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**DESCRIPTION OF LARVAE OF THREE ANASTREPHA SPECIES IN THE
FRATERCULUS GROUP (DIPTERA: TEPHRITIDAE)**

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Abstract.—The description of larval stages of *Anastrepha* species is a priority from both taxonomic and quarantine viewpoints. In this study, we describe and illustrate for the first time the second and third instar larvae of *Anastrepha amita* Zucchi, and the third instar larvae of *Anastrepha sororcula* Zucchi and *Anastrepha zenildae* Zucchi. All specimens analyzed herein were collected in Brazil. We also discuss character states that can be used to distinguish among the three species.

Key Words: fruit flies, larvae, posterior spiracle, cephalopharyngeal skeleton

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Anastrepha Schiner (Diptera: Tephritidae) species occur in tropical and subtropical environments from the southern United States to central Argentina (Aluja 1994). This is the most economically important genus of fruit flies in the Neotropical Region with over 300 species described (Norrbom et al. 1999, 2012, 2015, in press; Zucchi 2008; Uramoto et al. 2015). Many of these species were described based on trapped adults and therefore only about 50% of *Anastrepha* species have a known host.

Within this genus, 28 species groups have been proposed based on morphology

and host use. The majority of the most significant pest species belong to the *fraterculus* group, which currently comprises 38 species (Norrbom et al. 2012, in press).

Published descriptions for third instars are currently available for only 19 species of *Anastrepha* (Steck and Wharton 1988, Steck et al. 1990, Norrbom et al. 1999, Frías et al. 2009, Dutra et al. 2012, Dutra et al. 2018). So far, descriptions of larval stages are available for only eight species of the *fraterculus* group: *A. bahiensis* Lima, *A. coronilli* Carrejo & González, *A. distincta* Greene, *A. fraterculus* (Wiedemann), *A. ludens*

(Loew), *A. obliqua* (Macquart), *A. suspensa* (Loew) and *A. turpiniae* Stone (Lawrence 1979; Heppner 1984, 1991; Carroll and Wharton 1989; White and Elson-Harris 1992, Dutra et al. 2012).

Anastrepha amita has been reported from Trinidad and Tobago (Norrbon 2004) and Brazil (in 12 states) infesting only hosts in the family Verbenaceae: *Citharexylum myrianthum* Cham. (Souza Filho et al. 1999) and *C. poeppigii* Walp. (Marsaro Júnior et al. 2010). *Anastrepha sororcula* has been reported from Colombia, Ecuador, Peru, Paraguay (Norrbon 2004), and Brazil (in 21 states) infesting mainly hosts in the family Myrtaceae (Zucchi 2007, 2008). *Anastrepha zenildae* has been reported from Argentina (Norrbon 2004) and Brazil (in 18 states) infesting hosts mainly in the Myrtaceae (Zucchi 2007, 2008). *Anastrepha sororcula* and *A. zenildae* are of economic importance in Brazil (Zucchi 2000).

Morphological studies of larvae can contribute to the identification of pest species in infested fruit that are commonly intercepted in ports of entry and also to a better understanding of the phylogenetic relationships among *Anastrepha* species (Norrbon et al. 1999).

In this study, we describe and provide detailed SEM observations on the third instar of *A. amita*, *A. sororcula*, and *A. zenildae*. We also describe the second instar of *A. amita*.

MATERIALS AND METHODS

Larvae of *A. amita*, *A. sororcula*, and *A. zenildae* were dissected out of fruit collected in Campinas (22° 54' 09.2" S; 47° 01' 06.7" W) in the state of São Paulo (February 2011), in Mossoró (05°11'15" S; 37°20'39" W) in the state of Rio Grande do Norte (April 2011), and Limoeiro do Norte (05°08'44" S; 38°05'53" W) in the state of Ceará

(April 2011), respectively. In order to confirm species identities, some larvae were left to develop in each fruit species. The fruits were placed in 500 ml plastic containers with a layer of vermiculite and covered with voile cloth until larvae exited 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 until adults emerged. *Anastrepha amita* was the only species reared from *Cytherexylum myrianthum*, only *A. zenildae* was reared from *Ziziphus joazeiro* Mart. (Rhamnaceae), and both *A. sororcula* and *A. zenildae* were reared from *Psidium guajava* L. (Myrtaceae). Thus, we used PCR-RFLP to distinguish between specimens of the latter two species reared from *P. guajava* and compared with the results obtained for *A. zenildae* reared from *Z. joazeiro* (Dutra et al. unpublished data). Larvae were killed in hot water and preserved in 100% ethanol.

External measurements such as length and width were made using a Wild M3C stereomicroscope (Leica Geosystems, Heerbrugg, Switzerland) at the Laboratório de Entomologia Agrícola, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil. Some larvae were cleared in 10% potassium hydroxide at room temperature and placed in excavated slides with ethanol and glycerine (1:1). The cephalopharyngeal skeleton (CPS) was removed, placed in glycerine and measured at 100X magnification. Measurements on posterior and anterior spiracles were taken at 200X and 400X magnification, while antennomaxillary and stomal organs were measured at 400X magnification. These structures were photographed using a digital Olympus DP72 camera attached to an Olympus BX51 microscope (Olympus America, Melville,

New York) at the Laboratório de Citotaxonomia e Insetos Aquáticos (INPA). The CPS of each larva was placed in a Petri dish with ethanol and glycerine (1:1), photographed, measured and described using a digital camera (Leica DFC420) attached to a stereomicroscope (Leica M165C, Leica Geosystems, Heerbrugg, Switzerland) at the Laboratório de Prospecção de Bioativos de Insetos (INPA). Rows of dorsal and ventral spinules were counted at the middorsal and midventral lines, respectively. For *A. amita*, all of the structures mentioned above were photographed using a Leica DFC 295 digital camera attached to a Leica DM 5500B microscope and measuring software Leica Microsystems Framework version 4.2 (Leica Geosystems, Heerbrugg, Switzerland) at the Laboratório de Prospecção de Bioativos de Insetos (INPA), Manaus.

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 and the same abbreviations for thoracic and abdominal segments, cephalopharyngeal skeleton, posterior spiracular processes, and caudal tubercles as in Steck and Malavasi (1988) and Steck and Wharton (1988). Mandible measurements are shown in Figure 6. We use the term "occasional" to refer to values occurring at a frequency of $\leq 10\%$ of the specimens examined (Steck and Wharton 1988).

In the preparation for scanning electron microscopy (SEM), larvae were dehydrated in an ethanol series, then critical point dried in CO_2 for two hours, and sputter-coated with a gold layer. Larvae prepared this way were examined in a LEO 435 VP scanning electron microscope (LEO Electron Microscopy Ltd., Cambridge, England) at the Laboratório Temático de Microscopia Óptica e Eletrônica, at INPA.

Voucher specimens of larvae and associated females are deposited at the Coleção de Invertebrados, INPA. Statistical analyses followed Goyal et al. (2011). We used one-way analysis of variance (ANOVA) using R software version 3.1.2 to determine whether the measurements and ratios of measurements were affected by species (fixed variable). The Tukey test was used for means separation with $P < 0.05$.

