

INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

ISPM 27 DIAGNOSTIC PROTOCOLS

DP 9: Genus *Anastrepha* Schiner

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Contents

1. Pest Information	DP 9-2
2. Taxonomic Information	DP 9-3
3. Detection	DP 9-4
3.1 Inspection of fruits	DP 9-4
3.2 Inspection of traps	DP 9-4
4. Identification	DP 9-4
4.1 Preparation of adults for identification	DP 9-5
4.1.1 Rearing larvae to obtain adults	DP 9-5
4.1.2 Preparation of adults for microscopic examination	DP 9-5
4.2 Preparation of larvae for identification	DP 9-6
4.2.1 Storing the biological sample	DP 9-6
4.2.2 Preparation of larvae for microscopic examination	DP 9-6
4.3 Morphological identification of adults	DP 9-7
4.3.1 Identification of the genus <i>Anastrepha</i> Loew	DP 9-7
4.3.2 Key to adults of major economically important species of the genus <i>Anastrepha</i>	DP 9-7
4.4 Morphological identification of third instar larvae	DP 9-8
4.4.1 Key to third instar larvae of major economically important genera of Tephritidae in the Americas	DP 9-8
4.4.2 Key to third instar larvae of major economically important species of the genus <i>Anastrepha</i>	DP 9-9
5. Records	DP 9-15
6. Contact Points for Further Information	DP 9-15
7. Acknowledgements	DP 9-16
8. References	DP 9-16
9. Figures	DP 9-20

1. Pest Information

The family Tephritidae comprises about 4 450 species in 500 or so genera (Norrbon *et al.*, 1999a, 1999b; Norrbom, 2004b) (the figure was about 4 700 species in 2014 (A.L. Norrbom, personal communication, 2014)). 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 (Hernández-Ortiz, 1992; Foote *et al.*, 1993; Hernández-Ortiz and Aluja, 1993; Norrbom, 2004b; Norrbom *et al.*, 2012). At least seven 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. These seven species are *A. fraterculus* (Wiedemann); *A. grandis* (Macquart); *A. ludens* (Loew); *A. obliqua* (Macquart); *A. serpentina* (Wiedemann); *A. striata* Schiner; and *A. suspensa* (Loew). *A. fraterculus* (Wiedemann) has been recognized as a cryptic species complex (Hernández-Ortiz *et al.*, 2004, 2005; Selivon *et al.*, 2004, 2005; Vera *et al.*, 2006, Cáceres *et al.*, 2009). This diagnostic protocol for *Anastrepha* covers morphological identification of the genus and the species of major economic importance. For further general information about species of Tephritidae, see Norrbom (2010).

The length of the tephritid life cycle varies according to each species 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 (Pankopy and Rosenberg, 1984).

The relationship between *Anastrepha* species and their host plants is poorly understood. There are more than 330 host species from 48 families, however, most of them reported for a few generalist *Anastrepha* species (Norrbon and Kim, 1988; Norrbom, 2004a) 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).

The introduction of 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 reflective of their original host relationships. In this regard, the species *A. suspensa*, *A. fraterculus* and *A. striata* 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 (Norrbon, 2004a).

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 for the *dentata*, *leptozona*, *serpentina*, *daciformis*, *robusta* and *cryptostrepha* species groups. Myrtaceous fruits are also very important hosts. Almost 26 *Anastrepha* species, most belonging to the *A. fraterculus* species complex, have been reported feeding on plants of this family (Norrbon and Kim, 1988; Norrbom *et al.*, 1999c).

2. Taxonomic Information

Name: *Anastrepha* Schiner, 1868

Synonyms: *Acrotoxa* Loew, 1873; *Pseudodacus* Hendel, 1914; *Phobema* Aldrich, 1925; *Lucumaphila* Stone, 1939

Taxonomic position: Insecta: Diptera: Tephritidae, Trypetinae, Toxotrypanini

Common names: See Table 1.

