–0.33 mm, length b 0.19–0.24 mm, height c 0.19–0.23 mm, ratio a to c 1.3–1.4. Tooth long and sharp. Hypopharyngeal sclerite 0.17–0.21 mm long, 0.16–0.19 mm wide at ventral bridge. Epipharyngeal sclerite visible only in dorsal view, with medial lobe directed anteriorly. Labial sclerite short, robust, fairly sclerotized, and horseshoe-shaped in dorsal view. Parastomal bar extending almost the entire length of hypopharyngeal sclerite and arched. Ventral sclerite present below pharyngeal sclerite. Dorsal cornu usually with well-defined sclerotized area 0.42–0.52 mm long, length including hyaline area 0.57–0.85 mm. Dorsal bridge prominently projecting anteriorly from dorsal cornu and slightly sclerotized. Dorsal arch 0.35–0.46 mm high. Anterior sclerite irregularly shaped and sclerotized. Cornu notch index 0.6. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 8–9 ridges forming a series of grooves along length of ventral cornu. Ventral cornu 0.64–0.90 mm long from pharyngeal bar to posterior end of grooves. Ventral cornu 1.2 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 11–15 tubules (occasionally 10), in a single row (Fig. 2B, E). Tubule length 17.77–37.33 μm, distal width 15.87–26.76 μm, and basal width 12.96–21.39 μm. The anterior spiracle height at midline 120.81–178.11 μm,

distal width 193.26–271.82 μm, and basal width 85.78–133.42 μm at junction with trachea.

Fig. 2F illustrates the caudal segment with dorsal (D1 and D2), intermediate (I1 and I2), lateral (L1), and ventral (V1 and V2) tubercles and sensilla all well developed. D1 tubercle distinctly anterior to D2. Intermediate tubercles I1 and I2 more strongly developed, but associated sensilla weakly developed; I2 lateral and sometimes slightly ventral to I1. L1 and V all very weakly developed. D1 and I1 are associated with weakly developed sensilla. Some specimens have three lateral tubercles (V1, V2, and V3). Posterior spiracles located above horizontal midline. Anal lobe entire.

Posterior spiracle with openings with thick rimae and numerous trabeculae (Fig. 2C, G), 81.38–113.57 μm long, 15.87–25.85 μm width, and ratio length to width range 4.3–5.1. Ecdysial scar apparent. Felt chamber oval, 138.26–223.37 μm diameter at junction with trachea. Spiracular process SP-I comprising 16–25 trunks (occasionally 12 or 30) and 45–76 tips (occasionally 40), ratio tips to trunks 2.8–3.0, basal width 31.60–64.03 μm, and ratio basal width to length of spiracular opening ≈0.3–0.5. SP-II comprising 5–12 trunks and 18–45 tips (occasionally 16). SP-III comprising 10–15 trunks (occasionally 5–8) and 23–48 tips (occasionally 16). SP-IV comprising 17–25 trunks (occasionally 13–14) and 40–63 tips (occasionally 32), ratio tips to trunks 2.3–2.5, basal width 32.44–64.90 μm, ratio basal width to length of spiracular opening ≈0.3–0.5. Average bristle length ≈0.4 times length of spiracular opening.

Features of Second-instar Larvae. 4.18–5.37 mm long and 0.62–0.93 mm wide at the sixth abdominal segment. Shape and color as in third instar.

Spinules shaped and in discontinuous rows as in third instar. Spinule basal width 3.05–3.84 μm. Dorsal spinule pattern: T1 with 3–4 rows; T2 with 2–3 rows, the rows not completely traversing dorsum; T3, A1 to A8 lacking spinules. Ventral spinule pattern: T1 with 10–11 rows (occasionally 6–7), T2 with 4–7 rows (occasionally 10), T3 with 3–7 rows, A1 with 6–11 rows (occasionally 4–5), A2 with 10–14 rows, A3 with 11–14 rows (occasionally 8), A4 with 10–13 rows, A5 with 11–14 rows, A6 with 12–13 rows (occasionally 8), A7 with 11–13 rows, A8 with 11–14 rows. Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with 4–5 irregular rows anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with 12.99–18.22 μm outside diameter, apical knob with 9.94–11.43 μm diameter, and combined height 7.40–9.97 μm. Maxillary sensory organ weakly sclerotized, cylindrical, with 36.73–43.39 μm diameter, and height 11.33–14.85 μm. Stomal sensory organ cylindrical with 5.77–8.01 μm height. 6–7 oral ridges with the same shape as in third-instar larvae and accessory plates present.