RESULTS

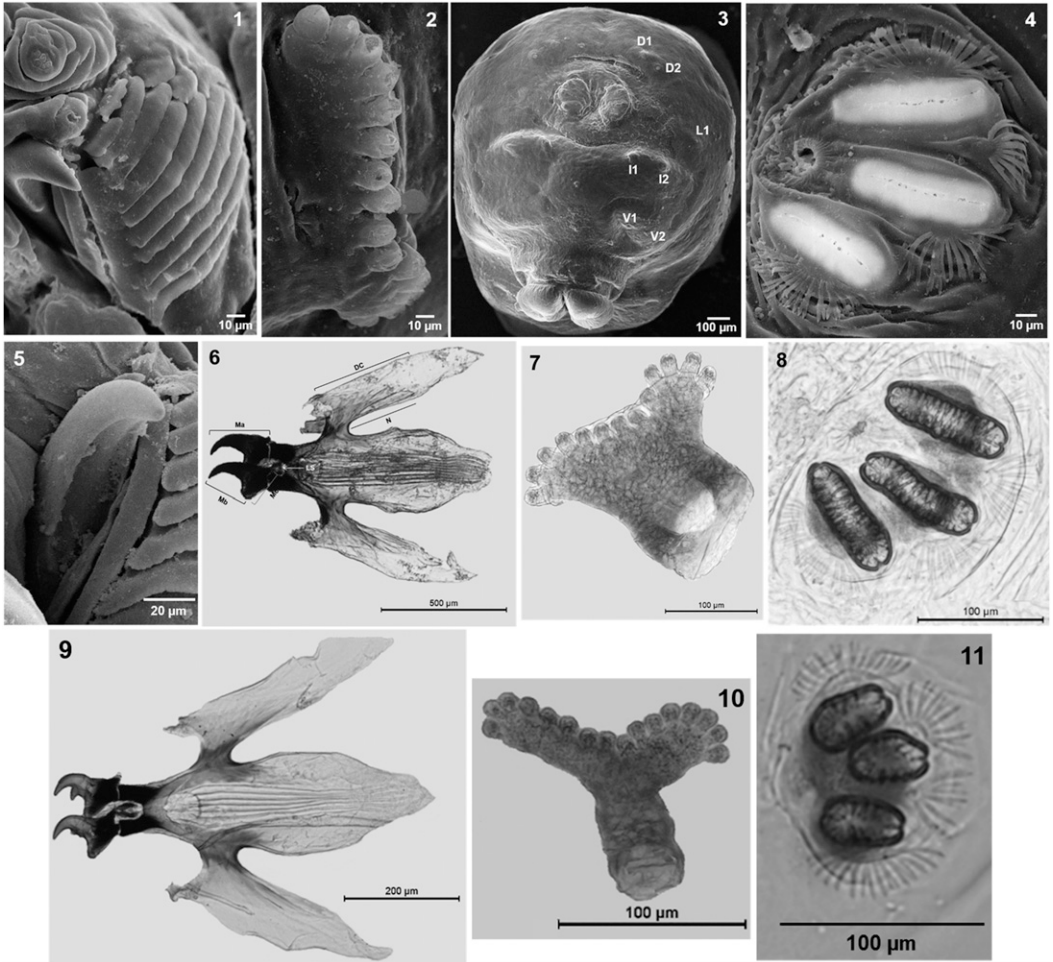
Anastrepha amita Zucchi

(Figs. 1–11)

Material examined.—A total of 40 larvae was examined (25 third instars and 15 second instars). Larvae were dissected out of fruit of *C. myrianthum* (locally known as “pombeiro”) a wild species collected in Campinas in the state of São Paulo, Brazil (February 2011).

Description of third instar.—7.23–10.85 mm (9.42 ± 1.19) long and 1.13–1.75 mm (1.51 ± 0.16) wide at the 6th abdominal segment. Elongate, cylindrical, tapered anteriorly (head and thoracic segments) and caudal end truncate (Table 1). Color creamy yellow.

On all segments, spinules in discontinuous rows of variable length dorsally and ventrally. Dorsal and ventral spinules conical, symmetrical to slightly curved posteriorly. Spinule basal width 2.18–3.99 μm (2.87 ± 0.51). Dorsal spinule pattern in rows as follows: T1 3–5 (3.8 ± 0.80); T2 1–3 (2.23 ± 0.72); T3 0–2 (0.77 ± 0.72); A1 0–2 (0.54 ± 0.66); A2 0–1 (0.23 ± 0.43); A3 0–1 (0.08 ± 0.27); A4 to A8 lacking spinules. Ventral spinule pattern in rows as follows: T1 6–13 (9.69 ± 2.05); T2 3–5 (4.0 ± 0.70); T3 2–5 (2.92 ± 0.86); A1 5–7 (6.38 ± 0.65); A2 10–13 (11.54 ± 0.96); A3 11–15 (12.0 ± 1.08); A4 11–15 (12.69 ± 1.18);



Figs. 1–11. Scanning electron microscopy (1–5) and optical microscopy views of third instar (6–8) and second instar (9–11) of *A. amita*. 1, Third instar, oral ridges. 2, 7, Anterior spiracle. 3, Caudal view of posterior segment. 4, 8, Posterior spiracle (dorsal at top). 5, Ventral surface of mandible. 6, Cephalopharyngeal skeleton. 9, Second instar, cephalopharyngeal skeleton. 10, Anterior spiracle. 11, Posterior spiracle. Abbreviations: D1 and D2, dorsal tubercles and sensilla; I1 and I2, intermediate tubercles and sensillum; L1, lateral tubercles and sensillum; V1 and V2, ventral tubercle and sensillum; Ma, mandible length a; Mb, mandible length b; Mc, mandible height c; LS, labial sclerite; DC, length of pigmented area of dorsal cornu; N, notch (Steck and Wharton 1988).

A5 11–14 (12.62 ± 1.12); A6 10–14 (12.15 ± 1.46); A7 11–13 (11.85 ± 0.68); A8 9–11 (10.54 ± 0.87). Ventral creeping welts present on third thoracic segment (T3) and on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with 3–4 (3.08 ± 0.27) irregular rows anterior and posterior to lobes (Table 1).

Antennal sensory organ slightly sclerotized, cylindrical basal collar with $12.34\text{--}22.63 \mu\text{m}$ outside diameter (18.61 ± 3.96); and apical knob $8.26\text{--}14.16 \mu\text{m}$ diameter (10.33 ± 2.88); combined height $5.16\text{--}15.54 \mu\text{m}$ (9.77 ± 3.73). Maxillary sensory organ cylindrical to slightly tapered, with $17.55\text{--}22.68 \mu\text{m}$ diameter (20.22 ± 2.07), $12.30\text{--}16.17 \mu\text{m}$ height (13.95 ± 1.64).

Table 1. Comparison of third instar larval measurements among three species of *Anastrepha*.

