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**Draft Annex to ISPM 27 ▪ 2006 – Genus *Anastrepha* (2004-015)**

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| **Status box** | |
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| This is not an official part of the standard and it will be modified after adoption | |
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| **Consultation on technical level** | This diagnostic protocol was written and corrected by the DP drafting group: lead author and co-authors:<br>• Mr Vicente **Hernández-Ortiz** (Instituto de Ecología, Red de Interacciones Multitróficas, México)<br>• Ms Norma Christina **Vaccaro** (Instituto Nacional de Tecnología Agrícola, Argentina)<br>• Ms Alicia **Basso** (Universidad de Buenos Aires, Facultad de Agronomía, Argentina).<br>The previous draft has also been reviewed and commented upon by:<br>• Allen L. **Norrbom** (SEL, United States Department of Agriculture. Smithsonian Institution, Washington DC, USA)<br>• Roberto A. **Zucchi** (Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, Brazil)<br>• Daniel **Frías** (Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile) |

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| **Main discussion points during development of the diagnostic protocol** | Taxonomic concepts for this genus are based mainly on adult morphology, and particularly on the female (Norrbom *et al.*, 2012); males of some species and the immature stages of the majority of the more than 260 described species cannot be distinguished reliably. Molecular data are being investigated, but for the majority of species the number of samples that have been sequenced remains limited. Several pest species of *Anastrepha* are believed to comprise multiple (yet to be described) cryptic species that are morphologically indistinguishable or require morphometric analysis for recognition (Hernández-Ortiz *et al*., 2004, 2012).<br><br>As a record, some progress was obtained through ITS1 analysis (e.g. Sonvico *et al*., 2004 GenBank: AY686689). This information was associated with morphological characterization of specimens and karyotipic analysis, along with crossings (Basso, 2003).<br><br>An international research project to describe the cryptic species in *Anastrepha fraterculus* complex is being coordinated by IAEA. As part of this project, molecular methods are being examined for diagnostic utility within this complex. Based on available data, methods such as DNA barcoding using the cytochrome oxidase I gene cannot reliably diagnose several important pest species.<br>Consequently, the identification methods included in this first version of the diagnostic protocol are based on morphological identification. The protocol includes diagnosis of the genus and some species of major economic concern belonging to *Anastrepha*. |
| **Notes** | 2014-02-25: Status box last modified |

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**Adoption**

*[4]* This protocol was adopted by the Commission on Phytosanitary Measures in 20--.

*[5]* **1. Pest Information**

*[6]* The family Tephritidae, members of which are commonly known as true fruit flies, comprises about 4 450 species in 500 or so genera (Norrbom *et al.,* 1999a, 1999b; Norrbom, 2004a) (the figure is about 4 700 species currently, A.L. Norrbom, pers. comm., XXXX). The Tephritidae are distributed worldwide in temperate, tropical

and subtropical regions. *Anastrepha* Schiner (Tephritidae: Toxotrypanini) is the largest genus of Tephritidae in the Americas, and is represented by more than 250 species that occur from the southern United States (Texas and Florida) to northern Argentina (Foote *et al.*, 1993; Hernández-Ortiz, 1992; Hernández-Ortiz and Aluja, 1993; Norrbom, 2004a; Norrbom *et al*., 2012). At least six species of *Anastrepha* are considered major economic pests because of the great importance of the cultivated fruits they attack (e.g. mango and citrus) and their wide host range; for example, the Mexican fruit fly*, A. ludens* (Loew); the West Indian fruit fly, *A. obliqua* (Macquart); the Caribbean fruit fly, *A. suspensa* (Loew)*;* the guava fruit fly, *A. striata* Schiner*;* the sapodilla fruit fly, *A. serpentina* (Wiedemann); the melon fruit fly, *A. grandis* (Macquart); and the South American fruit fly, *A. fraterculus* (Wiedemann). The latter has been recognized as a cryptic species complex (Hernández-Ortiz *et al*., 2004, 2012). This diagnostic protocol for *Anastrepha* covers morphological identification of the genus and the species of major economic concern. For further general information about species of Tephritidae, see Norrbom (2010).

*[7]* The length of the tephritid life cycle varies according to genotype as well as environmental and climatic conditions (Basso, 2003). Female *Anastrepha* deposit their eggs inside fruits. The number of eggs deposited per fruit is variable, and depends mainly on features of the host fruit such as size and ripeness (Malavasi *et al.*, 1983), but each species also seems to have innate limits on the number of eggs laid (Aluja *et al*., 1999). Within several days, deposited eggs hatch and larvae emerge. Larvae usually feed on fruit pulp, but in some cases also or exclusively on seeds. Mature larvae usually leave the fruit to pupate in the ground, but in certain cases pupation can take place within the fruit. Adults usually emerge after a pupal period of 16–25 days, and they require a period of sexual maturation of 5–20 days after emergence. During this process the flies obtain food from homopteran secretions, bird faeces, and juice produced by ripe fruits (Prokopy and Roitberg, 1984).

*[8]* The relationship between *Anastrepha* species and their host plants is poorly understood. There are more than 330 host species from 48 families, many of them reported for a few generalist *Anastrepha* species (Norrbom, 2004b; Norrbom and Kim, 1988), while food plants for many other *Anastrepha* species remain unknown. Furthermore, current information includes numerous doubtful records, and reports of infestations induced only under laboratory conditions. Restricting the host list to natural infestations, hosts are known for about 39.8% of *Anastrepha* species (Hernández-Ortiz and Aluja, 1993).

*[9]* The introduction of some cultivated exotic species such as *Mangifera indica* and *Citrus* spp. have allowed some pest species of *Anastrepha* to expand their original areas of distribution and enhance their reproductive potential. However, they still have marked preferences for certain native hosts, which is probably indicative of their original host relationships. In this regard, the species *A. suspensa, A. fraterculus* and *A. triata* breed mainly in hosts belonging to the family Myrtaceae, *A. ludens* in the Rutaceae, *A. obliqua* in the Anacardiaceae, *A. serpentina* in the Sapotaceae, and *A. grandis* in the Cucurbitaceae (Norrbom, 2004b).

*[10]* Among native hosts in the American tropics, there seems to be an ancestral association with plants that produce latex and particularly the family Sapotaceae. Sapotaceous fruits are frequent hosts of species of the *dentata, leptozona, serpentina, daciformis, robusta* and *cryptostrepha* groups. The Myrtaceae are also very important hosts: about 26 *Anastrepha* species, in particular belonging to the *fraterculus* group, have been reported in plants belonging to this family (Norrbom and Kim, 1988; Norrbom *et al*., 1999c).

*[11]* **2. Taxonomic Information**

*[12]* **Name**: *Anastrepha* Schiner, 1868

*[13]* **Synonyms**:

*[14]* *Acrotoxa* Loew, 1873

*[15]* *Pseudodacus* Hendel, 1914

*[16]* *Phobema* Aldrich, 1925

*[17]* *Lucumaphila* Stone, 1939

*[18]* **Taxonomic position**: Insecta: Diptera: Tephritidae, Trypetinae, Toxotrypanini

*[19]*

**Table 1.** Common names and synonyms of fruit fly species of major economic significance belonging to the genus *Anastrepha*

| Common name | *Anastrepha* species | Synonyms |
|---|---|---|
| South American fruit fly | ***Anastrepha fraterculus*** (Wiedemann, 1830) | *Tephritis mellea* Walker, 1837 |
| | | *Trypeta unicolor* Loew, 1862 |
| | | *Anthomyia frutalis* Weyenbergh, 1874 |
| | | *Anastrepha fraterculus* var. *soluta* Bezzi, 1909 |
| | | *Anastrepha peruviana* Townsend, 1913 |
| | | *Anastrepha braziliensis* Greene, 1934 |
| | | *Anastrepha costarukmanii* Capoor, 1954 |
| | | *Anastrepha scholae* Capoor, 1955 |
| | | *Anastrepha pseudofraterculus* Capoor, 1955 |
| | | *Anastrepha lambayecae* Korytkowski and Ojeda, 1968 |
| Melon fruit fly | ***Anastrepha grandis*** (Macquart, 1846) | *Anastrepha schineri* Hendel, 1914 |
| | | *Anastrepha latifasciata* Hering, 1935 |
| Mexican fruit fly | ***Anastrepha ludens*** (Loew, 1873) | *Anastrepha lathana* Stone |
| West Indian fruit fly | ***Anastrepha obliqua*** (Macquart, 1835) | *Anastrepha mombinpraeoptans* Sein, 1933 |
| | | *Anastrepha fraterculus* var. *ligata* Lima, 1934 |
| | | *Anastrepha trinidadensis* Greene, 1934 |
| Sapodilla fruit fly | ***Anastrepha serpentina*** (Wiedemann, 1830) | *Urophora vittithorax* Macquart, 1851 |
| Guava fruit fly | ***Anastrepha striata*** Schiner, 1868 | *Dictya cancellaria* Fabricius, 1805 |
| | | (see Norrbom *et al.*, 1999b) |
| Caribbean fruit fly | ***Anastrepha suspensa*** | *Anastrepha unipuncta* Sein, 1933 |

| | | |
|---|---|---|
| | (Loew, 1862) | *Anastrepha longimacula* Greene, 1934 |

[20] **3. Detection**

[21] Fruit flies can be detected by inspection as larvae inside fruits and as pupae in the containers in which the fruits are being transported, or they can be captured outdoors as adults by means of trapping systems.

[22] **Inspection of fruits.** Infested fruits can be found in imported or exported shipments, in baggage, and even on aeroplanes or terrestrial transportation vehicles. Fruits with soft areas, dark stains, rot, orifices or injuries that might have originated from female oviposition or larval feeding activities are targeted for inspection. In order to detect punctures made by female flies during oviposition, the test should be done under a microscope by a specialist. If larval exit holes are observed, the fruit containers should be inspected for pupae. Second and third instar larvae and pupae are not likely to occur when unripe fruits are collected and packed; however, these fruits might host eggs and first instar larvae, which are more difficult to detect. Potentially infested fruits that show typical punctures made by ovipositioning female flies should be opened to search for eggs or larvae inside. The success of phytosanitary measures depends on careful sampling and examination of fruits.