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

Common name	<i>Anastrepha</i> species	Synonyms
South American fruit fly	<i>Anastrepha fraterculus</i> (Wiedemann, 1830) species complex	<i>Tephritis mellea</i> Walker, 1837
		<i>Trypeta unicolor</i> Loew, 1862
		<i>Anthomyia frutalis</i> Weyenbergh, 1874
		<i>Anastrepha fraterculus</i> var. <i>ligata</i> Bezzi, 1934
		<i>Anastrepha peruviana</i> Townsend, 1913
		<i>Anastrepha brazilensis</i> Greene, 1934
		<i>Anastrepha costaricensis</i> Capoor, 1954
		<i>Anastrepha schorgeri</i> Capoor, 1955
		<i>Anastrepha pseudofraterculus</i> Capoor, 1955
		<i>Anastrepha lambertae</i> Korytkowski and Ojeda, 1968
Melon fruit fly	<i>Anastrepha grandis</i> (Macquart, 1846)	<i>Anastrepha solmeri</i> Hendel, 1914
		<i>Anastrepha latifasciata</i> Hering, 1935
Mexican fruit fly	<i>Anastrepha ludens</i> (Loew, 1862)	<i>Anastrepha lathana</i> Stone, 1942
West Indian fruit fly	<i>Anastrepha obliqua</i> (Macquart, 1835)	<i>Anastrepha fraterculus</i> var. <i>mombinpraeoptans</i> Sein, 1933
		<i>Anastrepha fraterculus</i> var. <i>ligata</i> Lima, 1934
		<i>Anastrepha trinidadensis</i> Greene, 1934
Sapodilla fruit fly	<i>Anastrepha serpentina</i> (Wiedemann, 1830)	<i>Urophora vittithorax</i> Macquart, 1851
Guava fruit fly	<i>Anastrepha striata</i> Schiner, 1868	<i>Dictya cancellaria</i> Fabricius, 1805 (see Norrbom <i>et al.</i> , 1999b)
Caribbean fruit fly	<i>Anastrepha suspensa</i> (Loew, 1862)	<i>Anastrepha unipuncta</i> Sein, 1933
		<i>Anastrepha longimacula</i> Greene, 1934

3. Detection

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.

3.1 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 visual examination should be done under a microscope by an expert. 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 ovipositing female flies should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits.

3.2 Inspection of traps.

Guidance on trapping *Anastrepha* fruit flies is given in Appendix 1 of ISPM 26 (*Establishment of pest free areas for fruit flies (Tephritidae)*). 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 applied internationally (e.g. ISPM 26). The specific methods of trap deployment and time of service of the traps must be in agreement with the national phytosanitary regulations.

4. Identification

The taxonomy of the genus *Anastrepha* is based on adult external morphology and characters of the female terminalia (Stone, 1942; Hernández-Ortiz, 1992; Zucchi, 2000; Norrbom *et al.*, 2012). 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 (Steck and Wharton, 1988; Steck *et al.*, 1990; Frías *et al.*, 2006, 2008, 2009; Dutra *et al.*, 2011a, 2011b, 2012, 2013; Figueira *et al.*, 2011). Identification keys for the larvae of the seven species of *Anastrepha* known to be of major economic importance (section 1; listed in Table 1) are available (Steck *et al.*, 1990; Carroll *et al.*, 2004) but should be used with consideration of their limits.

Although the third instar larvae of some *Anastrepha* species apparently can be discriminated (Berg, 1979; Steck and Wharton, 1988; Carroll and Wharton, 1989; Steck *et al.*, 1990; White and Elson-Harris, 1992; Carroll *et al.*, 2004; Frías *et al.*, 2006; Hernández-Ortiz *et al.*, 2010), 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.

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).

To study this idea further, the International Atomic Energy Agency (IAEA) has coordinated an international research project to describe the cryptic species in the *A. fraterculus* species complex. As part of this project, molecular methods have been examined for diagnostic utility within the genus. Based on available data, methods such as DNA barcoding using the *cytochrome oxidase I* gene cannot

reliably identify some dipteran species, including several important pest species (Will *et al.*, 2005; Meier *et al.*, 2006; Virgilio *et al.*, 2010; Lopes *et al.*, 2013). Some progress has been made by internal transcribed spacer (ITS)1 analysis (e.g. Sonvico *et al.*, 2004, GenBank accession number AY686689). This information was associated with morphological characterization of specimens and karyotypic analysis, along with cross-mating studies (Basso, 2003).

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

4.1 Preparation of adults for identification

4.1.1 Rearing larvae to obtain adults

The fruits 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 (96% ethanol for molecular studies of DNA) 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 abdomen with forceps, then squeeze the base and apex of the ovipositor to expose the aculeus tip (so that it does not need to be dissected later).

4.1.2 Preparation of adults for microscopic examination

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.

4.1.2.1 Aculeus

It is preferable to cut off the whole abdomen from a female to dissect the ovipositor (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 10% sodium hydroxide (NaOH) or 10% potassium hydroxide (KOH) solution and heating 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.

4.1.2.2 Wings

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).

4.2 Preparation of larvae for identification

4.2.1 Handling the biological sample

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).

Morphological examination of larvae (section 4.2.2) can be performed on unmounted larvae using a stereomicroscope, on slide-mounted larvae using a compound 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.

4.2.2 Preparation of larvae for microscopic examination

To prepare specimens for examination the larvae must be treated in hot water, which can be accomplished by placing live larvae in water at 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.

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 or 10% KOH 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).

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 lavender oil for 15 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.

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).

4.3 Morphological identification of adults

4.3.1 Identification of the genus *Anastrepha* Loew

Adults (Figure 1). Head (Figure 2-A): 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-B, 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.