Character	<i>A. amita</i>		<i>A. sororcula</i>		<i>A. zenilidae</i>		ANOVA		Tukey test		
	M*	SD**	M*	SD**	M*	SD**	DF	P	<i>A. amita</i> X <i>A. sororcula</i>	<i>A. amita</i> X <i>A. zenilidae</i>	<i>A. zenilidae</i> X <i>A. sororcula</i>
Total length larvae (mm)	9.42	1.193	8.97	0.478	9.67	0.575	2; 36	0.098	0.352	0.706	0.085
Total width larvae (mm)	1.51	0.166	1.28	0.161	1.45	0.132	2; 36	0.002	0.002	0.416	0.057
Number dorsal spinule rows:											
T1	3.85	0.800	4.38	0.506	4.77	0.599	2; 36	0.003	0.099	0.002	0.295
T2	2.23	0.725	3.92	0.493	4.31	0.480	2; 36	0.000	0	0	0.219
T3	0.77	0.725	1.69	0.854	0.69	0.947	2; 36	0.007	0.022	0.970	0.012
A1	0.54	0.660	0	0	0	0	2; 36	0	0.002	0.002	1
A2	0.23	0.438	0	0	0	0	2; 36	0.370	0.065	0.065	1
A3	0.08	0.277	0	0	0	0	2; 36	0.378	0.446	0.446	1
Number ventral spinule rows:											
T1	9.69	2.056	8.15	0.688	8.38	1.556	2; 36	0.032	0.039	0.091	0.923
T2	4.00	0.707	6.15	0.987	6.11	0.781	2; 36	0	0	0	0.969
T3	2.92	0.862	4.31	0.480	4.56	0.527	2; 36	0	0	0	1
A1	6.38	0.650	7.54	1.853	6.46	0.660	2; 36	0.032	0.048	0.985	0.069
A2	11.54	0.967	11.08	0.954	10.92	0.493	2; 36	0.162	0.346	0.159	0.885
A3	12.00	1.080	11.92	0.759	11.11	0.333	2; 36	0.370	0.967	0.050	0.085
A4	12.69	1.182	11.62	0.767	12.11	0.781	2; 36	0.014	0.015	0.067	0.805
A5	12.62	1.120	11.62	1.043	12.33	0.500	2; 36	0.029	0.023	0.540	0.216
A6	12.15	1.463	11.54	1.050	12.38	0.916	2; 36	0.344	0.371	0.984	0.466
A7	11.85	0.688	11.31	1.250	12.00	0.577	2; 36	0.129	0.282	0.898	0.130
A8	10.54	0.877	10.77	0.832	11.46	0.877	2; 36	0.026	0.775	0.025	0.115
Anal lobe:											
Number spinule rows	3.08	0.277	3.54	0.518	3.77	0.438	2; 36	0	0.022	0	0.357
Spinule width	2.87	0.513	3.00	0.520	2.92	0.314	2; 36	0.753	0.739	0.966	0.874
Antennal sensory organ:											
Diameter antennal collar (µm)	18.61	3.964	18.83	1.450	21.25	1.041	2; 12	0.223	0.988	0.257	0.314
Height apical knob (µm)	9.77	3.732	7.94	0.442	7.25	0.513	2; 12	0.211	0.409	0.203	0.872
Diameter apical knob (µm)	10.33	2.288	12.73	1.646	11.79	1.531	2; 15	0.111	0.096	0.386	0.658
Diameter sensory organ (µm)	20.22	2.070	31.22	0.760	22.91	1.668	2; 12	0	0	0.068	0
Height sensory organ (µm)	13.95	1.647	11.36	0.768	12.39	2.449	2; 9	0.168	0.149	0.453	0.698
Height stromal sensory (µm)	9.11	1.727	5.64	1.217	7.34	1.168	2; 9	0.020	0.016	0.225	0.248

Table 1. Continued.

Character	<i>A. amita</i>		<i>A. sororcula</i>		<i>A. zenilidae</i>		ANOVA		Tukey test			
	M*	SD**	M*	SD**	M*	SD**	DF	P	<i>A. amita</i> X <i>A. zenilidae</i>	<i>A. amita</i> X <i>A. sororcula</i>	<i>A. zenilidae</i> X <i>A. sororcula</i>	
Number of oral ridges	8.92	0.759	7.00	1.000	8.77	0.832	2; 36	0	0	0	0.894	0
Cephalopharyngeal skeleton:												
Total length CPS (mm)	1.09	0.075	1.13	0.079	1.25	0.137	2; 36	0	0	0.602	0	0.011
Number of ventral grooves	8.31	0.480	8.69	0.480	9.08	0.277	2; 36	0	0.066	0	0	0.066
Height dorsal arch (mm)	0.29	0.017	0.36	0.054	0.37	0.016	2; 36	0	0	0	0	0.565
Length dorsal cornu sclerotized (mm)	0.43	0.031	0.38	0.027	0.45	0.050	2; 36	0	0.015	0.259	0	0
Length dorsal cornu (mm)	0.64	0.086	0.65	0.062	0.72	0.067	2; 36	0.021	0.934	0.028	0.062	0.062
Length hypopharyngeal sclerite (mm)	0.17	0.013	0.18	0.017	0.19	0.007	2; 36	0.001	0.955	0.002	0.006	0.006
Width hypopharyngeal sclerite (mm)	0.17	0.014	0.20	0.025	0.21	0.018	2; 36	0	0	0	0.656	0.174
Length mandible a (mm)	0.23	0.019	0.25	0.017	0.23	0.040	2; 36	0.181	0.384	0	0.875	0.174
Length mandible b (mm)	0.14	0.014	0.18	0.010	0.19	0.014	2; 36	0	0	0	0	0.081
Length mandible c (mm)	0.15	0.010	0.17	0.014	0.19	0.011	2; 36	0	0.002	0	0	0.002
Notch index (mm)	0.31	0.039	0.25	0.032	0.30	0.031	2; 36	0	0	0	0.807	0.004
Length ventral cornu (mm)	0.66	0.057	0.66	0.063	0.75	0.079	2; 36	0.002	0.992	0.005	0.005	0.008
Anterior spiracle:												
Number of tubules	14.23	0.926	10.62	1.502	12.31	0.854	2; 36	0	0	0	0	0.001
Length tubule (µm)	26.62	2.905	22.62	3.098	22.75	4.75	2; 36	0.002	0.023	0.713	0.003	0.003
Distal width tubule (µm)	19.03	2.032	17.34	2.178	16.88	1.493	2; 36	0.017	0.077	0.019	0.819	0.819
Basal width tubule (µm)	15.75	2.513	12.42	2.580	13.86	1.930	2; 36	0.003	0.002	0.114	0.277	0.277
Height midline AE (µm)	166.89	9.752	136.95	15.612	147.79	17.267	2; 36	0	0	0	0.005	0.154
Distal width AE (µm)	254.49	26.183	187.16	17.124	240.71	23.011	2; 36	0	0	0	0.272	0
Basal width AE (µm)	134.45	17.984	96.93	12.036	117.28	9.884	2; 36	0	0	0	0.008	0.001
Posterior spiracle:												
Length chamber oval (µm)	85.03	5.356	81.45	6.557	94.93	7.682	2; 36	0	0.359	0.001	0	0
Width chamber oval (µm)	21.80	1.966	18.69	2.063	19.41	1.386	2; 36	0	0	0.005	0.576	0.576
Diameter PE (µm)	134.09	10.751	124.63	8.696	135.11	5.035	2; 36	0.005	0.019	0.949	0.009	0.009
Spiracular process:												
Basal width SP-I	32.08	4.171	30.98	5.446	30.49	5.220	2; 36	0.709	0.841	0.696	0.965	0.965
Number of tips SP-I	30.15	4.336	39.38	4.992	34.92	3.475	2; 36	0	0	0.020	0.032	0.032
Number of trunks SP-I	14.46	1.808	15.54	1.853	12.62	2.022	2; 36	0.001	0.327	0.046	0.001	0.001

Table 1. Continued.