[23] **Inspection of traps.** Guidance on trapping *Anastrepha* fruit flies for establishment of pest free areas is given in Appendix 1 of ISPM 26:2006. In general, monitoring systems established for the detection of fruit fly adults in trees, either in fruit-growing regions or in border areas between countries, require the utilization of McPhail traps baited with food attractants or synthetic lures. The baits, often with rich sources of ammonium, should be recognized and approved internationally (e.g. ISPM 26:2006). The specific methods of trap deployment and time of service of the traps must be in agreement with the phytosanitary regulations in use by each country.

[24] **4. Identification**

[25] The taxonomy of the genus *Anastrepha* is based on adult external morphology and characters of the female terminalia (Hernández-Ortiz, 1992; Norrbom *et al*., 2012; Stone, 1942; Zucchi, 2000). Because morphological characters of immature stages are not well documented for most *Anastrepha* species, these characters have a more limited utility in species recognition (White and Elson-Harris, 1992) in comparison with adult morphology. However, some information on egg structures and third instar larvae is available in the scientific literature and has diagnostic utility for certain species (Dutra *et al.*, 2011a, 2011b, 2012, 2013; Figueiredo *et al.*, 2011; Frías *et al*., 2006, 2008, 2009; Steck and Wharton, 1988; Steck *et al.*, 1990). Identification keys for the larvae of the seven species considered in Table 1 are available (Carroll *et al.*, 2004; Steck *et al.*, 1990) but should be used with consideration of their limits.

[26] Although the third instar larvae of some *Anastrepha* species apparently can be discriminated (Berg, 1979; Carroll and Wharton, 1989; Carroll *et al*., 2004; Frías *et al*., 2006; Hernández-Ortiz *et al.*, 2010; Steck and Wharton, 1988; Steck *et al.*, 1990; White and Elson-Harris, 1992), the available data are based on very limited sampling for most species that have been described. Studies of additional closely related species that have not yet been characterized may also reduce the reliability of the method. For this reason, experts should perform these diagnoses and evaluate all available information. The most reliable method for identification is rearing larvae to the adult stage.

[27] Several pest species of *Anastrepha* are believed to comprise multiple (yet to be described) cryptic species that are morphologically indistinguishable or require morphometric analysis for their recognition (Hernández-Ortiz *et al.*, 2004, 2012).

[28] To study this idea further, the International Atomic Energy Agency (IAEA) is coordinating an international research project to describe the cryptic species in the *Anastrepha fraterculus* complex. As part of this project, molecular methods are being examined for diagnostic utility within the genus. Based on available data, methods such as DNA barcoding using the *cytochrome oxidase* I gene cannot reliably diagnose several important pest species. Some progress was made by internal transcribed spacer (ITS)1 analysis (e.g. Sonvico *et al.*, 2004: GenBank AY686689). This information was associated with morphological characterization of specimens and karyotypic analysis, along with cross-mating studies (Basso, 2003).

[29] Consequently, the identification methods included in this diagnostic protocol are based on morphological characters.

*[30]* **4.1 Preparation of adults for identification**

*[31]* **4.1.1 Rearing larvae to obtain adults**

*[32]* The fruits to be examined are placed in cages covered with cloth or fine mesh and that have a sterile pupation medium (e.g. damp vermiculite, sand or sawdust) at the bottom. Once the larvae emerge from the fruit, they will move to the substratum for pupation. It is recommended to incubate each fruit separately. Each sample must be observed and pupae gathered daily. The pupae are placed in containers with the pupation medium, and the containers are covered with a tight lid that enables proper ventilation. Once the adults emerge, they must be kept alive for 48–72 h to ensure that the tegument and wings acquire the rigidity and characteristic coloration of the species. The adults are then killed and preserved by placing them in 70% ethanol, or they are killed with ethyl acetate or another agent and then mounted on pins. For female flies, immediately after killing them (before they harden) it is useful to gently squeeze the apical part of the preabdomen with forceps, then squeeze the base and apex of the oviscape to expose the aculeus tip (so that it does not need to be dissected later).

*[33]* **4.1.2 Preparation of adults for microscopic examination**

*[34]* For species recognition of adult stages, the entire specimen should be preserved – either dry (pinned) or in 70% ethanol. Examination of the wings and the aculeus is particularly important. Examination of the aculeus must be done at about 400× magnification. The wing and aculeus of each specimen can be mounted under two separate coverslips on the same slide. Dissection and mounting should be done only by someone with experience. Dissecting the female terminalia in *Anastrepha* is difficult and it is easy to damage useful parts.

*[35]* **4.1.2.1 Aculeus**

*[36]* It is preferable to cut off the whole abdomen from a female to dissect the oviscape (syntergosternite 7), the eversible membrane and the aculeus. For preserved dry (pinned) specimens, fine dissection scissors are recommended to remove the abdomen. The abdomen needs to be cleared. This can be accomplished by placing it in a 10% sodium hydroxide (NaOH) solution and heating it in a boiling water bath for 10–15 min, washing the structure with distilled water, and then removing internal contents under a stereomicroscope with the help of dissection forceps. The aculeus and the eversible membrane should be exposed. At this step it is possible to examine the aculeus directly in one or two drops of glycerine under a microscope. Afterwards, the structure can be transferred to a microvial with glycerine and pinned under the mounted dry specimen. For permanent slides, proceed as described in section 4.1.2. Mounting the aculeus permanently in the ventral position prevents the observation of some characters better seen in lateral view. For this reason, preservation in glycerine in a microvial is often preferable.

*[37]* **4.1.2.2 Wings**

*[38]* For permanent slides, proceed as described in section 4.1.2.1, avoiding the NaOH solution. Wing characters can usually be observed without mounting, so mounting is not recommended as a general practice. It may be necessary for morphometric studies, but it is not necessary for observation of the characters used in the key in section 4.3.2. If permanent mounts are made, it is recommended to cut off one of the wings from its base (the right wing is preferred because it facilitates comparison with images reported in the literature and this diagnostic protocol).

*[39]* **4.2 Preparation of larvae for identification**

*[40]* **4.2.1 Handling the biological sample**

*[41]* As noted in section 4, observation of adult characters may be necessary to make a definitive identification. If immature stages are found, it is recommended to preserve a few larvae for morphological examination by treating them in hot water (section 4.2.2) and then storing them in 70% ethanol. The remaining larvae and pupae are reared to obtain adult specimens for identification (section 4.1.1).

*[42]* Morphological examination of larvae (section 4.2.2) can be performed on unmounted larvae using a stereomicroscope, on slide-mounted larvae using a microscope, or on critical-point dried larvae using a scanning electron microscope (SEM). Slide mounting larvae can preclude subsequent analysis of morphological characters. On slide-mounted larvae it is possible to examine external morphology (e.g. anterior and posterior spiracles, oral ridges) as well as internal structures such as the cephalopharyngeal skeleton (Figures 21–44), using an optical microscope with objective 20×, 40× or higher. Detailed, high resolution observation of the external morphology of larvae is only possible using an SEM (Figures 45–61). It is therefore not recommended to slide mount all specimens representing a sample or the only larva available for diagnosis; unmounted larvae should be kept for future analysis.

*[43]* **4.2.2 Preparation of larvae for microscopic examination**

*[44]* To prepare specimens for examination the larvae must be treated in hot water, which can be accomplished by placing live larvae in water of approximately 65° C for 2–4 min. The larvae are cooled to room temperature and then immersed in 50% alcohol for 15–30 min. The specimens are transferred to a hermetic vial (15–25 ml) filled with 70% alcohol. It is advisable to include a label on the vial with all sampling information. These samples are ready for examination under a stereomicroscope or subsequent preparation for slide mounting or examining under an SEM.

*[45]* To prepare specimens for slide mounting, it is necessary to remove (clean) all the internal contents to allow observation of the cuticle, oral opening, cephalopharyngeal skeleton and anterior spiracles, as well as the posterior spiracular plate and anal lobes. This can be accomplished by making two transverse incisions in the larva, one behind the cephalic region and the anterior spiracles, and one before the caudal segment. The incised larva then needs to be immersed in a test tube containing 10% NaOH solution and heated in a boiling water bath for 10–15 min. The internal contents can then be carefully removed from the specimen using forceps and distilled water under a stereomicroscope (45× magnification or greater).

*[46]* Permanent slide mounts can be made using Canada balsam or Euparal. Before doing this, cleaned structures must be dehydrated by placing them for 25 min in each of 50%, 75% and 100% ethanol. For mounting with Canada balsam, the specimens should be transferred to absolute xylene for 3–5 min to clear them and then immediately mounted on a slide with one or two drops of Canada balsam. When Euparal is used as the mounting medium, structures should be transferred from 100% ethanol to clove oil for about 30 min to clear them before mounting. In both cases, slides must be allowed to dry for several days (the time can be reduced by using an oven), but they can be examined under the microscope at low magnification immediately after mounting. Slides should be labelled.

*[47]* For observation using an SEM, the specimens (stored in alcohol) must first be cleaned in their vials in distilled water with a drop of liquid soap added to serve as a surfactant. Then they should be rinsed thoroughly with distilled water and dehydrated by running through a series of ethanol baths: 70%, 80%, 95%, and three changes of absolute ethanol (15 min each bath). Specimens should then be critical-point dried and coated with gold-palladium (Carroll and Wharton, 1989). Similar techniques can be found elsewhere (e.g. Frías *et al.*, 2006, 2008, 2009).

*[48]* **4.3 Morphological identification of adults**

*[49]* **4.3.1 Identification of the genus *Anastrepha* Loew**

*[50]* **Adults** (Figure 1). Head (Figure 1A): Usually yellow with two to eight frontal and one or two orbital setae, sometimes posterior orbital seta absent; ocellar seta usually very weak or indistinct; postocellar, medial and lateral vertical setae present. Thorax (Figures 2, 3): Macrosetae of thorax usually black, red–brown or orange, rarely golden yellow; scutum usually yellow to orange, occasionally mostly dark brown or sometimes with dark brown or black stripes or spots, always with two to five yellow stripes; mesonotum with the following setae: one postpronotal, two notopleurals, one presutural supra-alar, one postsutural supra-alar, one postalar, one intra-alar, one dorsocentral, one acrostichal (rarely absent) and two scutellars.