Cephalopharyngeal skeleton with total length from tip of mandible to end of ventral cornu 754.71–834.01 μm (Fig. 2H). Mandible length a 178.97–206.24 μm, length b 111.99–125.21 μm, height c 132.64–163.45 μm, ratio a to c 1.2–1.3, apical half well sclerotized and basal half heavily sclerotized, teeth sharply pointed, and well-developed secondary tooth. Hypopharyngeal sclerite 112.16–154.92 μm long, anterior and apical half heavily sclerotized, posterior and basal half well sclerotized, and 72.21–89.78 μm wide at ventral bridge. Epipharyngeal sclerite, labial sclerite, and parastomal bar as in third instar. Dorsal cornu usually with well-defined sclerotized area 179.62–264.21 μm long, and length including hyaline area 343.54–492.15 μm. Dorsal arch 197.97–280.86 μm high. Anterior sclerite apparently absent. Cornu notch index 0.5. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 7–8 ridges forming a series of grooves along length of ventral cornu. Ventral cornu 400.20–488.33 μm long from pharyngeal bar to posterior end of grooves. Ventral cornu 1.8–2.2 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, and bearing 11–15 tubules, in a single row (Fig. 2I). Tubule length 10.60–17.08 μm, distal width 7.44–14.03 μm, and basal width 6.21–11.47 μm. The anterior spiracle height at midline 64.01–114.71 μm, distal width 105.33–128.00 μm, and basal width 21.48–46.85 μm at junction with trachea.

Caudal segment with sensilla-bearing tubercles arranged as in third instar. Posterior spiracles and anal lobe located as in third instar. Anal lobe entire.

Posterior spiracle with openings with thinner rimae and fewer trabeculae than in third instar (Fig. 2J), 33.32–48.71 μm long, 11.45–18.01 μm width, and ratio length to width range 2.7–2.9. Ecdysial scar apparent. Felt chamber oval, 64.87–80.19 μm diameter at junction with trachea. Spiracular process SP-I comprising 12–17 trunks and 31–40 tips (occasionally 23–26), ratio tips to trunks 2.3–2.5, basal width 15.93–30.27 μm, and ratio basal width to length of spiracular opening ≈0.4–0.6. SP-II comprising 5–9 trunks and 12–23 tips. SP-III comprising 7–11 trunks (occasionally 5) and 15–20 tips (occasionally 24). SP-IV comprising 12–15 trunks (occasionally 9–10) and 23–34 tips, ratio tips to trunks 1.9–2.2, basal width 14.08–25.62 μm, and ratio basal width to length of spiracular opening ≈0.4–0.5. Average bristle length ≈0.7 times length of spiracular opening.

Anastrepha turpiniae Stone
(Fig. 3)

Material Examined. In total, 34 larvae were examined (22 third-instar larvae and 12 second-instar larvae). Larvae were dissected out of fruit of *Terminalia cattapa* L., Combretaceae, an exotic tree introduced in Brazil.

Features of Third-Instar Larvae. 5.00–11.58 mm long and 1.00–2.06 mm wide at the sixth abdominal segment. Elongate, cylindrical, tapered thoracic segments, and truncate caudal end. Color creamy yellow.

On all segments spinules in discontinuous rows of variable length dorsally and ventrally. Spinules conical, symmetrical to slightly curved. Spinule basal width