Character	<i>A. amita</i>			<i>A. sororcula</i>			<i>A. zenildae</i>			ANOVA				Tukey test				
	M*	SD**		M*	SD**		M*	SD**		DF	P		<i>A. amita</i> X <i>A. zenildae</i>	<i>A. amita</i> X <i>A. sororcula</i>	<i>A. zenildae</i> X <i>A. sororcula</i>			
			F value															
Number of tips SP-II	17.85	3.848	15.23	2.586	13.85	2.444	5.85	2; 36	0.006			0.084	0.005	0.480				
Number of trunks SP-II	6.92	2.289	7.31	1.182	6.38	1.709	0.87	2; 36	0.425			0.847	0.724	0.394				
Number of tips SP-III	21.31	3.727	24.62	3.863	20.31	2.810	5.39	2; 36	0.008			0.053	0.748	0.009				
Number of trunks SP-III	10.77	1.423	10.38	1.043	7.77	1.739	16.92	2; 36	0			0.773	0	0				
Basal width SP-IV	36.74	6.413	27.17	4.044	26.27	3.875	18.09	2; 36	0			0	0	0.886				
Number of tips SP-IV	33.38	3.948	35.23	5.861	30.85	3.184	3.14	2; 36	0.055			0.549	0.328	0.044				
Number of trunks SP-IV	16.46	2.066	14.54	1.506	11.23	1.589	30.12	2; 36	0			0.020	0	0				

*M = mean

**SD = standard deviation

Stomal sensory organ cylindrical, 7.32–11.45 μm in height (9.11 ± 1.72), located apically on simple elongate lobe typical of most other *Anastrepha* larvae (Carroll et al. 2004). Oral ridges 8–10 (8.92 ± 0.75), with posterior margins entire, accessory plates present in series of two at ends of most oral ridges (Fig. 1, Table 1).

Cephalopharyngeal skeleton with shape and sclerotization as in Fig. 6. Total length from tip of mandible to end of ventral cornu 0.97–1.18 mm (1.09 ± 0.07). Mandible length a 0.21–0.26 mm (0.23 ± 0.01); length b 0.12–0.17 mm (0.14 ± 0.01); height c 0.14–0.17 mm (0.15 ± 0.01); ratio a:c 0.8–1.5. Tooth long, sharp or sometimes blunt. Mandible concave ventrally, surface of concavity smooth (Fig. 5). Hypopharyngeal sclerite 0.15–0.19 mm long (0.17 ± 0.01), 0.15–0.19 mm wide (0.17 ± 0.01) at ventral bridge. Epipharyngeal sclerite visible only in dorsal view, with medial lobe directed anteriorly. Labial sclerite short, robust, relatively well sclerotized, and horseshoe-shaped in dorsal view. Parastomal bar extending for almost entire length of hypopharyngeal sclerite, straight, sometimes slightly arched. Ventral sclerite present below pharyngeal sclerite. Dorsal cornu usually with well-defined sclerotized area 0.39–0.49 mm (0.43 ± 0.03); length including hyaline area 0.45–0.75 mm (0.64 ± 0.08). Dorsal bridge prominently projecting anteriorly from dorsal cornu and slightly sclerotized. Dorsal arch 0.26–0.32 mm high (0.29 ± 0.01). Anterior sclerite irregularly shaped and sclerotized. Cornu notch 0.25–0.37 mm (0.31 ± 0.03) long and cornu notch index 0.6–0.7 (N/DC (Steck and Wharton 1988)). Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 8–9 ridges (8.31 ± 0.48) forming series of grooves along length of ventral cornu. Ventral cornu 0.58–0.73 mm (0.66 ± 0.05) long

from pharyngeal bar to end of posterior grooves. Ventral cornu 1.5 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 13–18 tubules (14.23 ± 0.92), in single row (Figs. 2 and 7). Tubule length $20.54\text{--}29.42 \mu\text{m}$ (26.62 ± 2.90); distal width $14.80\text{--}24.01 \mu\text{m}$ (19.03 ± 2.03); basal width $9.69\text{--}19.20 \mu\text{m}$ (15.75 ± 2.51). Anterior spiracle length at midline $144.90\text{--}182.44 \mu\text{m}$ (166.89 ± 9.75); distal width $187.17\text{--}284.97 \mu\text{m}$ (254.49 ± 26.18); basal width $101.16\text{--}165.63 \mu\text{m}$ (134.45 ± 17.98) at junction with trachea (Table 1).

Caudal segment (Fig. 3) with dorsal (D1 and D2), intermediate (I1 and I2), lateral (L1), and ventral (V1 and V2) tubercles and sensilla mostly well developed; D1 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, V1 and V2 mostly very weakly developed. D1 and I1 associated with weakly developed sensilla. All specimens with two ventral tubercles (V1 and V2). Posterior spiracle located above horizontal midline. Anal lobe entire and protruding.

Posterior spiracular openings with thick rimae and numerous trabeculae (Figs. 4 and 8); $80.35\text{--}96.62 \mu\text{m}$ long (85.03 ± 5.35); $19.55\text{--}25.91 \mu\text{m}$ wide (21.80 ± 1.96); ratio length:width 3.7–4.1. Ecdysial scar apparent. Felt chamber oval, $120.54\text{--}154.74 \mu\text{m}$ (134.09 ± 10.75) in diameter at junction with trachea. Spiracular process SP-I comprising 12–17 trunks (14.46 ± 1.80) and 25–36 tips (30.15 ± 4.33); ratio tips / trunks 1.7–2.1; basal width $25.31\text{--}39.80 \mu\text{m}$ (32.08 ± 4.17); ratio basal width:length of spiracular opening 0.3–0.4. SP-II comprising 5–13 trunks (6.92 ± 2.28) and 12–24 tips (17.85 ± 3.84). SP-III comprising 8–4 trunks (10.77 ± 1.42)

and 15–28 tips (21.31 ± 3.72). SP-IV comprising 14–21 trunks (16.46 ± 2.06) and 27–41 tips (33.38 ± 3.94); ratio tips / trunks 2.1–2.2; basal width $25.04\text{--}45.08 \mu\text{m}$ (36.74 ± 6.41) (Table 1); ratio basal width / length of SP-IV spiracular opening about 0.3–0.4. Average spiracular process length 0.3 times length of spiracular opening. The main diagnostic characters for the larvae of this species are shown in Table 2.

Description of second instar.—2.25–5.43 mm long (3.98 ± 0.91) and 0.37–0.93 mm wide (0.65 ± 0.15) at 6th abdominal segment. Shape and color as in third instar.