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 postcostal margin, and continuing to apex of wing; 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 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 in section 4.3.2).

Male terminalia (Figure 5): Epandrium broad in lateral view, lateral surstylus short or elongated; medial surstylus shorter than lateral surstylus with two or three blackish prenisetae apically; proctiger membranous, weakly sclerotized laterally and ventrally; aedeagus elongated, usually longer than length of oviscapae of female; glans weakly sclerotized with a small apical T-shaped sclerite, glans sometimes absent in non-pest species.

Female terminalia (Figure 6): Oviscapae tube-like, variable in length; eversible membrane (usually inverted inside oviscapae) apically with dorsal group of hook-like sclerotized plates (also named rasps); aculeus (usually inverted inside eversible membrane and oviscapae) well sclerotized, tip sometimes serrated on lateral margin.

4.3.2 Key to adults of major economically important species of the genus *Anastrepha*

Key adapted from Fernández-Ortiz *et al.* (2010). For additional information on morphological structures and other *Anastrepha* species, see Norrbom *et al.* (2012). See Tables 2 and 4 for diagnostic morphological characters of the genus *Anastrepha*.

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 **2**
 - 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.)..... ***Anastrepha grandis* (Macquart)**
2. Scutum predominantly dark brown with brown to black stripes **3**
 - Scutum yellow or orange, without dark brown markings except sometimes along scuto-scutellar suture **4**

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.) *Anastrepha serpentina* (Wiedemann)

– 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.) *Anastrepha striata* Schiner

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.....5

– 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, tip 0.19–0.22 mm long, 0.10–0.13 mm wide, lateral margins serrate on distal 0.50–0.65 (Figure 17). *Anastrepha suspensa* (Loew)

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 variable.....6

– Female aculeus length more than 2.5 mm (usually 3.0–5.8 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.) *Anastrepha ludens* (Loew)

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.) *Anastrepha obliqua* (Macquart)

– 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 to 14 teeth on distal two-fifths to three-fifths (Figure 20); wing pattern variable (Figure 13). *Anastrepha fraterculus* (Wiedemann) species complex

4.4 Morphological identification of third instar larvae

4.4.1 Key to third instar larvae of major economically important genera of Tephritidae in the Americas

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

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). *Toxotrypana Gerstaecker*

- 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) **2**
- 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..... ***Rhagoletis* Loew**
- 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. **3**
- 3. Posterior region of mandible without distinct neck (Figure 23). Caudal ridge lacking (Figure 59)..... ***Anastrepha* Schiner**
- Posterior region of mandible with distinct neck (Figures 22, 24). Caudal ridge present (Figure 60). **4**
- 4. Oral ridges with shorter round teeth (Figure 48)..... ***Ceratitis* McLeay**
- Oral ridges with long, sharply pointed teeth (not as above)..... ***Bombus* Macquart**

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

Key adapted from Steck *et al.* (1990). See Table 3 for diagnostic morphological characters of third instar larvae of *Anastrepha* species.

Geographic distribution and hosts are quoted only as additional information of the common source of origin for the species.

- 1. Posterior spiracles prominently raised from the body surface; or most body segments with conspicuous setae or processes; or posterior spiracular openings sinuous..... **Not Tephritidae**
- Posterior spiracles nearly flush with body surface; tubercles, if present, on caudal segment only; posterior spiracular slits elongate oval (Figures 49–50) (Tephritidae) **2**
- 2. Prominent chitinous preoral teeth (=somal 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 ridges lacking and caudal sensilla strongly reduced. **Other Tephritidae (not *Anastrepha*)**
- Preoral teeth (=somal guards) lacking, and dental sclerite lacking or inconspicuous (Figure 48); caudal tubercles moderately developed **(*Anastrepha*) 3**
- 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) **4**
- Dorsal spinules absent on all abdominal segments, or if present, only in abdominal segment A1 (some specimens of *A. ludens*)..... **5**
- 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.) ***Anastrepha grandis***
- 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.) .. ***Anastrepha striata***
- 5. Dorsal spinules present on thoracic segment T3 (Figure 61) **6**

- Dorsal spinules absent on thoracic segment T3 (not as above)7
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 in United States to Panama.)*Anastrepha ludens*
- 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 in United States and Antilles.)*Anastrepha suspensa*
7. Posterior spiracular processes SP-I and SP-IV with 5 to 11 short basal trunks (average, 8) (Figure 36); oral ridges usually in 12 to 14 rows; anterior spiracle with 13 to 18 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.)*Anastrepha serpentina*
- Posterior spiracular processes SP-I and SP-IV with 8 to 18 long basal 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)8
8. Posterior spiracular processes SP-II usually with three to six basal 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.)*Anastrepha obliqua*
- Posterior spiracular processes SP-II usually with four to nine basal trunks; posterior spiracular slits 2.5–4.0 times longer than wide (Figure 46). (Distribution: tropical Americas.)*Anastrepha fraterculus* (Weidemann) species complex