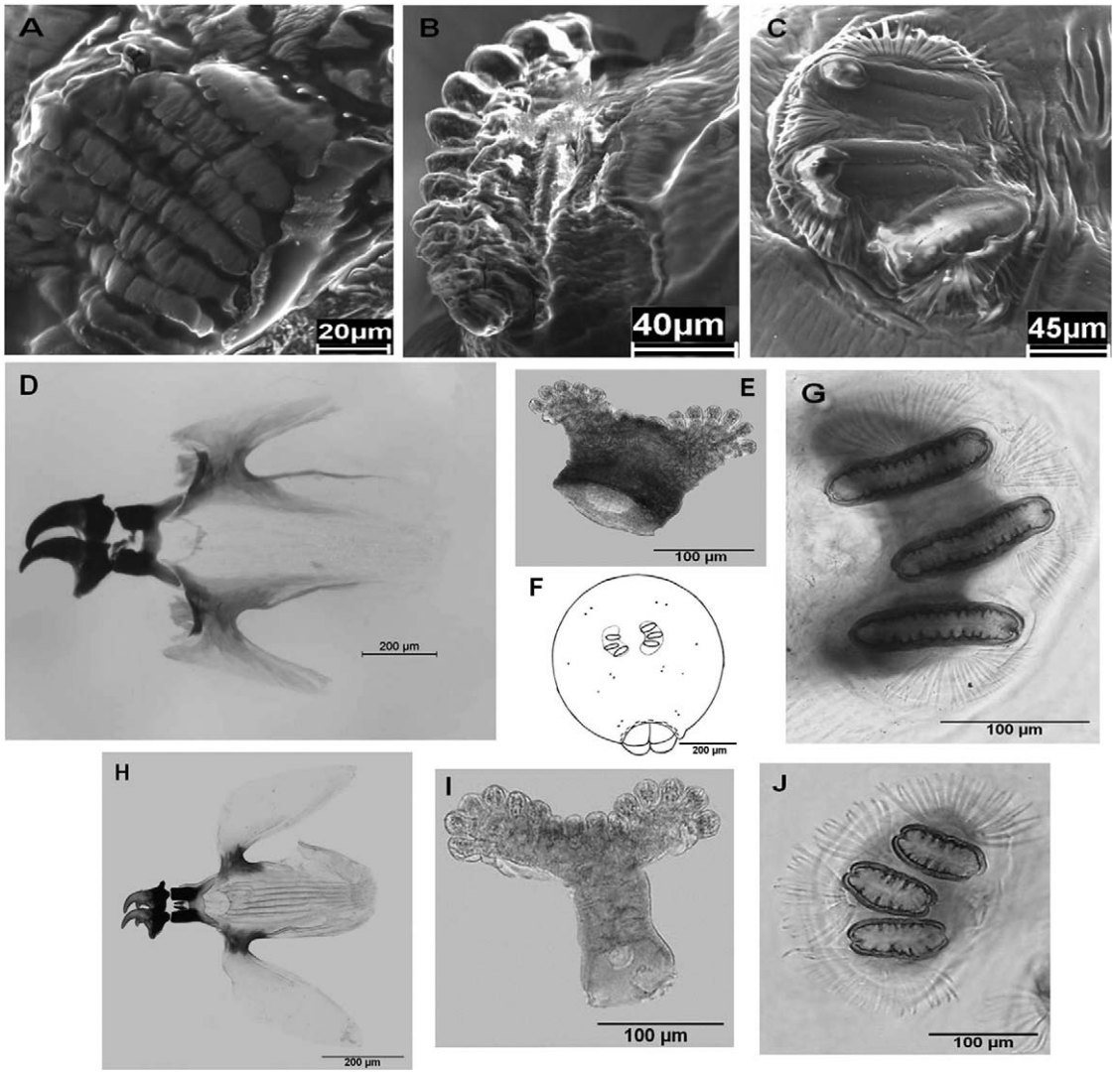

Fig. 3. Scanning electron microscopy (A to C) and optical microscopy views of third-instar (D to G) and second-instar (H to J) larvae of *A. turpiniae*. Third-instar larvae (A) Oral ridges. (B and E) Anterior spiracle. (C and G) Posterior spiracle. (D) Cephalopharyngeal skeleton. (F) Caudal view of posterior segment. Second-instar larvae (H) Cephalopharyngeal skeleton. (I) Anterior spiracle. (J) Posterior spiracle.