*[51]* Wings (Figure 4): Subcostal break present; crossvein *r-m* placed distal to mid-length of discal cell (*dm*); basal cubital cell (*bcu*) with a well-developed posteroapical extension; vein *M* usually conspicuously curved forwards apically (strongly so in all pest species) and not meeting costa at a 90° angle. Wing pattern with orange to brown coloured bands forming a typical pattern as follows: costal (C)-band on basal costal margin including all

of vein $R_1$, subcostal cell and the pterostigma; S-band extending from apex of cell *bcu* across cell *dm* and crossvein *r-m,* reaching costal margin, and continuing to apex of wing; and V-band forming an inverted V shape, comprising the proximal arm (subapical band) along vein *dm-cu* and the distal arm (posterior apical band) arising from cell *m*, both are convergent in cell $R_{4+5}$; distal arm frequently incomplete or absent. The typical wing pattern is modified in some economically important species (see key to species).

*[52]* Male terminalia (Figure 5): Epandrium broad in lateral view with lateral surstylus short or elongated; medial surstylus shorter than lateral surstylus with two stout blackish prensisetae apically; proctiger membranous, weakly sclerotized laterally and ventrally; phallus elongated, usually longer than length of oviscape of female; glans weakly sclerotized with an apical T-shaped sclerite, glans sometimes absent in non-pest species.

*[53]* Female terminalia (Figure 6): Oviscape tube-like, variable in length: eversible membrane (usually inverted inside oviscape) apically with dorsal group of hook-like sclerotized plates (also named raspers); aculeus (usually inverted inside eversible membrane and oviscape) well sclerotized, tip sometimes serrated on lateral margins.

*[54]* **4.3.2 Key to adults of the species of major economic concern of the genus *Anastrepha***

*[55]* Key adapted from Hernández-Ortiz *et al*. (2010). For additional information on morphological structures and other *Anastrepha* species, see Norrbom *et al.* (2012).

*[56]* 1- Wing with C-band interrupted just at end of vein $R_1$ by a well-delimited hyaline mark in cell $r_1$; anterior and posterior orbital setae present; distal arm of V-band usually present at least partially, but if absent, wing pattern dark brown to black.

*[57]* ..... **2**

*[58]* Wing with C-band uninterrupted from wing base to apex, sometimes diffuse in cell $r_1$; posterior orbital seta often absent; distal arm of V-band absent. All following characters must be present: basal half of S-band continuous from apex of cell *bcu* through crossvein *R-M* and connecting with C-band; cell $r_{2+3}$ completely pigmented in entire length; vein $R_{2+3}$ almost straight in entire length; cell *br* broadly hyaline between veins *BM-Cu* and *R-M* (Figure 7); abdominal tergites yellow; scutum with dark brown dorsocentral stripes; aculeus of female extremely long (5.3–6.2 mm) and usually greater than 0.10 mm wide, aculeus tip with V-shaped ridges, lateral margins non-serrate (Figure 14); glans of male present. (Larvae infest melons.)

*[59]* .....***Anastrepha grandis* (Macquart)**

*[60]* 2- Scutum predominantly dark brown with brown to black stripes.

*[61]* ..... **3**

*[62]* Scutum yellow or orange, without dark brown markings except sometimes along scuto-scutellar suture.

*[63]* ..... **4**

*[64]* 3- Wing pattern mostly dark brown; distal arm of V-band completely absent (Figure 8); abdominal tergites mostly dark brown with T-shaped medial white mark; thoracic pleuron mostly brown, strongly contrasting with yellow markings; female aculeus 2.6–3.8 mm long, aculeus tip 0.37–0.46 mm long, 0.14–0.17 mm wide, lateral margins finely serrated on distal 0.5–0.7 (Figure 15). (Larvae infest sapotaceous fruits.)

*[65]* .....***Anastrepha serpentina* (Wiedemann)**

*[66]* Wing pattern mostly orange and moderate brown; distal arm of V-band usually present (Figure 9); abdominal tergites and pleuron yellow or orange; scutum with two broad dorsocentral stripes connected on posterior

margin to form U-shaped mark, without setulae in a small area along transverse suture, but with dense white microtrichia contrasting with black setulae; female aculeus 1.95–2.30 mm long, tip broad, 0.24–0.31 mm long, 0.17–0.20 mm wide (Figure 16). (Larvae infest guavas.)

*[67]* .....
***Anastrepha striata* Schiner**

*[68]* 4- Anterior apical band of wing (=distal section of S-band) narrow to moderately broad, never reaching apex of vein *M*; V-band with arms separated anteriorly or if joined, with large hyaline mark between them and vein *M*. Scuto-scutellar suture with or without brown spot medially; aculeus variable.

*[69]* ..... **5**

*[70]* Anterior apical band of wing (=distal section of S-band) extremely wide, reaching apex of vein *M*; V-band broad and complete, with arms widely connected anteriorly, hyaline mark between them and vein *M* small or absent (Figure 10); scuto-scutellar suture usually with large rounded brown spot medially; female aculeus 1.4–1.6 mm long (Figure 17), tip 0.19–0.23 mm long, 0.10–0.13 mm wide, lateral margins serrate on distal 0.50–0.65.

*[71]* .....
***Anastrepha suspensa* (Loew)**

*[72]* 5- Female aculeus length less than 2.0 mm (usually 1.4–1.9 mm), tip short and wide with large teeth on sides; other characters are variable.

*[73]* .....**6**

*[74]* Female aculeus length more than 2.5 mm (usually 3.3–5.8 mm); aculeus tip length 0.28–0.42 mm, with a moderate constriction near mid-length; lateral margins non-serrate or finely serrate on distal 0.55 or less (Figure 18); brown lateral markings of subscutellum always evident and sometimes extended onto mediotergite (Figure 3B); wing pattern as in Figure 11. (Larvae commonly infest citrus and mango.)

*[75]* .....***Anastrepha ludens* (Loew)**

*[76]* 6- Subscutellum entirely yellow, only mediotergite with brown markings on sides (Figure 3C); brown spot on scuto-scutellar suture absent; aculeus tip 0.16–0.20 mm long, with lateral serrations on distal two-thirds or four-fifths (Figure 19); wing pattern as in Figure 12. (Larvae commonly infest mangos or *Spondias* fruits.)

*[77]* .....***Anastrepha obliqua* (Macquart)**

*[78]* Both mediotergite and subscutellum with broad dark brown to black markings on sides (Figure 3A); brown spot on scuto-scutellar suture usually present. aculeus 1.4–1.9 mm long; aculeus tip 0.20–0.28 mm long; lateral margins with 8 to14 teeth on distal two-fifths to three-fifths (Figure 20); wing pattern variable (Figure 13).

*[79]* .....***Anastrepha fraterculus* (Wiedemann) *sensu lato***

*[80]* **4.4 Morphological identification of third instar larvae**

*[81]* **4.4.1 Key to third instar larvae of major economically important genera of Tephritidae in the Americas**

*[82]* Key adapted from Frías *et al*. (2006). For additional information on larval morphology of this genera and related species, see Frías *et al*. (2006, 2008), White and Elson-Harris (1992) and Carroll *et al*. (2004).

*[83]* 1- Mandible more than 0.3 mm long. Ventral apodeme of mandible broad and rounded apically (Figure 26). Spiracular hairs shorter than width of medial spiracular slit (Figure 49).

*[84]* .....***Toxotrypana*** **Gerstaecker**

*[85]* Mandible less than 0.3 mm long. Ventral apodeme of mandible sharp apically (Figures 22–24). Spiracular hairs longer than width of medial spiracular slit (as in Figure 50).

*[86]* .....**2**

*[87]* 2- Hypopharyngeal bridge narrow at subapical area of hypopharyngeal sclerite (Figure 21). Preoral and oral teeth present (Figure 47); oral ridges usually non-serrate (Figures 45, 47). Dorsolateral sensilla group equidistant from antenna and maxillary palpus.

*[88]* .....***Rhagoletis*** **Loew**

*[89]* Hypopharyngeal bridge narrow at middle of hypopharyngeal sclerite (as in Figures 27–32). Preoral and oral teeth absent; oral ridges usually with serrated margins (Figure 48). Dorsolateral sensilla group closer to maxillary palpus than to antenna.

*[90]* .....**3**

*[91]* 3- Posterior region of mandible without distinct neck (Figure 23). Caudal ridge lacking (Figure 59).

*[92]* .....***Anastrepha*** **Schiner**

*[93]* Posterior region of mandible with distinct neck (Figures 22, 24). Caudal ridge present (Figure 60).

*[94]* ..... **4**

*[95]* 4- Oral ridges with shorter round teeth (Figure 48).

*[96]* .....***Ceratitis*** **McLeay**

*[97]* Oral ridges with long, sharply pointed teeth (not as above).

*[98]* .....***Bactrocera*** **Macquart**

*[99]* **4.4.2 Key to third instar larvae of major economically important species of the genus *Anastrepha***

*[100]* Key adapted from Steck *et al*. (1990). *Geographic distribution and hosts are quoted only as additional information of the common source of origin for the species.

*[101]* 1- Posterior spiracles prominently raised from the body surface; or most body segments with conspicuous setae or processes; or posterior spiracular openings sinuous.

*[102]* .....**Not Tephritidae**

*[103]* Posterior spiracles nearly flush with body surface; tubercles, if present, on caudal segment only; posterior spiracular slits elongate or oval (Figures 49–50) (Tephritidae).

*[104]* .....**2**

*[105]* 2- Prominent chitinized preoral teeth (=stomal guards) adjacent to oral opening, or dental sclerite conspicuous (Figures 45, 47); and/or caudal tubercles strongly developed; or larva taken from papaya with caudal tubercles lacking and caudal sensilla strongly reduced.

*[106]* .....**Other Tephritidae** (**not *Anastrepha***)

*[107]* Preoral teeth (=stomal guards) lacking, and dental sclerite lacking or inconspicuous (Figure 48); caudal tubercles at most moderately developed.

*[108]* .....***(Anastrepha)* 3**

*[109]* 3- Dorsal spinules present on at least two or more abdominal segments, separate, conical, in fewer than five to six rows on thoracic segments T2 and T3 (Figure 61); posterior spiracular processes SP-I and SP-IV (Figure 46) with average of six or more trunks with bristle length one-third or more times length of spiracular opening (Figures 40, 44).

*[110]* .....**4**

*[111]* Dorsal spinules absent on all abdominal segments, or if present, only in segment A1 (some specimens of *A. ludens*).