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 notopleural, one presutural supra-alar, one postsutural supra-alar, one postalar, one intralar, one dorsal ventral, one acrostichal (rarely absent) and two scutellars
	Wings	Veins: Vein <i>M</i> usually conspicuously curved forwards apically (strongly so in all pest species) and meeting cubita without 90° angle; crossvein <i>r-m</i> placed distal to mid-length of discal cell (<i>dm</i>); basal cubital cell (<i>bcu</i>) with well-developed posteroapical extension
		Wing pattern: C-band on basal costal margin; S-band (from apex of cell <i>bcu</i> across cell <i>m</i> and cell <i>m-cu</i> to <i>r-m</i>); V-band forming an inverted V shape comprising the proximal arm (subapical band) on <i>dm-cu</i> and distal arm (posterior apical band) arising from cell <i>m</i> , both convergent in cell <i>R</i> ₄₊₅
	Male genitalia	Lateral surstylus short or elongate; medial surstylus shorter than lateral surstylus with two prenisetae 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	Ovipositor tube-like, variable in length; eversible membrane apically with dorsal hook-like sclerotized plates (also named rasps); aculeus well sclerotized, length variable, tip sometimes serrated on lateral margins

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

Species	Structure	Description
<i>Anastrepha fraterculus</i> species complex	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
<i>Anastrepha grandis</i>	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
<i>Anastrepha ludens</i>	Oral ridges	11 to 17 rows, marginal 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
<i>Anastrepha obliqua</i>	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
<i>Anastrepha serpentina</i>	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 six to nine short trunks

Species	Structure	Description
	Anal lobes	Usually bilobed (occasionally entire)
<i>Anastrepha striata</i>	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
<i>Anastrepha suspensa</i>	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 T1
	Posterior spiracles	Slits 2.5–3.5 times longer than wide
	Anal lobes	—

Table 4. Diagnostic morphological characters of adults of *Anastrepha* species

Species	Structure	Description
<i>Anastrepha fraterculus</i> species complex	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 <i>M</i> ; 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
<i>Anastrepha grandis</i>	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 <i>b_{cu}</i> through crossvein <i>R</i> and connecting with C-band above; cell <i>r</i> ₂₊₃ completely pigmented in entire length; vein <i>R</i> ₂₊₃ almost straight; cell <i>br</i> broadly hyaline between <i>bm-cu</i> and <i>r-m</i>
	Female genitalia	Aculeus extremely long (5.3–8.2 mm) and usually greater than 0.10 mm wide; aculeus tip with V-shaped ridge; lateral margins non-serrate
<i>Anastrepha ludens</i>	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 weakly serrate on distal 0.55 or less
<i>Anastrepha obliqua</i>	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 <i>M</i> ; 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
<i>Anastrepha serpentina</i>	Head chaetotaxy	Posterior orbital seta present
	Thorax	Thorax mostly brown or red-brown contrasting with yellow markings; scutum

Species	Structure	Description
		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
<i>Anastrepha striata</i>	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 and 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
<i>Anastrepha suspensa</i>	Head chaetotaxy	Posterior orbital seta present
	Thorax	Scuto-scutellar suture with large brown spot medially; mediotergite entirely yellow or with brown markings on sides
	Wings	Anterior apical band (=distal section of the S-band) extremely wide, reaching the apex of vein <i>M</i> ; 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

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

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

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

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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 on Diagnostic Protocols (TPDP).

7. Acknowledgements

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In addition, the following experts were significantly involved in the development of this protocol:

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V. Balmès (Anses, Laboratoire de la santé des végétaux, Unité entomologie et plantes invasives, France).

8. References

The present standard refers to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at <https://www.ippc.int/core-activities/standards-setting/ispms>.