1.94–3.09 μm. Dorsal spinule pattern: T1 with 4–5 rows (occasionally 2–3), T2 with 4–5 rows (occasionally 3), T3 with 0–2 rows, and A1 to A8 lacking spinules. Ventral spinule pattern: T1 with 4–6 rows (occasionally three or eight), T2 with 4–6 rows (occasionally three), T3 with 2–4 rows (occasionally 5), A1 with 6–8 rows, A2 with 10–13 rows, A3 with 12–14 rows, A4 with 11–14 rows, A5 with 12–14 rows (occasionally 10), A6 and A7 with 11–14 rows, A8 with 10–13 rows. Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with 3–4 irregular rows anteriorly and posteriorly.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with 12.18–19.49 μm outside diameter, apical knob with 9.49–13.34 μm diameter, and combined height 6.62–14.13 μm. Maxillary sensory organ cylindrical to slightly tapered with 12.70–19.71 μm diameter, and height 6.82–12.31 μm. Stomal sensory organ cylindrical with 4.29–9.22 μm height. 7–8 oral ridges with posterior margin irregularly serrated, accessory plates present (Fig. 3A).

Cephalopharyngeal skeleton with shape and sclerotization as in Fig. 3D. Total length from tip of mandible to end of ventral cornu 0.95–1.44 mm. Mandible length a 0.23–0.28 mm, length b 0.14–0.19 mm, height c 0.15–0.20 mm, ratio a to c 1.4–1.5. Tooth long and sharp. Hypopharyngeal sclerite 0.17–0.21 mm long, 0.12–0.16 mm wide at ventral bridge. Epipharyngeal sclerite visible only in dorsal view, with medial lobe directed anteriorly. Labial sclerite short, robust, moderately sclerotized, and horseshoe-shaped in dorsal

view. Parastomal bar extending almost entire length of hypopharyngeal sclerite and arched. Ventral sclerite present below pharyngeal sclerite. Dorsal cornu usually with well-defined sclerotized area 0.29–0.48 mm long, length including hyaline area 0.40–0.74 mm. Dorsal bridge prominently projecting anteriorly from dorsal cornu and slightly sclerotized. Dorsal arch 0.28–0.36 mm high. Anterior sclerite irregularly shaped and sclerotized. Cornu notch index 0.6–0.7. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 7–9 ridges forming a series of grooves along length of ventral cornu. Ventral cornu 0.58–0.99 mm long from pharyngeal bar to posterior end of grooves. Ventral cornu 2.0 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 12–16 tubules, in a single row (Fig. 3B, E). Tubule length 19.25–33.09 μm, distal width 12.75–25.79 μm, and basal width 10.36–22.31 μm. The anterior spiracle height at midline 112.67–160.60 μm, distal width 196.02–262.80 μm, and basal width 97.25–139.78 μm at junction with trachea.

Fig. 3F illustrates the caudal segment with dorsal (D1 and D2), intermediate (I1 and I2), lateral (L1), and ventral (V1 and V2) tubercles and sensilla all well developed. D1 tubercle distinctly anterior to D2. Intermediate tubercles I1 and I2 more strongly developed, but associated sensilla weakly developed, I2 lateral and sometimes slightly ventral to I1. L1 and V all very weakly developed. D1 and I1 are associated with weakly developed sensilla. Some specimens have three lateral tubercles (V1, V2, and V3). Posterior spiracles located above horizontal midline. Anal lobe bifid.

Posterior spiracle with openings with thick rimae and numerous trabeculae (Fig. 3C, G), 71.86–98.86 μm long, 15.06–23.85 μm width, and ratio length to width range 4.1–4.7. Ecdysial scar apparent. Felt chamber oval, 126.55–174.03 μm diameter at junction with trachea. Spiracular process SP-I comprising 10–32 trunks and 13–42 tips, ratio tips to trunks 1.3, basal width 20.76–39.86 μm, and ratio basal width to length of spiracular opening ≈0.2–0.4. SP-II comprising 3–12 trunks and 12–24 tips. SP-III comprising 4–10 trunks and 9–28 tips. SP-IV comprising 10–21 trunks (occasionally 7–8) and 18–46 tips; ratio tips to trunks 1.8–2.1, basal width 20.56–37.30 μm, and ratio basal width to length of spiracular opening ≈0.2–0.3. Average bristle length ≈0.4 times length of spiracular opening.

Features of Second-Instar Larvae. 3.50–4.50 mm long and 0.50–0.87 mm wide at the sixth abdominal segment. Shape as in third instar. Color ranging from creamy white to creamy yellow.