*[112]* .....**5**

*[113]* 4- Anterior spiracle with 28–37 tubules (Figure 43); cephalopharyngeal skeleton as in Figure 32. (Main hosts: larvae breed in fruits of Cucurbitaceae; distribution: Panama to Argentina.)

*[114]* .....***Anastrepha grandis***

*[115]* Anterior spiracle with 12 to 23 tubules (Figure 39); cephalopharyngeal skeleton as in Figure 31. (Main hosts: larvae breed in fruits of Myrtaceae; distribution: tropical Americas.)

*[116]* .....***Anastrepha striata***

*[117]* 5- Dorsal spinules present on thoracic segment T3 (Figure 61).

*[118]* .....**6**

*[119]* Dorsal spinules absent on thoracic segment T3 (not as above).

*[120]* .....**7**

*[121]* 6- Oral ridges in 11 to 17 rows, usually with margins entire; anterior spiracles with 12 to 20 tubules (Figures 33, 51); posterior spiracular slits 3.1–4.6 times longer than wide (Figure 34). Cephalopharyngeal skeleton as in Figure 27. (Main hosts: larvae breed in fruits of *Citrus* spp. (Rutaceae) or *Mangifera indica*; distribution: southern Texas, USA to Panama.)

*[122]* .....***Anastrepha ludens***

*[123]* Oral ridges in 8 to 11 rows with stout, bluntly rounded, widely spaced teeth; anterior spiracles with 9 to 15 tubules (Figure 41); posterior spiracular slits 2.5–3.5 times longer than wide (Figure 42). Cephalopharyngeal skeleton as in Figure 29. (Main hosts: larvae breed in fruits of Myrtaceae; distribution: Florida, USA and Antilles.)

*[124]* .....***Anastrepha suspensa***

*[125]* 7- Posterior spiracular processes SP-I and SP-IV with 5 to 11 short trunks (average, 8) (Figure 36); oral ridges usually in 12 to 14 rows; anterior spiracle with 13 to 19 tubules in a single row (Figure 35); anal lobes usually bilobed (as in Figure 57). Cephalopharyngeal skeleton as in Figure 30. (Main hosts: larvae breed in fruits of Sapotaceae; distribution: tropical Americas.)

*[126]* .....***Anastrepha serpentina***

*[127]* Posterior spiracular processes SP-I and SP-IV with 8 to 18 long trunks (average, 13); oral ridges in 7 to 10 rows; anterior spiracle with 9 to 18 tubules in a single row (as in Figure 34); anal lobes complete or bilobed (Figures 57, 58).

*[128]* .....**8**

*[129]* 8- Posterior spiracular processes SP-II usually with three to six trunks; posterior spiracular slits 3.0–4.9 times longer than wide (Figure 38). Cephalopharyngeal skeleton as in Figure 28. (Main hosts: larvae breed in fruits of the Anacardiaceae; distribution: tropical Americas, including Antilles.)

*[130]* .....***Anastrepha obliqua***

*[131]* Posterior spiracular processes SP-II usually with four to nine trunks; posterior spiracular slits 2.5–4.0 times longer than wide (Figure 46). (Distribution: tropical Americas.)

*[132]* .....***Anastrepha fraterculus* (species complex)**

*[133]*

**Table 2.** Diagnostic morphological characters of the genus *Anastrepha* used in the keys of this protocol

| Biological stage | Structure | Description |
|---|---|---|
| **Larva** | Mandible | Less than 0.3 mm long; posterior region without distinct neck; preapical tooth lacking |
| | Posterior spiracles | Spiracular hairs longer than width of medial spiracular slits |
| | Hypopharyngeal bridge | Narrow, located at middle of hypopharyngeal sclerite |
| | Preoral and oral teeth | Absent |
| | Oral ridges | Usually serrated |

| | | |
|---|---|---|
| | Stomal sensory organ | Enlarged |
| **Adult** | Head chaetotaxy | Two to eight frontal and one or two orbital setae; ocellar setae very weak or indistinct; postocellars unicolorous |
| | Mesonotum chaetotaxy | One postpronotal, two notopleurals, one presutural supra-alar, one postsutural supra-alar, one postalar, one intra-alar, one dorsocentral, one acrostichal (rarely absent) and two scutellars |
| | Wings | Veins: Vein *M* usually conspicuously curved forwards apically (strongly so in all pest species) and meeting costa without 90° angle; crossvein *r-m* placed distal to mid-length of discal cell (*dm*); basal cubital cell (*bcu*) with well-developed posteroapical extension |
| | | Wing pattern: C-band on basal costal margin; S-band (from apex of cell *bcu* across cell *dm* and crossvein *r-m*); V-band forming an inverted V shape comprising the proximal arm (subapical band) on *dm-cu* and distal arm (posterior apical band) arising from cell *m*, both convergent in cell $R_{4+5}$ |
| | Male genitalia | Lateral surstylus short or elongate; medial surstylus shorter than lateral surstylus with two prensisetae apically; proctiger weakly sclerotized laterally and ventrally; glans weakly sclerotized with an apical T-shaped sclerite, glans sometimes absent in non-pest species |
| | Female genitalia | Oviscape tube-like, variable in length; eversible membrane apically with dorsal hook-like sclerotized plates (also named raspers); aculeus well sclerotized, length variable, tip sometimes serrated on lateral margins |

*[134]*

**Table 3.** Diagnostic morphological characters of third instar larvae of *Anastrepha* species

| Species | Structure | Description |
|---|---|---|
| *Anastrepha fraterculus* | Oral ridges | 7 to 10 rows |
| | Anterior spiracle | 9 to 18 tubules in a single row |
| | Dorsal spinules | Abdominal segments absent |
| | | Thoracic segments absent on T3 |
| | Posterior spiracles | SP-I and SP-IV with 10 to 17 long trunks; SP-II usually with 6 to 9 trunks; slits 2.5–3.5 times longer than wide |
| | Anal lobes | Entire in some populations, bifid in others |
| *Anastrepha grandis* | Oral ridges | 8 to 13 rows |
| | Anterior spiracle | 28 to 37 tubules |
| | Dorsal spinules | Abdominal segments present on two or more segments |
| | | Thoracic segments present on T2 and T3 |
| | Posterior spiracles | SP-I and SP-IV with six or more trunks with bristle length one-third times length of spiracular opening |
| | Anal lobes | Bilobed |
| *Anastrepha ludens* | Oral ridges | 11 to 17 rows; margins entire |
| | Anterior spiracle | 12 to 20 tubules |

| | | |
|---|---|---|
| | Dorsal spinules | Abdominal segments present on A1 |
| | | Thoracic segments present on T3 |
| | Posterior spiracles | Slits 3.1–4.6 times longer than wide |
| | Anal lobes | Bilobed |
| *Anastrepha obliqua* | Oral ridges | 7 to 10 rows |
| | Anterior spiracle | 9 to 18 tubules in a single row |
| | Dorsal spinules | Abdominal segments absent |
| | | Thoracic segments absent on T3 |
| | Posterior spiracles | SP-I and SP-IV with 10 to 17 long trunks; SP-II usually with 3 to 6 trunks; slits 3–4.5 times longer than wide |
| | Anal lobes | Entire |
| *Anastrepha serpentina* | Oral ridges | 12 to 18 rows |
| | Anterior spiracle | 13 to 19 tubules in a single row |
| | Dorsal spinules | Abdominal segments absent |
| | | Thoracic segments absent on T3 |
| | Posterior spiracles | SP-I and SP-IV with 6 to 9 short trunks |
| | Anal lobes | Usually bilobed (occasionally entire) |
| *Anastrepha striata* | Oral ridges | 5 to 8 rows |
| | Anterior spiracle | 12 to 23 tubules |
| | Dorsal spinules | Abdominal segments present on two or more segments; thoracic segments present on T2 and T3 |
| | Posterior spiracles | SP-I and SP-IV with six or more trunks, length of hairs one-third or more of the length of the spiracular opening |
| | Anal lobes | Entire or partially bilobed |
| *Anastrepha suspensa* | Oral ridges | 8 to 11 rows; margins with stout, bluntly rounded, widely spaced teeth |
| | Anterior spiracle | 9 to 15 tubules |
| | Dorsal spinules | Abdominal segments absent |
| | | Thoracic segments present on T3 |
| | Posterior spiracles | Slits 2.5–3.5 times longer than wide |
| | Anal lobes | --------- |

*[135]* **Table 4.** Diagnostic morphological characters of adults of *Anastrepha* species

*[136]*

| Species | Structure | Description |
|---|---|---|

| | | |
|---|---|---|
| *Anastrepha fraterculus* | Head chaetotaxy | Posterior orbital seta present |
| | Thorax | Both mediotergite and subscutellum with broad brown markings on sides; scuto-scutellar suture usually with medial brown spot |
| | Wings | Distal arm of S-band normally developed, never reaching apex of vein *M*; V-band connected to or separated from S-band anteriorly |
| | Female genitalia | Aculeus 1.4–1.9 mm long; aculeus tip 0.20–0.28 mm long; lateral margins with 8 to 14 teeth occupying distal two-fifths to three-fifths |
| *Anastrepha grandis* | Head chaetotaxy | Posterior orbital seta usually absent |
| | Thorax | Scutum with dark brown dorsocentral stripes |
| | Wings | C-band uninterrupted along costal vein; basal half of S-band (on discal cell) continuous from apex of cell *bcu* through crossvein *R-M* and connecting with C-band above; cell $r_{2+3}$ completely pigmented in entire length; vein $R_{2+3}$ almost straight; cell *br* broadly hyaline between veins *bm-cu* and *r-m* |
| | Female genitalia | Aculeus extremely long (5.3–6.2 mm), and usually greater than 0.10 mm wide; aculeus tip with V-shaped ridges, lateral margins non-serrate |
| *Anastrepha ludens* | Head chaetotaxy | Posterior orbital seta present |
| | Thorax | Subscutellum always with brown marks laterally, sometimes extending onto mediotergite |
| | Wings | V-band usually not connected to S-band, and with arms separated anteriorly |
| | Female genitalia | Aculeus usually 3.3–5.8 mm long; aculeus tip 0.28–0.42 mm long, 0.12–0.14 mm wide, with a moderate constriction near mid-length; lateral margins non-serrate or finely serrate on distal 0.55 or less |
| *Anastrepha obliqua* | Head chaetotaxy | Posterior orbital seta present |
| | Thorax | Subscutellum entirely yellow, only mediotergite with brown markings on sides; scuto-scutellar suture without medial brown spot |
| | Wings | Distal arm of S-band normally developed, never reaching apex of vein *M*; V-band usually connected anteriorly to S-band |
| | Female genitalia | Aculeus less than 2.0 mm long; aculeus tip 0.16–0.20 mm long, with lateral serrations on distal two-thirds to four-fifths |
| *Anastrepha serpentina* | Head chaetotaxy | Posterior orbital seta present |
| | Thorax | Thorax mostly brown or red–brown contrasting with yellow markings; scutum mostly brown with three yellow stripes |
| | Wings | Wing pattern mostly dark brown; distal arm of V-band completely absent |
| | Female genitalia | Aculeus 2.6–3.8 mm long; aculeus tip 0.37–0.46 mm long, 0.14–0.17 mm wide, lateral margins finely serrated on distal 0.5–0.7 |
| *Anastrepha striata* | Head chaetotaxy | Posterior orbital seta present |