Spinules shaped and in discontinuous rows as in third instar. Spinule basal width $3.04\text{--}3.95 \mu\text{m}$. Dorsal spinule pattern in rows as follows: T1 3–7 (4.88 ± 1.36); T2 3–4 (3.27 ± 0.46); T3 1–3 (2.22 ± 0.83); A1 0–5 (2.50 ± 1.35); A2 4–5 (4.5 ± 0.54); A3 3–5 (4 ± 0.57); A4 2–5 (3.88 ± 1.05); A5 0–4 (2.22 ± 1.39); A6 to A8 lacking spinules. Ventral spinule pattern in rows as follows: T1 6–10 (8 ± 1.51); T2 2–3 (2.5 ± 0.57); T3 2–4 (2.75 ± 0.95); A1 6–9 (6.55 ± 1.01); A2 10–15 (11.11 ± 1.61); A3 11–15 (11.89 ± 1.45); A4 11–13 (11.77 ± 0.66); A5 10–12 (11.70 ± 0.67); A6 10–13 (11.77 ± 1.30); A7 10–13 (11.60 ± 1.17); A8 10–12 (10.67 ± 0.70). Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with 3–4 irregular rows (3.25 ± 0.46) anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with $12.09\text{--}20.46 \mu\text{m}$ outside diameter (15.23 ± 2.58); apical knob with $7.67\text{--}12.28 \mu\text{m}$ diameter (9.70 ± 1.50); combined height $7.24\text{--}13.28 \mu\text{m}$ (9.50 ± 1.72). Maxillary sensory organ cylindrical to slightly tapered with $14.98\text{--}27.13 \mu\text{m}$ diameter (17.94 ± 3.41), $11.68\text{--}14.58 \mu\text{m}$ height (13.32 ± 1.25).

Table 2. Diagnoses of larvae of the three *Anastrepha* species analyzed in this study.

<i>Anastrepha</i>	Rows of spinules		Oral ridges	CPS mandible b (mm) [*]	CPS mandible c (mm) [*]	CPS length (mm) [*]	N° of tubules on AS ^{**}	Posterior spiracle
	Dorsal	Ventral						N° spiracular process tips
<i>amita</i>	Present on A1 to A3	Different number on all	8–10	0.12–0.17	0.14–0.17	0.97–1.18	13–18	SP-I: 25–36 SP-IV: 27–41
<i>sororcula</i>	Absent on A1 to A3	Same number on A5–A7	6–9	0.16–0.19	0.14–0.19	0.95–1.23	9–12	SP-I: 36–48 SP-IV: 22–47
<i>zenilidae</i>	Absent on A1 to A3	Different number on all	8–10	0.17–0.21	0.17–0.21	1.09–1.59	11–16	SP-I: 30–43 SP-IV: 26–35

*CPS = cephalopharyngeal skeleton

**AS = anterior spiracle

Stomal sensory organ cylindrical with 3.89–4.76 μm height (4.31 ± 0.43). Oral ridges 6–8 (7.36 ± 0.67).

Cephalopharyngeal skeleton with total length from tip of mandible to end of ventral cornu 554.64–648.38 μm (618.73 ± 28.17) (Fig. 9). Mandible length a 108.56–144.90 μm (125.22 ± 7.76); length b 64.77–80.14 μm (71.51 ± 4.83); height c 87.81–99.48 μm (96.49 ± 3.91); ratio a:c 1.2–1.4; apical half moderately sclerotized and basal half heavily sclerotized, base with two openings; primary tooth sharply pointed and well-developed secondary tooth present. Hypopharyngeal sclerite 90.63–127.15 μm long (113.62 ± 11.18), anterior / apical half heavily sclerotized and posterior / basal half moderately sclerotized, 64.08–92.17 μm wide at ventral bridge (72.08 ± 7.19). Epipharyngeal sclerite, labial sclerite and parastomal bar as in third instar. Dorsal cornu usually with well-defined sclerotized area, length 183.97–277.60 μm (222.43 ± 23.09); length including hyaline area 316.32–419.06 μm (356.73 ± 34.69). Dorsal arch 148.26–179.17 μm high (162.49 ± 8.23). Anterior sclerite apparently absent. Cornu notch 137.15–181.66 μm long (166.14 ± 14.32) and cornu notch index 0.6–0.7. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 7–8 ridges (7.20 ± 0.41) forming series of grooves along length of ventral cornu. Ventral cornu 297.61–399.84 μm long (360.22 ± 28.77) from pharyngeal bar to posterior end of grooves. Ventral cornu 1.4–1.6 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 13–16 tubules (14.13 ± 1.06), in single row (Fig. 10). Tubule length 12.03–18.26 μm (15.49 ± 1.92); distal width 9.92–12.42 μm (10.81 ± 0.75); basal width 5.31–9.97 μm ($7.16 \pm$

1.14). Anterior spiracle length at midline 81.39–110.97 μm (95.68 ± 7.91); distal width 98.69–131.16 μm (117.70 ± 10.40); basal width 30.65–50.67 μm (41.37 ± 6.32) 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 entire.

Posterior spiracle openings with thinner rimae and fewer trabeculae than in third instar (Fig. 11); 23.34–30.64 μm long (28.01 ± 2.17); 12.75–17.27 μm wide (14.27 ± 1.28); ratio length:width 1.7–1.8. Ecdysial scar apparent. Felt chamber oval, 48.46–65.87 μm in diameter at junction with trachea (55.35 ± 5.17). Spiracular process SP-I comprising 7–11 trunks (8.92 ± 1.18) and 15–21 tips (17.58 ± 2.10); ratio tips / trunks 1.8–2.1; basal width 8.91–15.52 μm (11.38 ± 1.92); ratio basal width / length of spiracular opening about 0.3–0.5. SP-II comprising 4–9 trunks (6.18 ± 1.32) and 7–18 tips (11.45 ± 4.05). SP-III comprising 5–8 trunks (6 ± 1.29) and 8–15 tips (11.08 ± 1.89). SP-IV comprising 8–11 trunks (9.62 ± 0.76) and 15–22 tips (17.92 ± 2.43); ratio tips / trunks 1.8–2.0; basal width 8.87–14.88 μm (10.89 ± 1.55); ratio basal width / length of spiracular opening about 0.3–0.4. Average spiracular process length 0.7–0.8 times length of spiracular opening.

Anastrepha sororcula Zucchi

(Figs. 12–19)

Material examined.—A total of 13 third instars was examined. Larvae were dissected from fruit of *P. guajava* (guava). Fruit were collected in Mossoró in the state of Rio Grande do Norte, Brazil (April 2011).