Spinules shaped and in discontinuous rows as in third instar. Spinule basal width 1.84–3.03 μm. Dorsal spinule pattern: T1 with 4–5 rows, T2 with 3–5 rows, T3 with 2–3 rows, A1 with 0–3 rows, A2 with 2–5 rows (occasionally 0), A3 with 4–6 rows, A4 with 4–5 rows, A5 with five rows (occasionally 0), A6 with 0–3 rows, A7 and A8 lacking spinules. The rows of dorsal spinules do not completely traverse dorsum. Ventral spinule pattern: T1 and T2 with 4–5 rows, T3 with 1–3 rows (occasionally 0 or 5), A1 with 5–10 rows, A2 with 10–11 rows (occasionally 12), A3 with 10–12 rows, A4 with 11–13 rows, A5 with 10–12 rows (occasionally 9), A6 with 11–12 rows (occasionally 10 or 13), A7 with 11–12 rows (occasionally 13), A8 with 9–11 rows (occasionally 12). Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with three irregular rows anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with 8.44–11.88 μm outside diameter, apical knob with 6.59–8.48 μm diameter, and combined height 4.43–5.94 μm. Maxillary sensory organ weakly sclerotized, cylindrical, with 10.21–18.26 μm diameter, and height 6.53–7.10 μm. Stomal sensory organ cylindrical with 3.76–5.29 μm height. 6–8 oral ridges with the same shape as in third-instar larvae and accessory plates present.

Cephalopharyngeal skeleton with total length from tip of mandible to end of ventral cornu 622.04–784.97 μm (Fig. 3H). Mandible length a 123.32–160.60 μm, length b 77.05–94.07 μm, height c 97.19–122.70 μm, ratio a to c 1.2–1.3, apical half well sclerotized and basal half heavily sclerotized, some mandibles show two openings at base, teeth sharply pointed, and well-developed secondary tooth. Hypopharyngeal sclerite 105.57–163.11 μm long, anterior and apical half heavily sclerotized and posterior and basal half well sclerotized, 72.10–83.86 μm wide at ventral bridge. Epipharyngeal sclerite, labial sclerite, and parastomal bar as in third instar. Dorsal cornu usually with well-defined sclerotized area 162.22–236.21 μm long; length including hyaline area 224.10–396.84 μm. Dorsal arch 129.70–199.34 μm high. Anterior sclerite apparently absent. Cornu notch index 0.5. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and seven ridges forming a series of grooves along length of ventral cornu. Ventral cornu 328.55–497.61 μm long from pharyngeal bar to posterior end of grooves. Ventral cornu 2.0–2.1 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 13–15 tubules, in a single row (Fig. 3I). Tubules length 11.50–16.52 μm, distal width 8.01–11.46 μm, and basal width 5.00–7.87 μm. The anterior spiracle height at midline 76.72–91.68 μm, distal width 98.49–127.65 μm, and basal width 33.27–91.68 μm at junction with trachea.

Caudal segment with sensilla-bearing tubercles arranged as in third instar. Posterior spiracles and anal lobe located as in third instar. Anal lobe bifid.

Posterior spiracle with openings with thinner rimae and fewer trabeculae than in third instar (Fig. 3J); 27.00–34.17 μm long, 11.58–18.34 μm width, ratio length to width range 1.8–2.3. Ecdysial scar apparent. Felt chamber round, 60.19–77.83 μm diameter at junction with trachea. Spiracular process SP-I comprising 11–13 trunks (occasionally 10) and 22–29 tips (occasionally 19–20), ratio tips to trunks 2.0–2.2, basal width 11.42–15.49 μm, ratio basal width to length of spiracular opening ≈0.4. SP-II comprising 4–6 trunks and

8–13 tips (occasionally 15). SP-III comprising 6–9 trunks and 14–18 tips. SP-IV comprising 10–12 trunks (occasionally 8) and 19–27 tips (occasionally 37), ratio tips to trunks 1.9–2.2, basal width 10.25–14.33 μm, and ratio basal width to length of spiracular opening ≈0.3–0.4. Average bristle length ≈0.5–0.7 times length of spiracular opening.

Discussion

Consistent size differences were verified between the third instars and second instars examined for the three species. Third instars are about twice as large as second instars for most measurements, and all second-instar larvae had one subapical tooth on the mouthhook, whereas third instars had none. This characteristic can be used to differentiate second- and third-instar larvae of *Anastrepha* species. However, some structures such as the number of oral ridges and the shape of the anal lobe remained constant.