| | | |
|---|---|---|
| | Thorax | Scutum with two broad dorsocentral stripes connected on posterior margin forming a U-shaped mark, without setulae in a small area along transverse suture |
| | Wings | Wing pattern mostly orange and brown; distal arm of V-band present or absent |
| | Female genitalia | Aculeus 1.95–2.30 mm long; aculeus tip broad, 0.24–0.31 mm long, 0.17–0.20 mm wide |
| *Anastrepha suspensa* | Head chaetotaxy | Posterior orbital seta present |
| | Thorax | Scuto-scutellar suture with large rounded brown spot medially; mediotergite entirely yellow or with brown mark on sides |
| | Wings | Anterior apical band (=distal section of the S-band) extremely wide, reaching the apex of vein *M*; V-band broad and complete, with arms widely connected anteriorly |
| | Female genitalia | Aculeus 1.4–1.6 mm long; aculeus tip 0.19–0.23 mm long, 0.10–0.13 mm wide, lateral margins serrate on distal 0.50–0.65 |

*[137]* **5. Records**

*[138]* Records and evidence should be retained as described in section 2.5 of ISPM 27:2006.

*[139]* In cases where other contracting parties may be adversely affected by the diagnosis, the records and evidence (in particular, preserved or slide-mounted specimens and photographs of distinctive taxonomic structures, as appropriate) should be deposited in a museum or other permanent collection.

*[140]* **6. Contact Points for Further Information**

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[149] A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel to develop Diagnostic Protocols (TPDP).

[150] **7. Acknowledgements**

[151] This protocol was written by Vicente Hernández-Ortiz (Instituto de Ecología A.C., Red de Interacciones Multitróficas, Xalapa, México) with the collaboration of Norma Vaccaro (Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Concordia, Argentina) and Alicia Basso (Universidad de Buenos Aires, Facultad de Agronomía, Buenos Aires, Argentina).

[152] In addition, the following colleagues reviewed and improved the protocol:

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[158] - Ana Lía Terra, Andrea Listre and Odile Volonterio (Ministerio de Ganadería, Agricultura y Pesca, Dirección General de Servicios Agrícolas, Montevideo, Uruguay)

[159] - Mallik Malipatil (La Trobe University, Bioprotection, Biosciences Research Division, Department of Primary Industries, Knoxfield Centre, Melbourne, Victoria, Australia).

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[198] **9. Figures**

[199] **Figure 1.** General habitus of the adult female of *Anastrepha ludens* (Mexican fruit fly) in dorsal view.

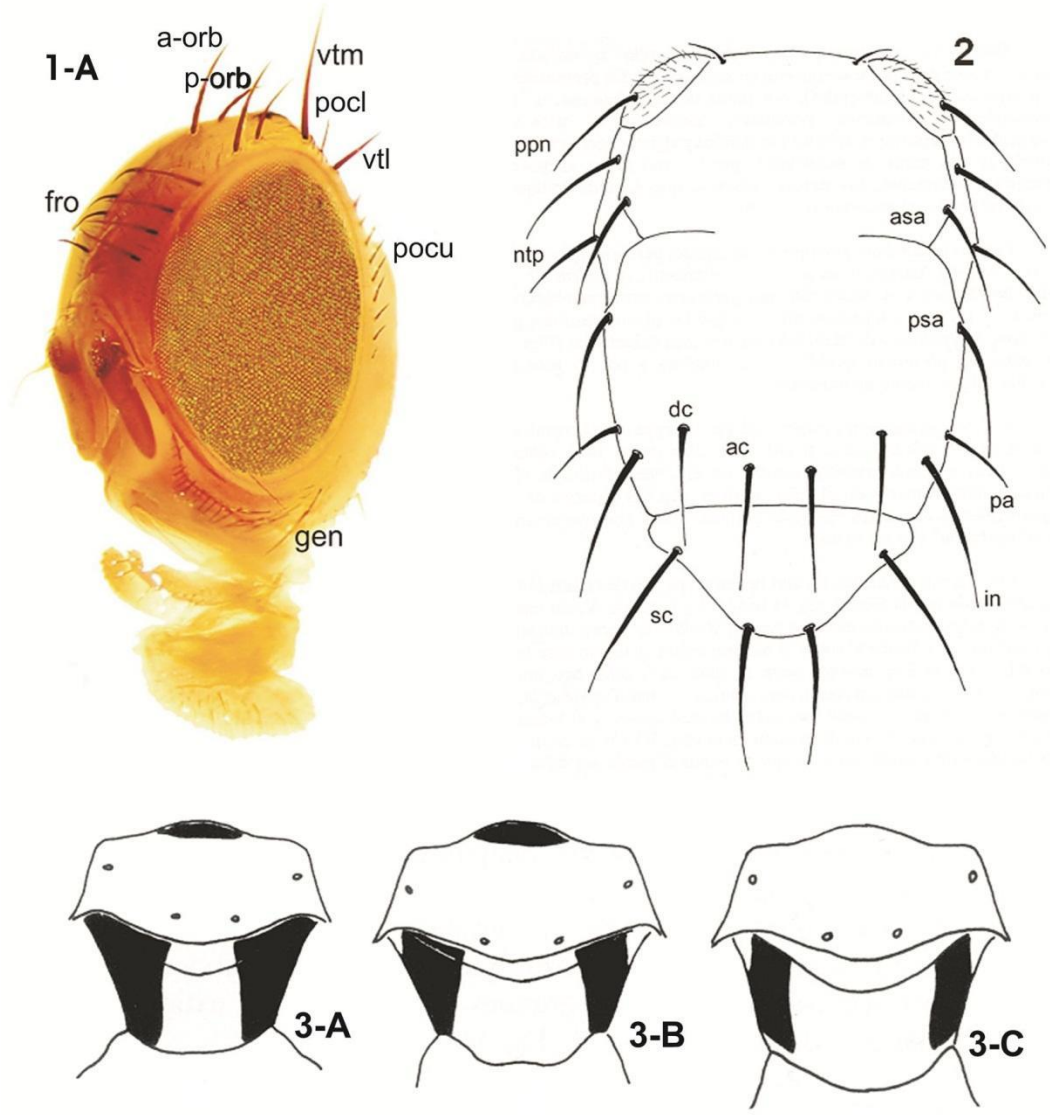
[200] Micrograph courtesy V. Hernández-Ortiz.

[201]

[202] **Figures 1(A)–3. (1A)** Morphology of the head of *Anastrepha* species, fronto-lateral view. *a-orb,* anterior orbital setae; *fro,* frontal setae; *gen,* gena; *pocl,* postocellar setae; *pocu,* postocular setae; *p-orb,* posterior orbital seta; *vtl,* vertical lateral seta; *vtm,* vertical medial seta. **(2)** Thorax dorsal view and chaetotaxy. *ac,* acrostichal; *asa,* supra-alar presutural; *dc,* dorsocentral; *in,* intra-alar; *ntp,* notopleurals; *pa,* postalar; *ppn,* postpronotal; *psa,* supra-alar postsutural; *sc,* scutellars. **(3)** Mediotergite and subscutellum, postero-dorsal view: (A) *A. fraterculus*; (B) *A. ludens*; (C) *A. obliqua.*

[203] Source: Figure 1 adapted from Hernández-Ortiz *et al.* (2010); Figures 2 and 3 adapted from Hernández-Ortiz (1992).

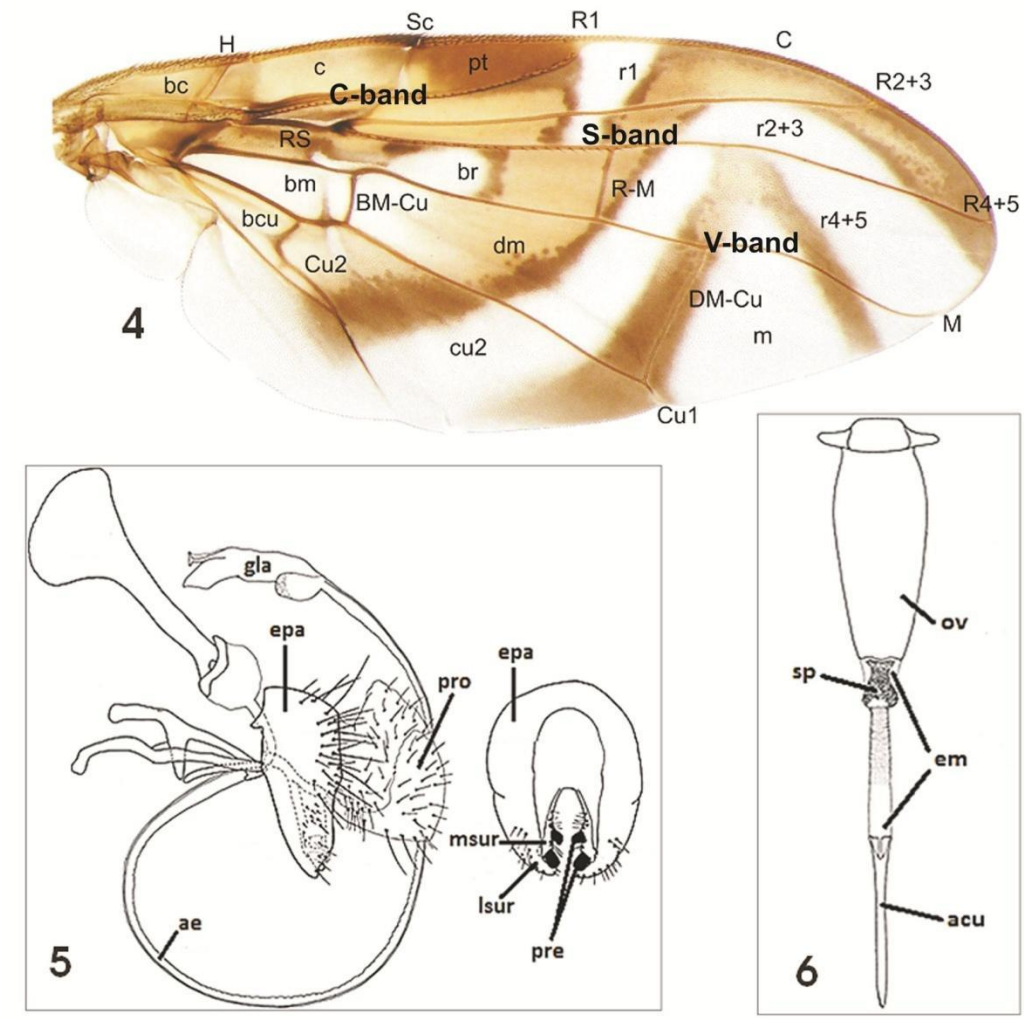
[204]