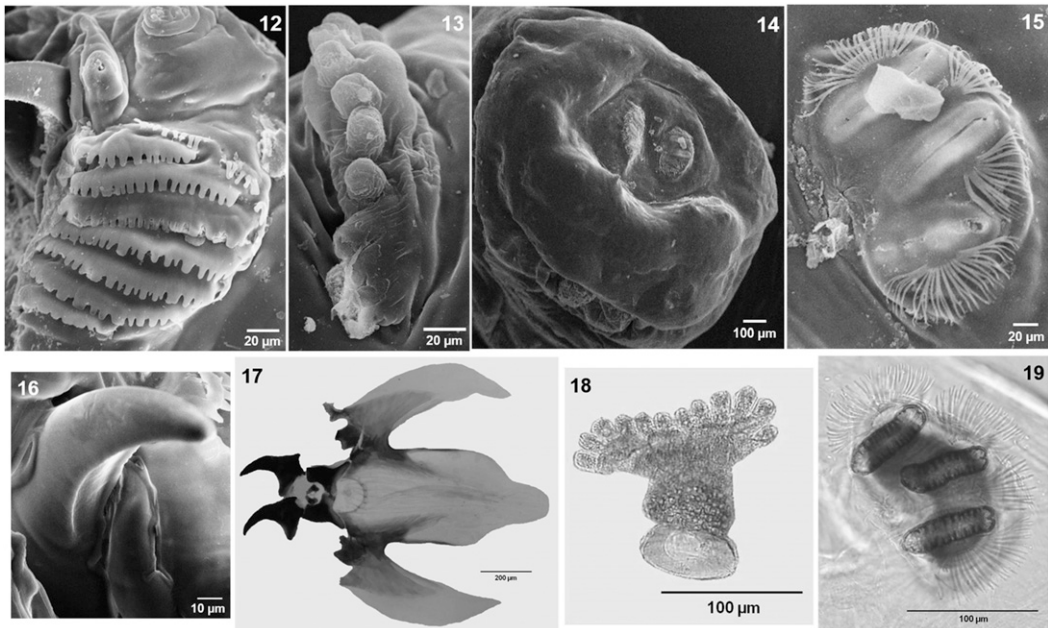
Description third instar.—8.47–10.02 mm (8.97 ± 0.47) long and 1.03–1.55 mm (1.28 ± 0.16) wide at 6th abdominal

segment. Elongate, cylindrical, thoracic segments tapered, and caudal end truncate (Table 1). Color creamy white.

On all segments, spinules in discontinuous rows of variable length dorsally and ventrally. Spinules conical, symmetrical to slightly curved posteriorly. Spinule basal width 2.06–3.78 μm (3.0 ± 0.52). Dorsal spinule pattern in rows as follows: T1 4–5 (4.38 ± 0.50); T2 3–5 (3.92 ± 0.49); T3 0–3 (1.69 ± 0.85); A1 to A8 lacking spinules. Ventral spinule pattern in rows as follows: T1 7–9 (8.15 ± 0.68); T2 4–7 (6.15 ± 0.98); T3 4–5 (4.31 ± 0.48); A1 5–10 (7.54 ± 1.85); A2 10–13 (11.08 ± 0.95); A3 11–13 (11.92 ± 0.75); A4 11–13 (11.62 ± 0.76); A5 10–13 (11.62 ± 1.04); A6 10–13 (11.54 ± 1.05); A7 10–13 (11.31 ± 1.25); A8 10–13 (10.77 ± 0.83) (Table 1). Ventral creeping welts present on all abdominal segments (A1 to A8). Additional band of spinules surrounding anal lobes with 3–4 irregular rows (3.54 ± 0.51) anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with 17.34–20.63 μm outside diameter (18.83 ± 1.45); apical knob 10.34–14.61 μm in diameter (12.73 ± 1.64); combined height 7.46–8.60 μm (7.94 ± 0.44). Maxillary sensory organ cylindrical to slightly tapered with 30.39–32.20 μm diameter (31.22 ± 0.76), height 10.66–12.10 μm (11.36 ± 0.76). Stomal sensory organ cylindrical, 4.33–6.92 μm in height (5.64 ± 1.21), located apically on simple elongate lobe typical of most other *Anastrepha* larvae (Carroll et al. 2004). Oral ridges 6–9 (7.0 ± 1.0), posterior margins irregularly serrated, accessory plates present in series of two at ends of most oral ridges (Fig. 12, Table 1).

Cephalopharyngeal skeleton with shape and sclerotization as in Fig. 17. Total length from tip of mandible to end of



Figs. 12–19. Scanning electron microscopy (12 to 16) and optical microscopy views (17 to 19) of third instar of *A. sororcula*. (12) Oral ridges. (13 and 18) Anterior spiracle. (14) Caudal view of posterior segment. (15 and 19) Posterior spiracle. (16) Ventral surface of mandible. (17) Cephalopharyngeal skeleton.

ventral cornu 0.95–1.23 mm (1.13 ± 0.07). Mandible length a 0.21–0.27 mm (0.25 ± 0.01); length b 0.16–0.19 mm (0.18 ± 0.01); height c 0.14–0.19 mm (0.17 ± 0.01); ratio a:c 1.5. Primary tooth long and sharp, some specimens with small secondary tooth. Mandible concave ventrally, surface of concavity smooth (Fig. 16). Hypopharyngeal sclerite 0.14–0.22 mm long (0.18 ± 0.01), 0.18–0.23 mm wide (0.20 ± 0.02) at ventral bridge measured in squashed preparations as seen in Fig. 17. Epipharyngeal sclerite visible only in dorsal view, with medial lobe directed anteriorly. Labial sclerite short, robust, fairly well sclerotized, and horseshoe-shaped in dorsal view. Parastomal bar extending for almost entire length of hypopharyngeal sclerite and arched slightly. Ventral sclerite present below pharyngeal sclerite. Dorsal cornu usually with well-defined

sclerotized area 0.35–0.43 mm long (0.38 ± 0.02); length including hyaline area 0.50–0.75 mm (0.65 ± 0.06). Dorsal bridge prominently projecting anteriorly from dorsal cornu and slightly sclerotized. Dorsal arch 0.20–0.43 mm high (0.36 ± 0.05). Anterior sclerite irregularly shaped and sclerotized. Cornu notch 0.20–0.29 mm long (0.25 ± 0.03) and cornu notch index 0.6. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 8–9 ridges (8.69 ± 0.48) forming series of grooves along length of ventral cornu. Ventral cornu 0.55–0.75 mm (0.66 ± 0.06) long from pharyngeal bar to posterior end of grooves (Table 1). Ventral cornu 1.6–1.7 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 9–12 tubules (10.62 ± 1.50), in single row (Figs. 13 and 18). Tubule

length 19.02–27.85 μm (22.62 ± 3.09); distal width 14.16–20.18 μm (17.34 ± 2.17); basal width 9.07–16.77 μm (12.42 ± 2.58). Anterior spiracle height at midline 119.13–160.64 μm (136.95 ± 15.61); distal width 155.58–215.84 μm (187.16 ± 17.12); basal width 73.13–113.96 μm (96.93 ± 12.03) at junction with trachea (Table 1).

Caudal segment (Fig. 14) with dorsal (D1 and D2), intermediate (I1 and I2), lateral (L1), and ventral (V1 and V2) tubercles and sensilla mostly well developed; D1 distinctly anterior to D2. Intermediate tubercles I1 and I2 more strongly developed and associated sensilla weakly developed; I2 lateral and sometimes slightly ventral to I1. L1 and V1 and V2 most very weakly developed. D1 and I1 associated with weakly developed sensilla. Posterior spiracles located above horizontal midline. Anal lobe entire and protruding.