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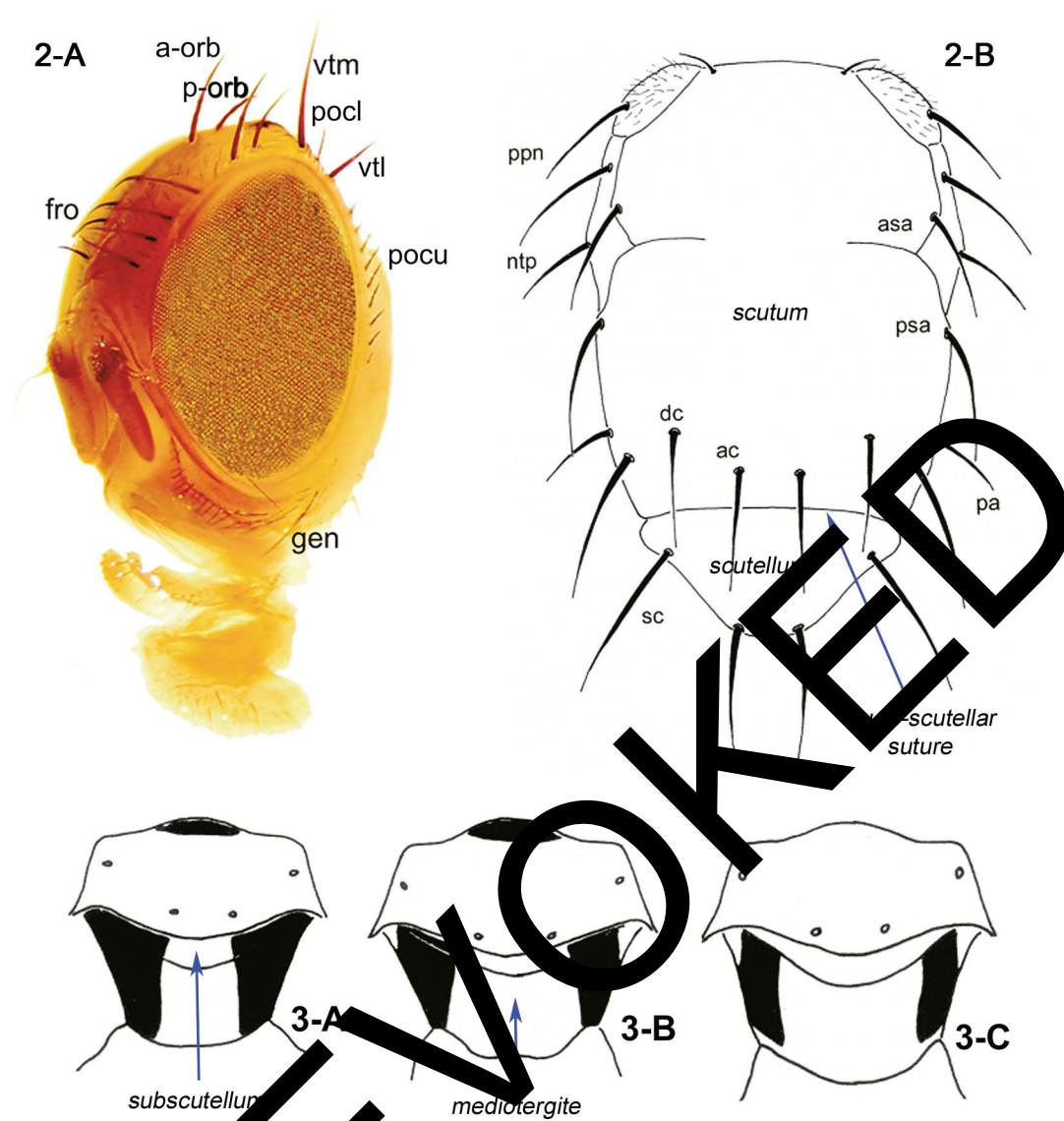
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9. Figures