[205] **Figures 4–6. (4)** Wing pattern of *Anastrepha* and nomenclature of veins and cells (dorsal view). **(5)** Male terminalia in *Anastrepha* species. *ae,* aedeagus; *epa,* epandrium; *gla,* glans; *lsur,* lateral surstylus; *msur,* medial surstylus; *pre,* prensisetae; *pro,* proctiger. **(6)** Female terminalia in *Anastrepha* species. *acu,* aculeus; *em,* eversible membrane; *ov,* oviscape; *sp,* sclerotized plates (rasper).

[206] Source: Figure 4 adapted from Hernández-Ortiz *et al.* (2010); Figures 5 and 6 adapted from Norrbom *et al.* (2012).

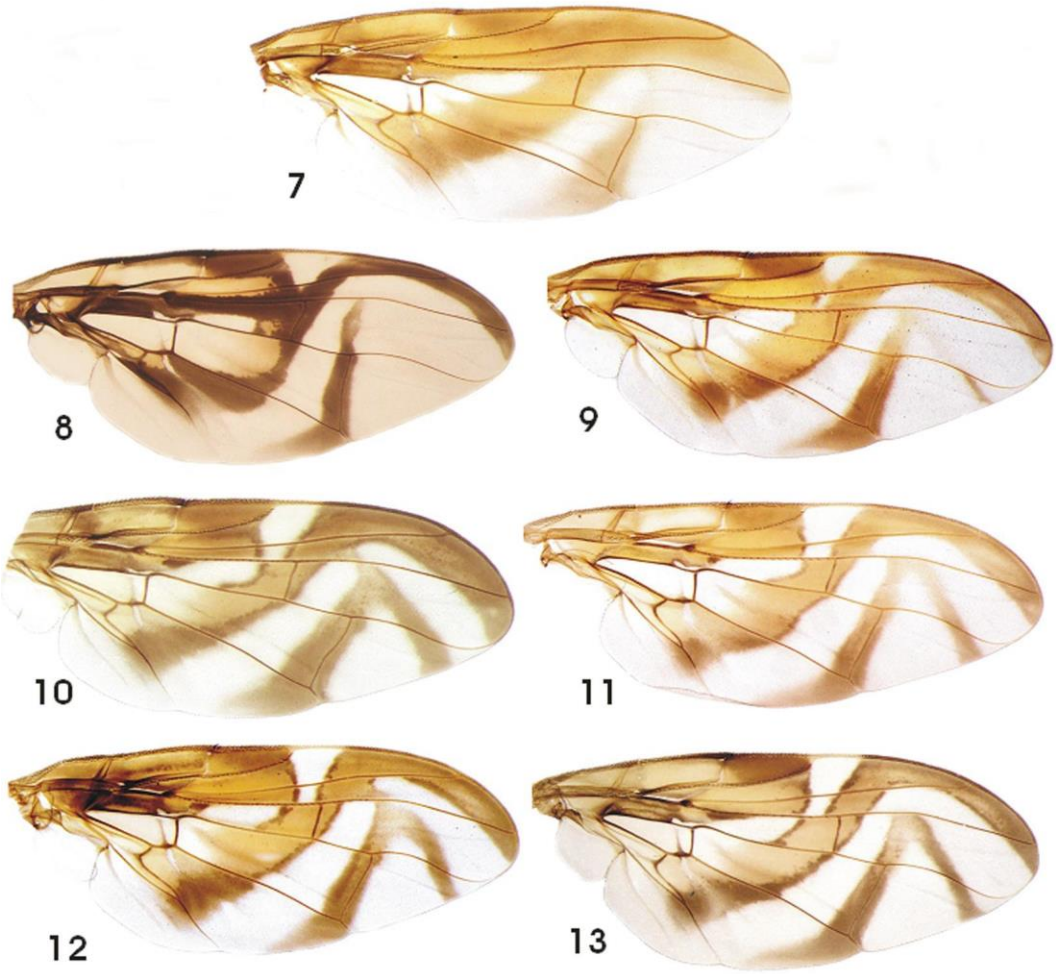
[207]




[208] **Figures 7–13.** Wing pattern of *Anastrepha* species: **(7)** *A. grandis*; **(8)** *A. serpentina*; **(9)** *A. striata*; **(10)** *A. suspensa*; **(11)** *A. ludens*; **(12)** *A. obliqua*; **(13)** *A. fraterculus* (Brazil).

[209] Source: All figures adapted from Hernández-Ortiz *et al.* (2010).

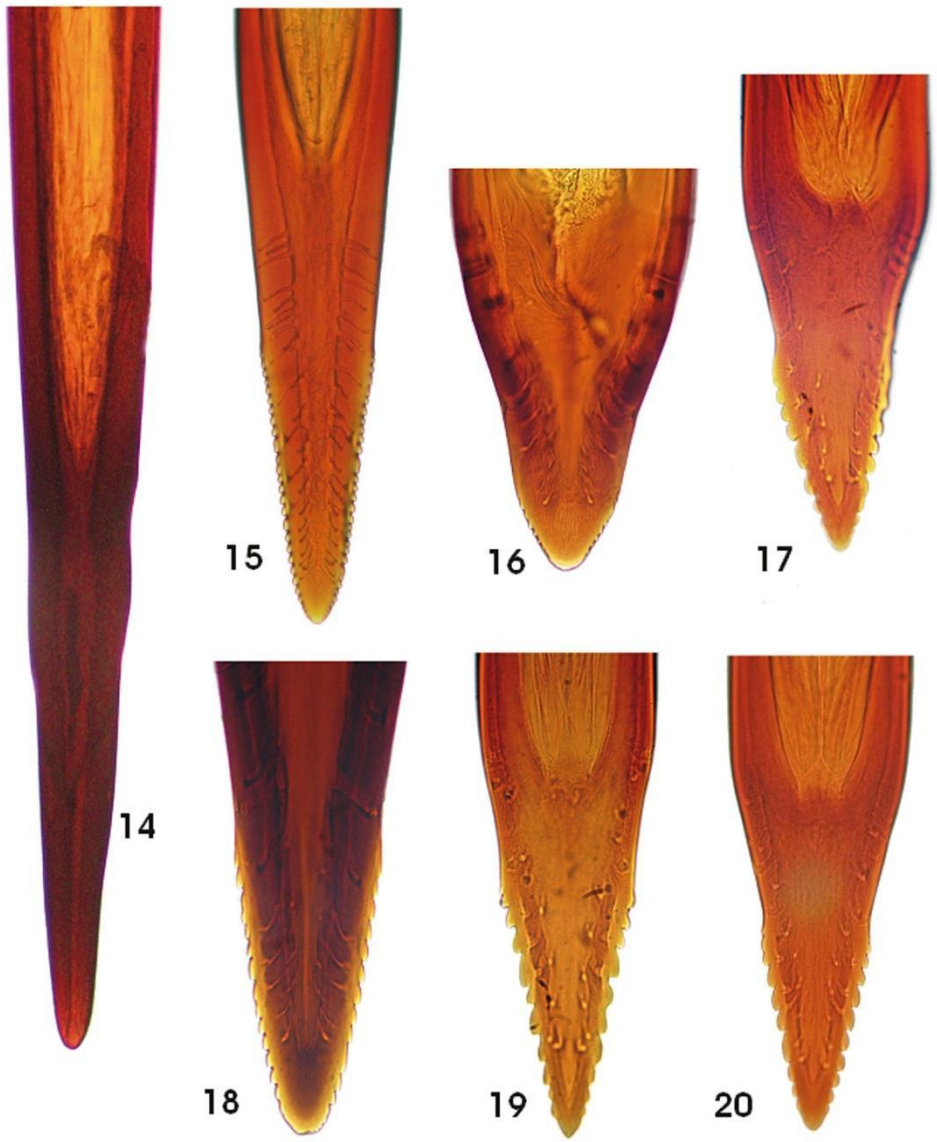
[210]




[211] **Figures 14–20.** Morphology of the aculeus tip in *Anastrepha* species of major economic significance: **(14)** *A. grandis*; **(15)** *A. serpentina*; **(16)** *A. striata*; **(17)** *A. suspensa*; **(18)** *A. ludens*; **(19)** *A. obliqua*; **(20)** *A. fraterculus* (Brazil).