Other differences between instars include the number of grooves in the pharyngeal filter in *A. bahiensis;* more spinule rows ventrally on segment T1 in second instars, and twice the number of tips on the posterior spiracle in third instars as compared with second instars in *A. coronilli.* In *A. turpiniae*, second instars have dorsal spinule rows on segments A1 to A6, whereas they are absent in third instars, and the number of tubules on the anterior spiracle also can be different between second and third instars in this species.

Third instars of *A. bahiensis* can be distinguished from the other two species studied here on the basis of the smaller size of the mandible and shorter length of the cephalopharyngeal skeleton. Third instars of *A. coronilli* are separated easily on the basis of their larger number of tips of trunks on the posterior spiracle; it has entire anal lobes, whereas the other two species have bifid anal lobes; larger size of the mandible and longer length of the cephalopharyngeal skeleton. The distinguishing characters in *A. turpiniae* are the presence of dorsal spinules on segment T3, which occasionally can be absent; same number of spinule rows in ventral segments A4, A6, and A7; lower ratio of tips to trunks in the SP-1 of the posterior spiracle and smaller number of grooves in the pharyngeal filter.

It should be noted that characters such as the number of oral ridges, the number of tubules on the anterior spiracles, and the shape of the anal lobes can be observed with unmounted specimens with a dissecting microscope. However, for the spinules and the spiracular bristles it is necessary to clear and prepare the specimens on slides for examination with a compound microscope to count the tips.

When we ran third-instar larvae of *A. bahiensis, A. coronilli,* and *A. turpiniae* through the larval key of Steck et al. (1990), all three species were erroneously identified as *Anastrepha serpentina* (Weidemann), *A. fraterculus* (Weidemann), or *A. suspensa* (Loew). None of the species ran consistently through the key, as the numbers of anterior spiracular tubules overlaps the alternative ranges given in couplet 8, thus producing more than one result. Pending description of larvae of additional species of *Anastrepha*, a revised key to *Anastrepha* larvae will be presented at a later date. A revised larval key may newly incorporate features of the cephalopharyngeal skeleton and fine structure of the oral ridges.

The presence of a ventral sclerite in third-instar larvae of *Anastrepha* was reported for the first time for *Anastrepha leptozona* Hendel by Frías et al. (2009) and the authors stated that this trait "could be used for the recognition of this species". However, we observed a ventral sclerite under optical microscope in some of the third-instar larvae of all three species analyzed in this study and in five other *Anastrepha* species (Dutra et al., unpublished data). Thus, the presence of this structure cannot be used as a diagnostic character. The ventral sclerite of third-instar larvae has probably not been reported in other species because of its difficult visualization as it is a translucent and fragile structure.

Most of the characters described for the three species studied here overlap with those of the other five species already described in the *fraterculus* group: *Anastrepha distincta* Greene, *Anastrepha fraterculus* (Wiedemann), *Anastrepha ludens* (Loew), *Anastrepha obliqua* (Macquart), and *Anastrepha suspensa* (Loew) (Steck et al. 1990, White and Elson-Harris 1992, Norrbom et al. 1999). For instance, the number of oral ridges ranges from six to 10 in the third instars of the three species described here and ranges from seven to 12 in *A. fraterculus*, *A. obliqua*, *A. suspensa*, and *A. distincta* (Steck et al. 1990, White and Elson-Harris 1992) and only *A. ludens* has a higher number with >16 (Carroll and Wharton 1989). One useful character to distinguish among some species in the *fraterculus* group would be the number of tips on the posterior spiracular bristles of SP-I, as *A. coronilli* has 45–76 tips, the largest number of tips described so far, whereas *A. ludens* has 17–28 tips and the other species have from 10 to 17 tips.

Several *Anastrepha* species in the *fraterculus* group infest *Psidium* (Myrtaceae) (Araujo and Zucchi 2003, Norrbom 2004). Thus far, however, there is taxonomic information on the larvae of only five species besides the three species described herein. Because of the considerable overlap of characters among species within this group, it may be necessary to develop other methods that will effectively allow separation of these species such as multivariate, molecular approaches, or both.

Descriptions of larvae from a large selection of species in the *fraterculus* group might be very useful to help understand the level of variation among species within this group. Moreover, such descriptions will most likely help interpret the level of variation among larvae of the several putative species within the *fraterculus* complex.

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