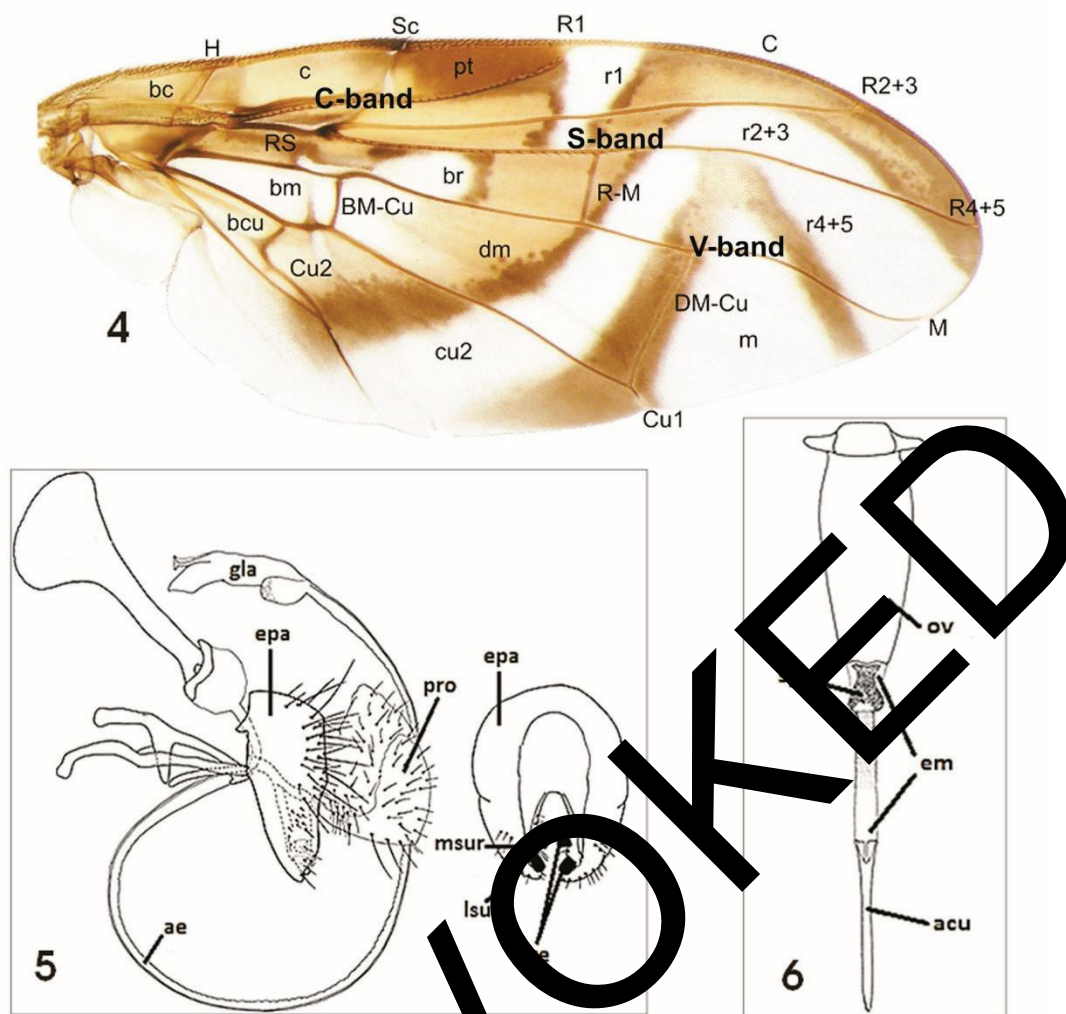


Figure 1. General habits of the adult female of *Anastrepha ludens* (Mexican fruit fly) in dorsal view.
Micrograph courtesy V. Hernández-Ortiz.