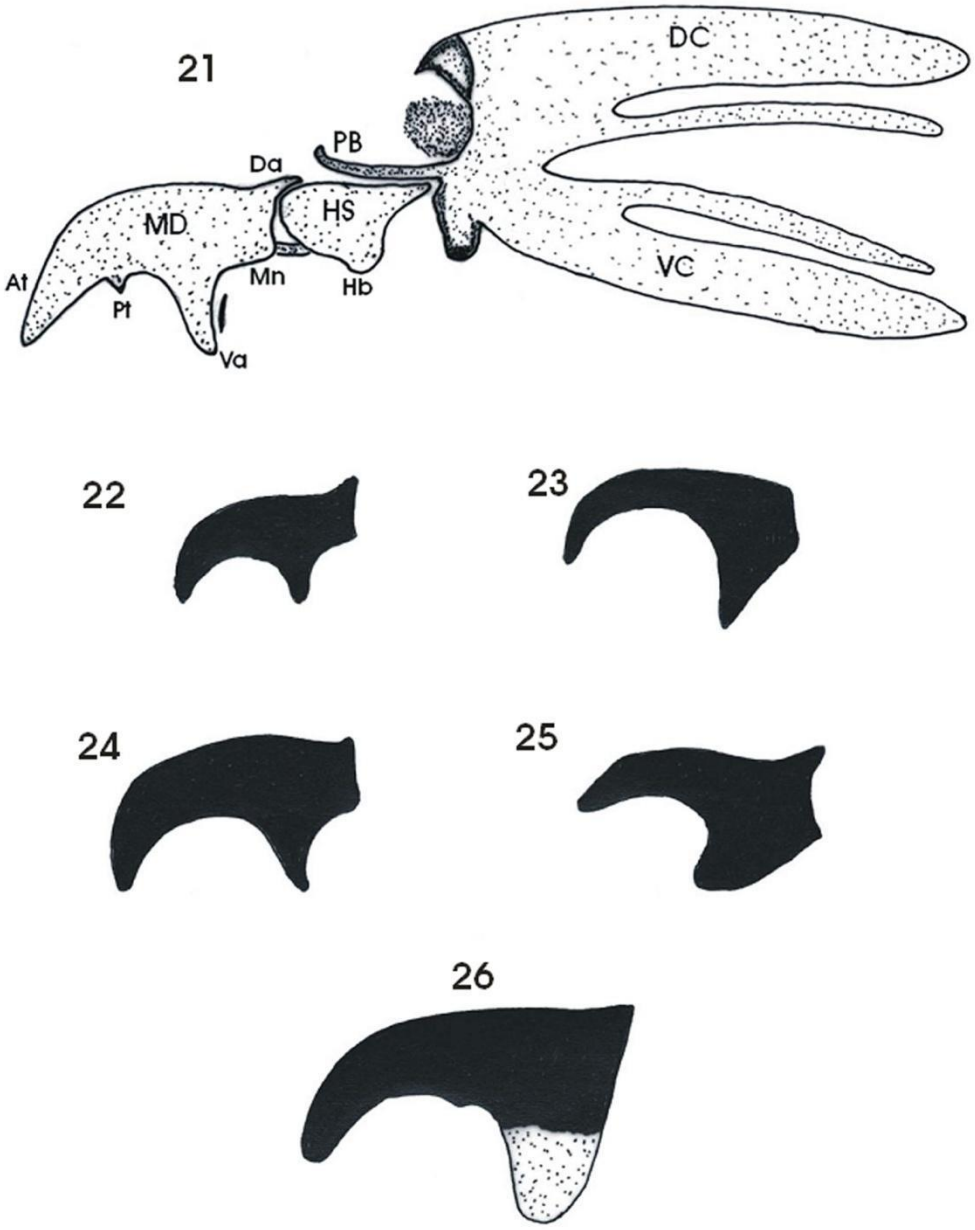
[212] Source: All figures adapted from Hernández-Ortiz *et al.* (2010).

*[213]*




*[214]* **Figures 21–26. (21)** Morphology of the cephalopharyngeal skeleton of third instar larvae. Mandible hook of third instar larvae, lateral view: **(22)** *Ceratitis capitata*; **(23)** *Anastrepha obliqua*; **(24)** *Bactrocera dorsalis*; **(25)** *Rhagoletis tomatis*; **(26)** *Toxotrypana* sp. *At,* apical tooth; *DC,* dorsal cornu; *Hb,* hypopharyngeal bridge; *HS,* hypopharyngeal sclerite; *MD,* mandible; *Mn,* mandibular neck; *PB,* parastomal bar; *Pt,* preapical tooth; *Va,* ventral Apodeme; *VC,* ventral cornu.

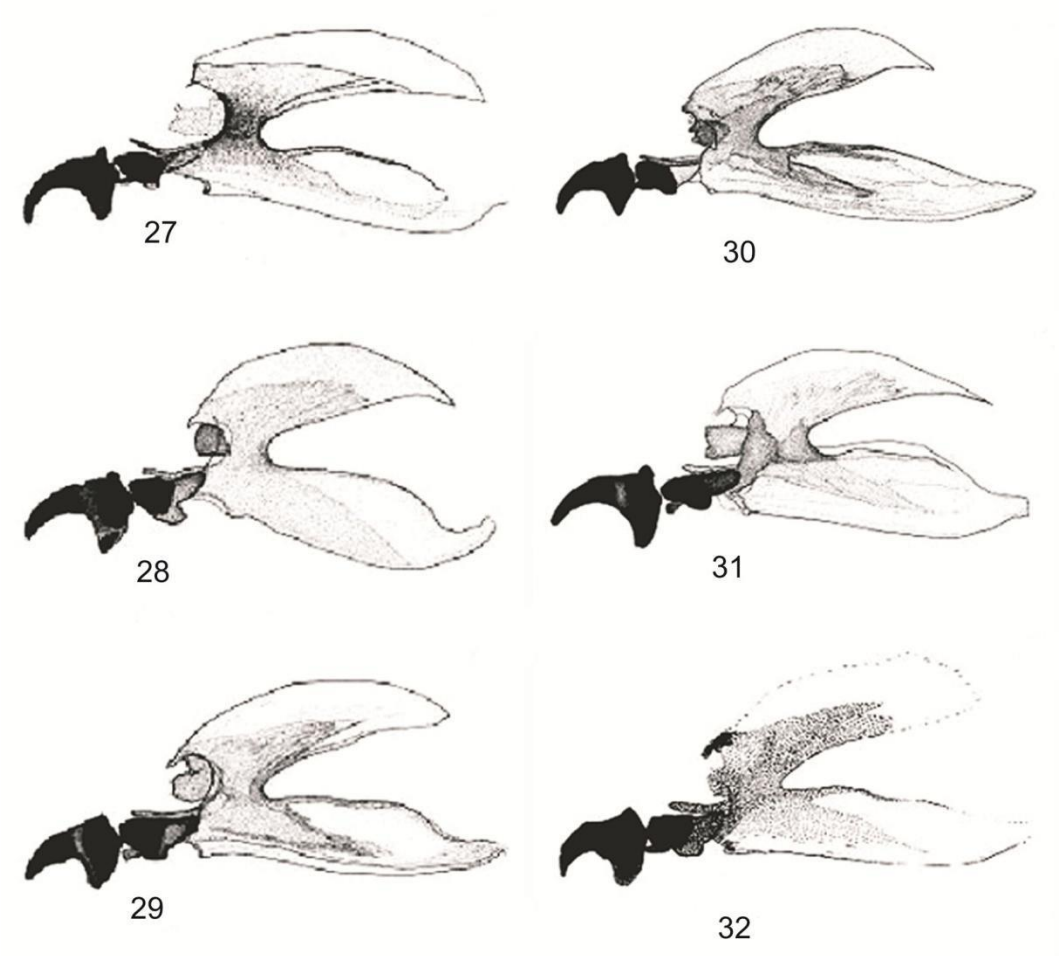
Source: All figures adapted from Frías *et al.* (2006).




*[215]* **Figures 27–32.** Cephalopharyngeal skeleton of third instar larvae of *Anastrepha* species: **(27)** *A. ludens*; **(28)** *A. obliqua*; **(29)** *A. suspensa*; **(30)** *A. serpentina*; **(31)** *A. striata*; **(32)** *A. grandis*.

*[216]* Source: All figures adapted from Carroll *et al.* (2004).

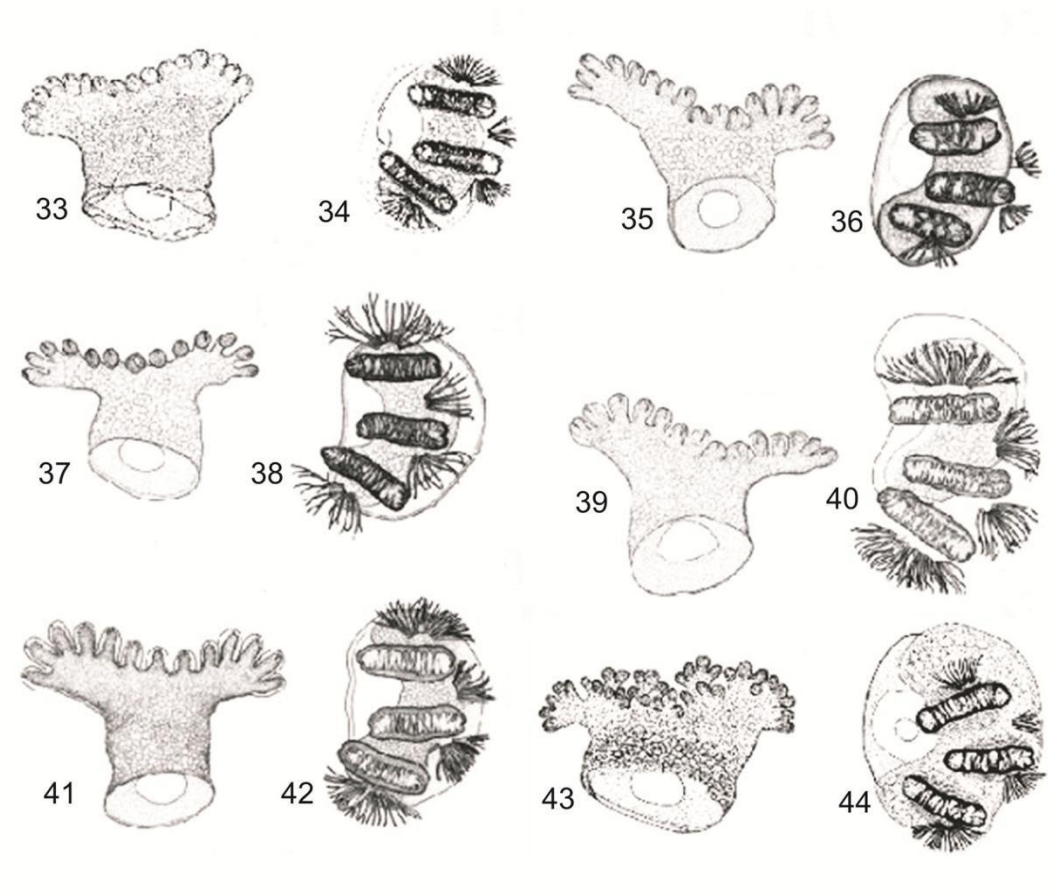
*[217]*




*[218]* **Figures 33–44.** Anterior and posterior spiracles of third instar larvae of *Anastrepha* species: **(33, 34)** *A. ludens*; **(35, 36)** *A. serpentina*; **(37, 38)** *A. obliqua*; **(39, 40)** *A. striata*; **(41, 42)** *A. suspensa*; **(43, 44)** *A. grandis.*

*[219]* Source: All figures adapted from Carroll *et al.* (2004).