Posterior spiracle openings with thick rimae and numerous trabeculae (Figs. 15 and 19); 71.02–92.47 μm long (81.45 ± 6.55); 16.17–21.71 μm wide (18.69 ± 2.06); ratio length / width 4.3–4.4. Ecdysial scar apparent. Felt chamber oval, 109.37–139.91 μm (124.63 ± 8.69) in diameter at junction with trachea. Spiracular process SP-I comprising 13–19 trunks (15.54 ± 1.85) and 36–48 tips (39.98 ± 4.99); ratio tips / trunks 2.7–3.0; basal width 22.29–37.90 μm (30.98 ± 5.44); ratio basal width / length of spiracular opening 0.3–0.4. SP-II comprising 6–9 trunks (7.31 ± 1.18) and 13–21 tips (15.23 ± 2.58). SP-III comprising 9–12 trunks (10.38 ± 1.04) and 19–31 tips (24.62 ± 3.86). SP-IV comprising 12–17 trunks (14.54 ± 1.50) and 22–47 tips (35.23 ± 5.86); ratio tips / trunks 2.6; basal width 21.33–32.87 μm (27.17 ± 4.04) (Table 1); ratio basal width / length of spiracular opening 0.3–0.4. Average spiracular process length

about 0.5 times length of spiracular opening. The main diagnostic characters for the larva of this species are shown in Table 2.

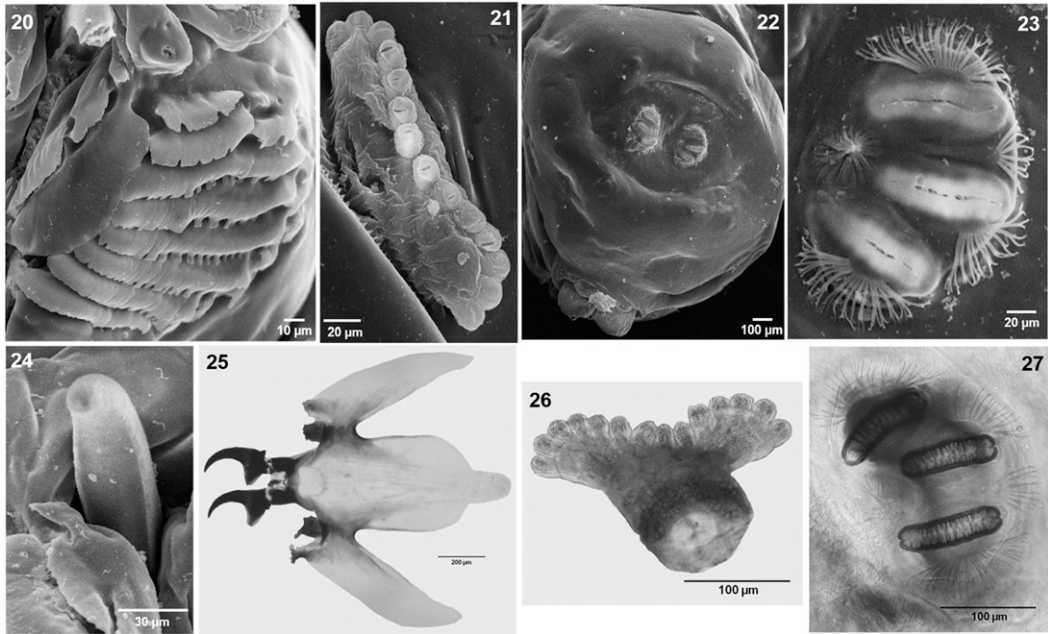
Anastrepha zenildae Zucchi

(Figs. 20–27)

Material examined.—A total of 24 third instars was examined. Larvae were dissected from fruit of *Z. joazeiro*, locally known as “juá,” a native tree in the Caatinga biome in the Brazilian semiarid northeastern region and fruit of *P. guajava* (guava). Fruits were collected in Limoeiro do Norte in the state of Ceará and in Mossoró in the state of Rio Grande do Norte, Brazil, respectively (April 2011).

Description of third instar.—8.78–10.54 mm long (9.67 ± 0.57) and 1.24–1.65 mm wide (1.45 ± 0.13) at 6th abdominal segment (Table 1). Elongate, cylindrical, thoracic segments tapered, and caudal end truncate. 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 posteriorly. Spinule basal width 2.28–3.42 μm (2.92 ± 0.31). Dorsal spinule pattern in rows as follows: T1 4–6 (4.77 ± 0.59); T2 4–5 (4.31 ± 0.48); T3 0–2 (0.69 ± 0.94); A1 to A8 lacking spinules. Ventral spinule pattern in rows as follows: T1 6–11 (8.38 ± 1.55); T2 5–7 (6.11 ± 0.78); T3 3–5 (4.56 ± 0.52); A1 6–8 (6.46 ± 0.66); A2 10–12 (10.92 ± 0.49); A3 11–12 (11.11 ± 0.33); A4 11–14 (12.11 ± 0.78); A5 12–13 (12.33 ± 0.50); A6 11–14 (12.38 ± 0.91); A7 11–13 (12.0 ± 0.57); A8 11–13 (11.46 ± 0.87) (Table 1). Ventral creeping welts present on third thoracic segment (T3) and on all abdominal segments (A1 to A8). Additional band of spinules



Figs. 20–27. Scanning electron microscopy (20 to 24) and optical microscopy views (25 to 27) of third instar of *A. zenildae*. (20) Oral ridges. (21 and 26) Anterior spiracle. (22) Caudal view of posterior segment. (23 and 27) Posterior spiracle. (24) Ventral surface of mandible. (25) Cephalopharyngeal skeleton.

surrounding anal lobes with 3–4 irregular rows (3.77 ± 0.43) anterior and posterior to lobes.

Antennal sensory organ slightly sclerotized, cylindrical basal collar with $20.15\text{--}22.78\ \mu\text{m}$ outside diameter (21.25 ± 1.04); apical knob $10.02\text{--}13.52\ \mu\text{m}$ in diameter (11.79 ± 1.53); combined height $6.54\text{--}7.59\ \mu\text{m}$ (7.25 ± 0.51). Maxillary sensory organ cylindrical to slightly tapered with $22.17\text{--}25.28\ \mu\text{m}$ diameter (22.91 ± 1.66), $10.26\text{--}15.64\ \mu\text{m}$ height (12.39 ± 2.44). Stomal sensory organ cylindrical, $5.68\text{--}8.37\ \mu\text{m}$ in height (7.34 ± 1.16), located apically on simple elongate lobe typical of most other *Anastrepha* larvae (Carroll et al. 2004). Oral ridges 8–10 (8.77 ± 0.83) with posterior margins irregularly serrated, accessory plates present in series of two at ends of most oral ridges (Fig. 20, Table 1).