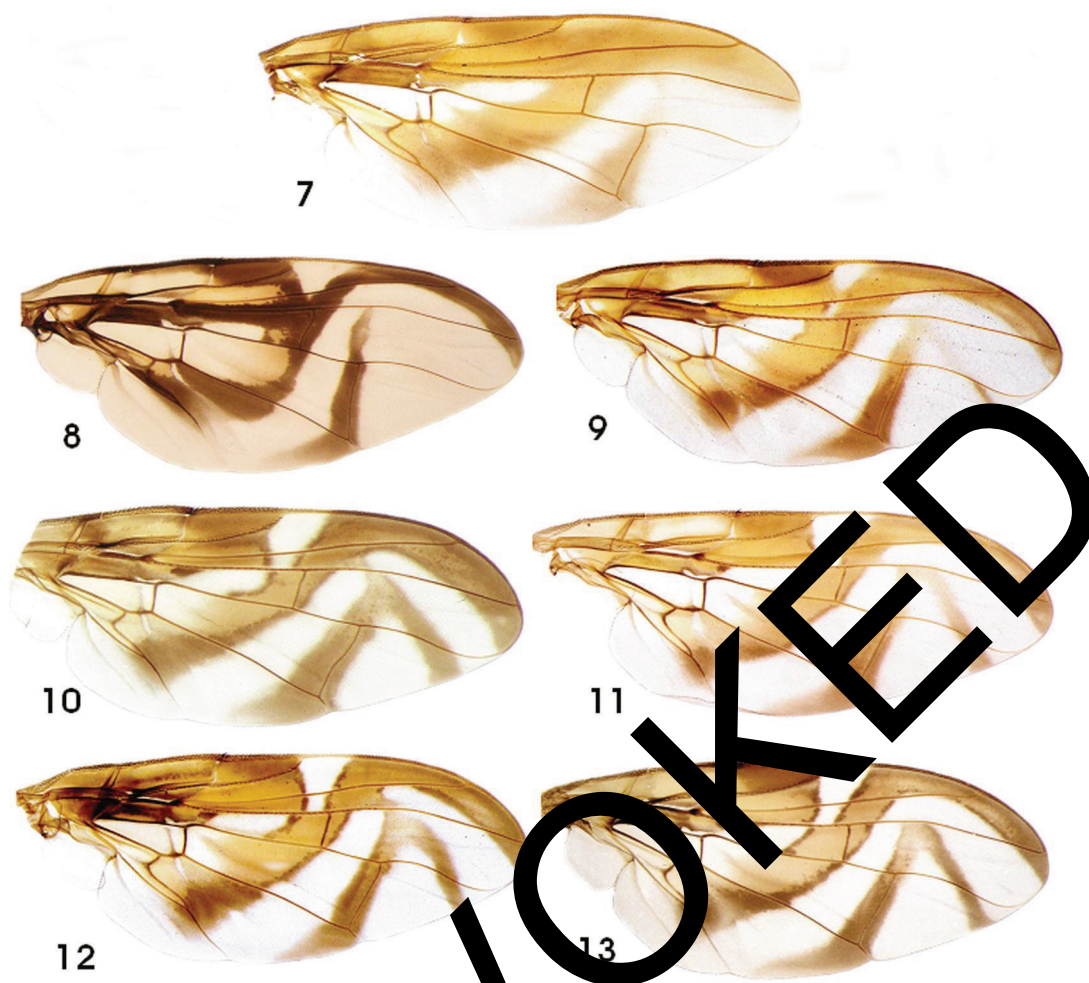


Figures 2–3. (2-A) Morphology of the head of *Anastrepha* species, fronto-lateral view. *a-orb*, anterior orbital setae; *fro*, frontal setae; *gen*, gena; *pocl*, postocellar setae; *pocu*, postocular setae; *p-orb*, posterior orbital seta; *vtl*, vertical lateral seta; *vtm*, vertical medial seta.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

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



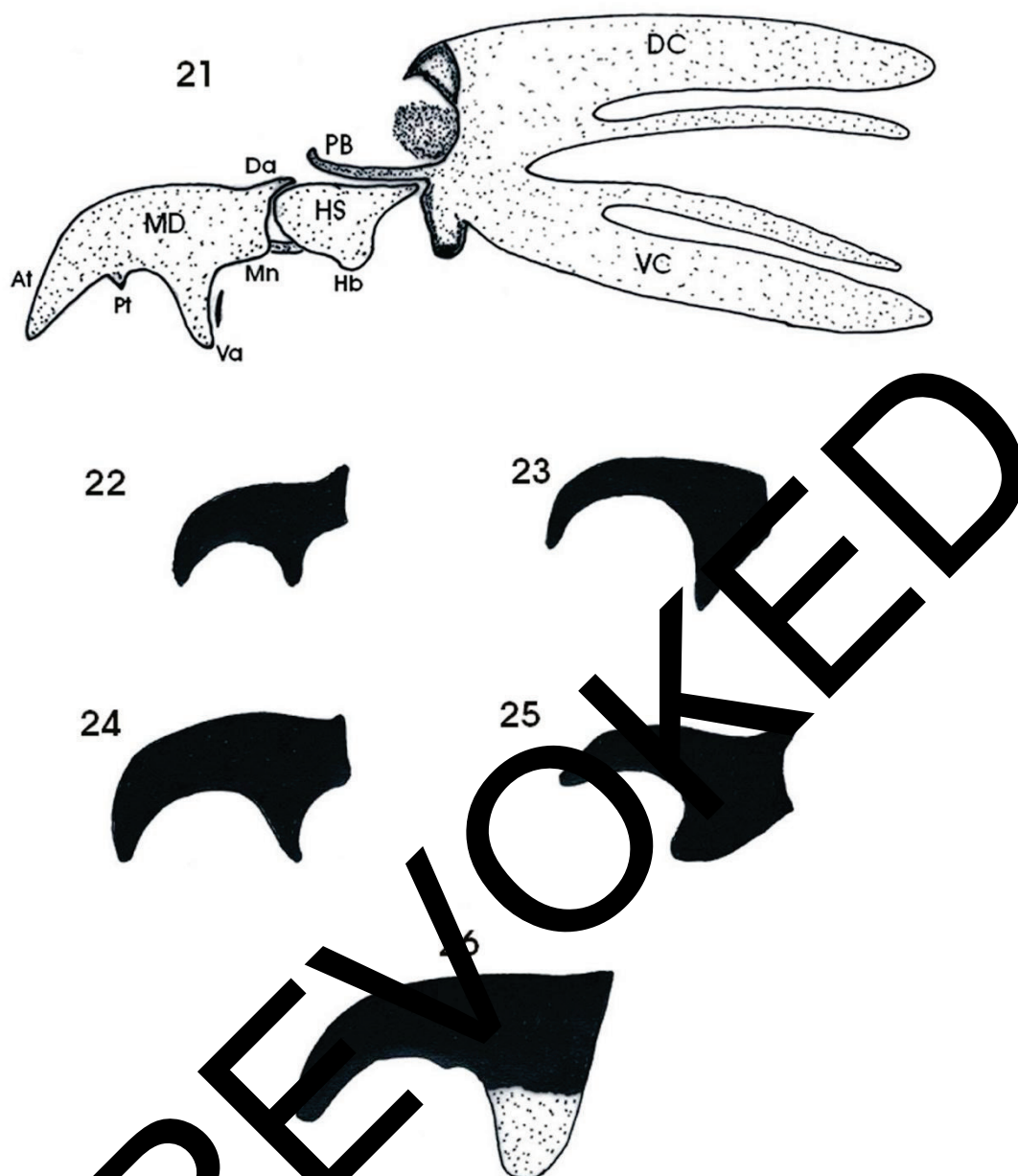
Figures 4–6. (4) Wing pattern of *Anastrepha* and nomenclature of veins and cells (dorsal view). (5) Male terminalia in *Anastrepha* species. ae, aedeagus; epa, epandrium; gla, glans; lsu, lateral surstylus; msur, medial surstylus; pro, proctiger; pt, proctiger. (6) Female terminalia in *Anastrepha* species. acu, aculeus; em, eversible membrane; ov, ovipositor; sp, sclerotized plates (rasper).
Source: Figure 4 adapted from Hernandez-Ortiz et al. (2010); Figures 5 and 6 adapted from Norrbom et al. (2012).