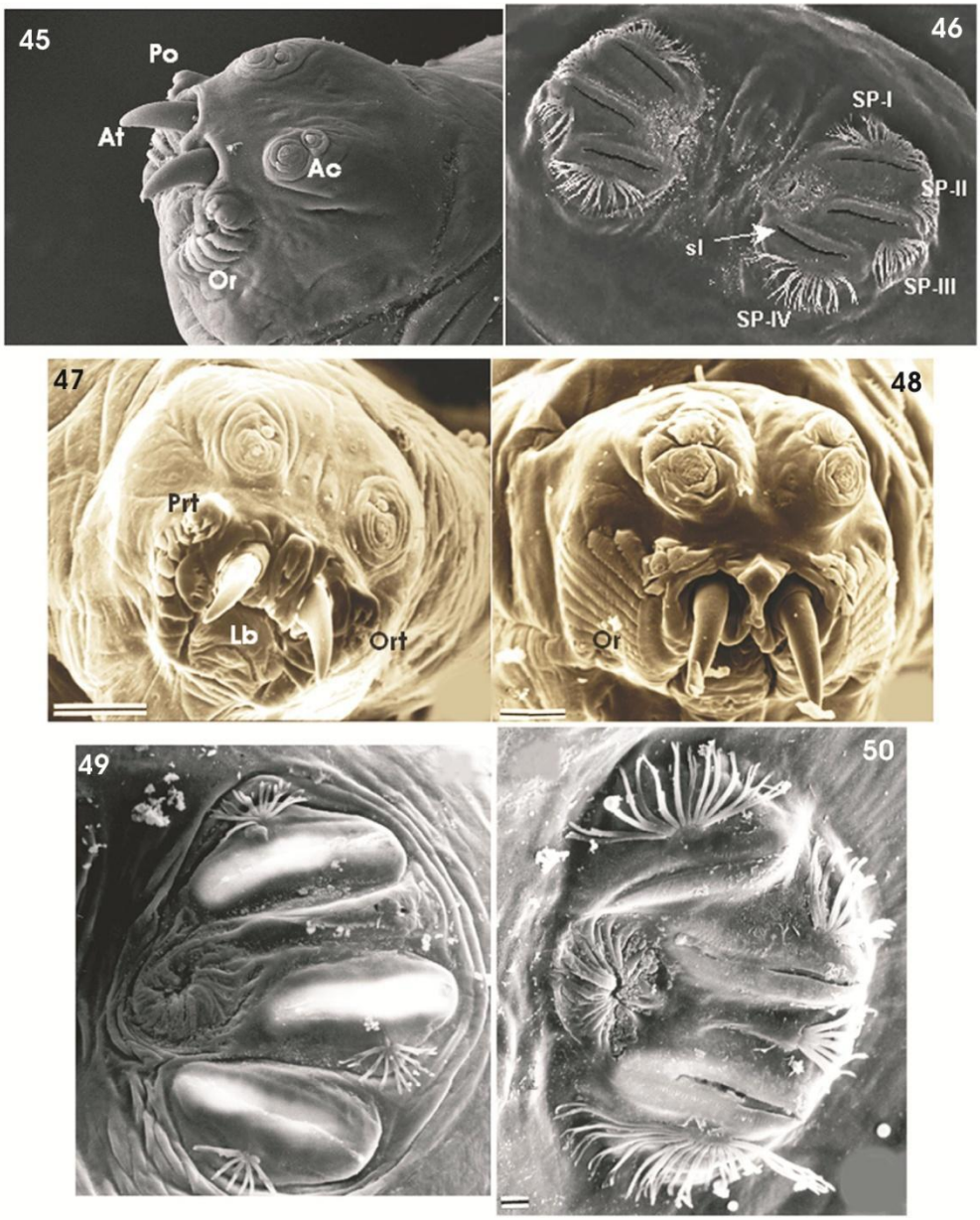
*[220]*




*[221]* **Figures 45–50. (45, 47, 48)** Cephalic segment of third instar larvae. **(46, 49, 50)** Spiracular plates of caudal segment. **(45)** *Rhagoletis sp.* **(46)** *Anastrepha fraterculus.* **(47)** *Rhagoletis brncici.* **(48)** *Ceratitis capitata.* **(49)** *Toxotrypana* sp. **(50)** *Anastrepha obliqua*. *Ac,* anteno-maxillary complex; *At,* apical tooth; *Lb,* labium; *Or,* oral ridges; *Ort,* oral teeth; *Po,* preoral organ; *Prt,* preoral teeth; *sl,* spiracular slits. Spiracular processes (=spiracular hairs): *SP-I* dorsal, *SP-II* and *SP-III* medials, *SP-IV* posterior.

*[222]* Source: Figures 45 and 47–50 adapted from Frías *et al.* (2006); Figure 46 adapted from Hernández-Ortiz *et al.* (2010).

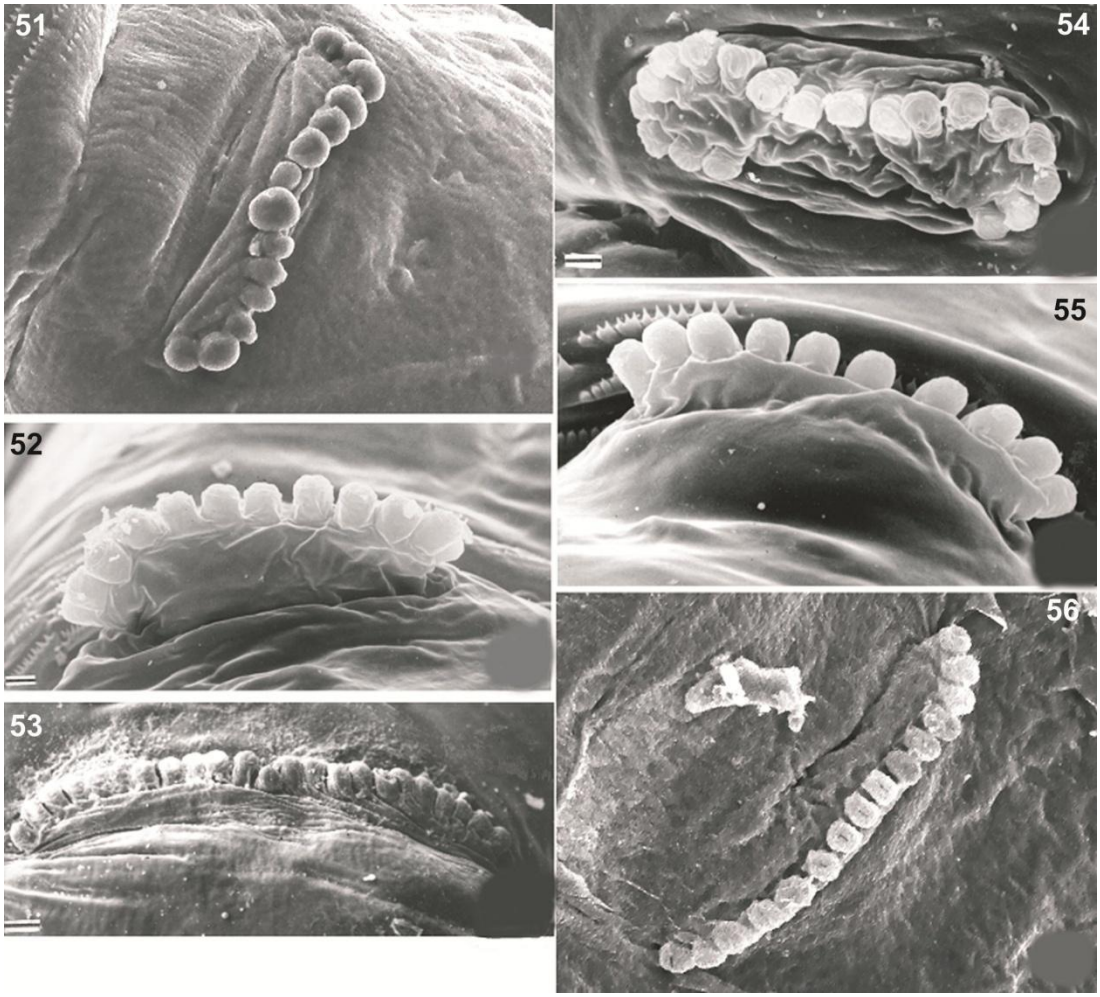
[223]




[224] **Figures 51–56.** Anterior spiracles of the first thoracic segment, third instar larvae: **(51)** *Anastrepha ludens*; **(52)** *Anastrepha fraterculus*; **(53)** *Toxotrypana curvicauda*; **(54)** *Rhagoletis conversa*; **(55)** *Ceratitis capitata*; **(56)** *Bactrocera cucurbitae*.

[225] Source: Figures 52–55 adapted from Frías *et al.* (2006); Figures 51 and 56 adapted from Hernández-Ortiz *et al.* (2010).

[226]




[227] **Figures 57–61. (57)** Anal lobes bifids, *Anastrepha striata*; **(58)** Anal lobes entire, *Anastrepha obliqua*; **(59)** caudal ridges absent, *Anastrepha suspensa*; **(60)** caudal ridges present, *Bactrocera carambolae*; **(61)** *Anastrepha striata*, dorsal view of third instar larva showing rows of dorsal spinules.

[228] Micrographs courtesy G. Steck.

*[229]*