Cephalopharyngeal skeleton with shape and sclerotization as in Fig. 25. Total length from tip of mandible to end of ventral cornu $1.09\text{--}1.59\ \text{mm}$ (1.25 ± 0.13). Mandible length a $0.16\text{--}0.28\ \text{mm}$ (0.23 ± 0.04); length b $0.17\text{--}0.21\ \text{mm}$ (0.19 ± 0.01); height c $0.17\text{--}0.21\ \text{mm}$ (0.19 ± 0.01); ratio a : c $0.9\text{--}1.3$. Tooth long, sharp, and heavily sclerotized, occasionally short and rounded. Mandible concave ventrally and surface of concavity smooth (Fig. 24). Hypopharyngeal sclerite $0.18\text{--}0.20\ \text{mm}$ long (0.19 ± 0), $0.19\text{--}0.23\ \text{mm}$ wide (0.21 ± 0.01) at ventral bridge measured on squashed preparations as seen in Fig. 25. Epipharyngeal sclerite visible only in dorsal view, with medial lobe directed anteriorly. Labial sclerite short, robust, fairly well sclerotized, and horseshoe-shaped in dorsal view. Parastomal bar extending for almost entire

length of hypopharyngeal sclerite and straight. Ventral sclerite present below pharyngeal sclerite. Dorsal cornu usually with well-defined sclerotized area 0.36–0.53 mm long (0.45 ± 0.05); length including hyaline area 0.60–0.81 mm (0.72 ± 0.06). Dorsal bridge prominently projecting anteriorly from dorsal cornu and slightly sclerotized. Dorsal arch 0.36–0.41 mm high (0.37 ± 0.01). Anterior sclerite irregularly shaped and sclerotized. Cornu notch 0.27–0.31 mm long (0.30 ± 0.03) and cornu notch index 0.7–0.8. Ventral cornu with poorly defined sclerotized area. Pharyngeal filter with weakly sclerotized anterior bar and 9–10 ridges (9.08 ± 0.27) forming series of grooves along length of ventral cornu. Ventral cornu 0.66–0.91 mm (0.75 ± 0.07) long from pharyngeal bar to posterior end of grooves (Table 1). Ventral cornu 1.7–1.8 times as long as sclerotized area of dorsal cornu.

Anterior spiracle bilobed, symmetrical, bearing 11–16 tubules (12.31 ± 0.85), in single row (Figs. 21 and 26). Tubule length 19.39–31.38 μm (22.75 ± 4.75); distal width 14.60–19.67 μm (16.88 ± 1.49); basal width 10.80–17.19 μm (13.86 ± 1.93). Anterior spiracle height at midline 121.05–179.73 μm (147.79 ± 17.26); distal width 192.87–261.10 μm (240.71 ± 23.01); basal width 106.17–137.07 μm (117.28 ± 9.88) at junction with trachea (Table 1).

Caudal segment (Fig. 22) with dorsal (D1 and D2), intermediate (I1 and I2), lateral (L1), and ventral (V1 and V2) tubercles and sensilla mostly well developed; D1 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, V1 and V2 most very weakly developed. D1 and I1 are associated with weakly developed sensilla. Posterior spiracles

located above horizontal midline. Anal lobe entire and protruding.

Posterior spiracle openings with thick rimae and numerous trabeculae (Figs. 23 and 27); 85.25–108.37 μm long (94.93 ± 7.68); 17.37–21.76 μm wide (19.41 ± 1.38); ratio length / width 4.5–4.9. Ecdysial scar apparent. Felt chamber oval, 127.26–148.24 μm (135.11 ± 5.03) in diameter at junction with trachea. Spiracular process SP-I comprising 9–16 trunks (12.62 ± 2.02) and 30–43 tips (34.92 ± 3.47); ratio tips / trunks 2.5–2.6; basal width 22.92–34.40 μm (30.49 ± 5.22); ratio basal width / length of spiracular opening about 0.3. SP-II comprising 5–9 trunks (6.38 ± 1.70) and 10–17 tips (13.85 ± 2.44). SP-III comprising 5–11 trunks (7.77 ± 1.73) and 17–24 tips (20.31 ± 2.81). SP-IV comprising 9–14 trunks (11.23 ± 1.58) and 26–35 tips (30.85 ± 3.18); ratio tips / trunks 2.5–2.8; basal width 20.28–33.86 μm (26.27 ± 3.87) (Table 1); ratio basal width / length of spiracular opening about 0.3. Average spiracular process length about 0.4 times length of spiracular opening. The main diagnostic characters for the larva of this species are shown in Table 2.

DISCUSSION

The second instar has been described for only eight species of *Anastrepha*. At least for the species for which second instars have been described, the second and third instars can be distinguished easily by non-overlapping size differences of the sclerotized structures and presence of a large secondary tooth on the mandible of second instars which is lacking in the third instars of most species (that of *A. sororcula* sometimes has a small secondary tooth) (White and Elson-Harris 1992). This has been confirmed in *A. amita* and is true for three other species within the *fraterculus* group: *A. bahiensis* Lima, *A. coronilli*

Carrejo & González, and *A. turpiniae* Stone (Dutra et al. 2012). Additionally, second and third instars of *A. amita* can be differentiated by the absence of dorsal spinules on abdominal segments A4 and A5, the number of tips on spiracular processes SP-I and SP-IV and the pattern of sclerotization of the cephalopharyngeal skeleton.

Third instars of *Anastrepha* show many similarities across species, however, it is still possible to differentiate among the three species described here by the presence or absence of dorsal spinules and the number of rows of ventral spinules on some segments, plus some characteristics of the cephalopharyngeal skeleton (Table 2).

Larvae of *A. amita* can be distinguished from larvae of *A. sororcula* by the presence of dorsal spinules on abdominal segments A1 to A3 and the number of tubules on the anterior spiracle (13–18 in *A. amita* vs. 9–12 in *A. sororcula*).

Larvae of *A. amita* can be differentiated from larvae of *A. zenildae* by the presence of dorsal spinules on abdominal segments A1 to A3.

Larvae of *A. sororcula* and *A. zenildae* cannot be distinguished using morphological characters, but a PCR-RFLP method has been developed to separate them.

In the larval key of Steck et al. (1990), both *A. amita* and *A. zenildae* run to either *Anastrepha striata* Schiner or *Anastrepha bistrigata* Bezzi, whereas *A. sororcula* runs to either *A. sororcula* or *A. suspensa*. Therefore, a LucID key to *Anastrepha* larvae with recently described species is necessary and will be presented at a later date. Additional characters such as the shape of the posterior margin of the oral ridges and measurements of the cephalopharyngeal skeleton can improve the key.

Many of the structures measured for larvae of *Anastrepha* show overlap, mainly among species within the same group, such as in this study. For instance, the number of tubules on the anterior spiracle ranges from 9 to 18 for the species described here whereas they range from 9 to 20 for the other species within the *fraterculus* group previously described (Lawrence 1979; Heppner 1984, 1991; Carroll and Wharton 1989; White and Elson-Harris 1992, Dutra et al. 2012). Still, it is possible to distinguish among species within the *fraterculus* group on the basis of the absence / presence of rows of dorsal spinules on abdominal segments, the number of trunks / tips on spiracular processes of the posterior spiracle, and characteristics of the cephalopharyngeal skeleton. The cephalopharyngeal skeleton has been described for only four species in the *fraterculus* group besides the three in the present study: *A. bahiensis*, *A. coronilli*, *A. ludens*, and *A. turpiniae* (Carroll and Wharton 1989, Dutra et al. 2012).

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