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).
Source: All figures adapted from Hernández-Ortiz et al. (2010).



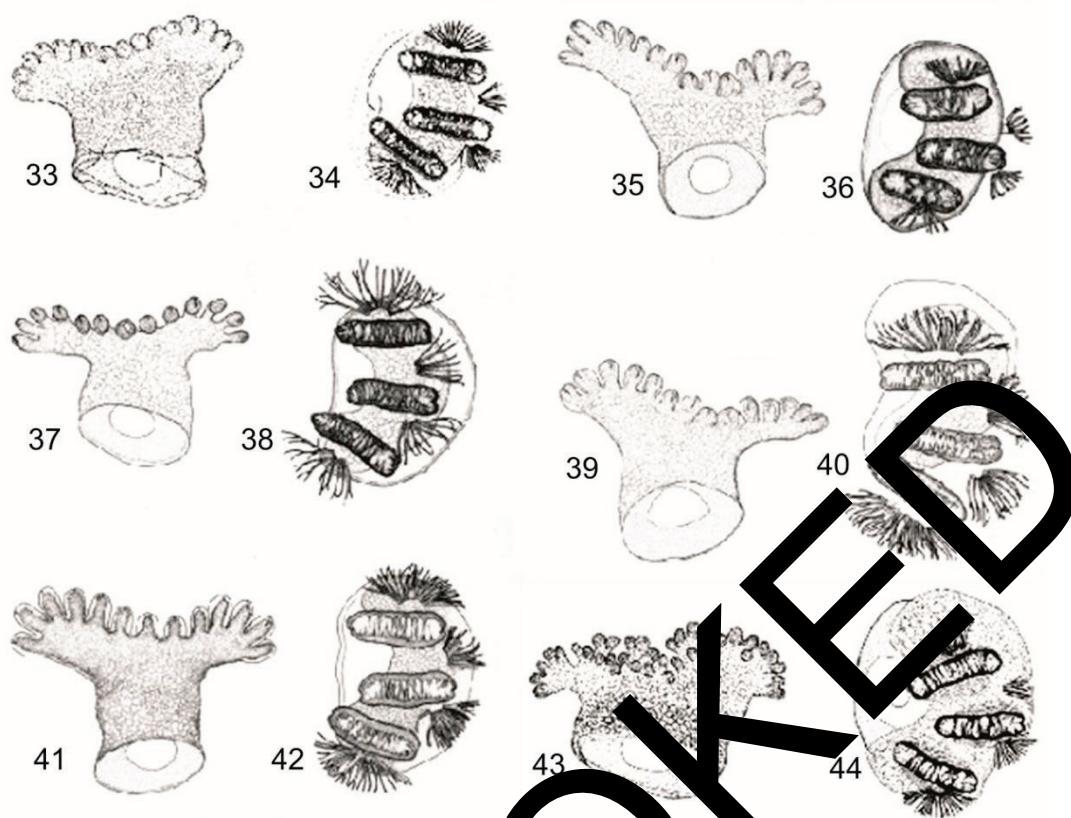
Figures 14-20. Morphology of the aculeus tip in *Anastrepha* species of major economic importance: (14) *A. grandis*; (15) *A. serpentina*; (16) *A. striata*; (17) *A. suspensa*; (18) *A. ludens*; (19) *A. obliqua*; and (20) *A. fraterculus* (Brazil).
Source: All figures adapted from Hernández-Ortiz et al. (2010).



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 torquatus*; and (26) *Toxotrypana* sp. At, apical tooth; DC, dorsal cornu; DS, dental sclerite; 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).



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*; and (32) *A. grandis*.
Source: All figures adapted from Carron et al. (2004).



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*; and (43, 44) *A. grandis*.
 Source: All figures adapted from Carroll et al. (2004).

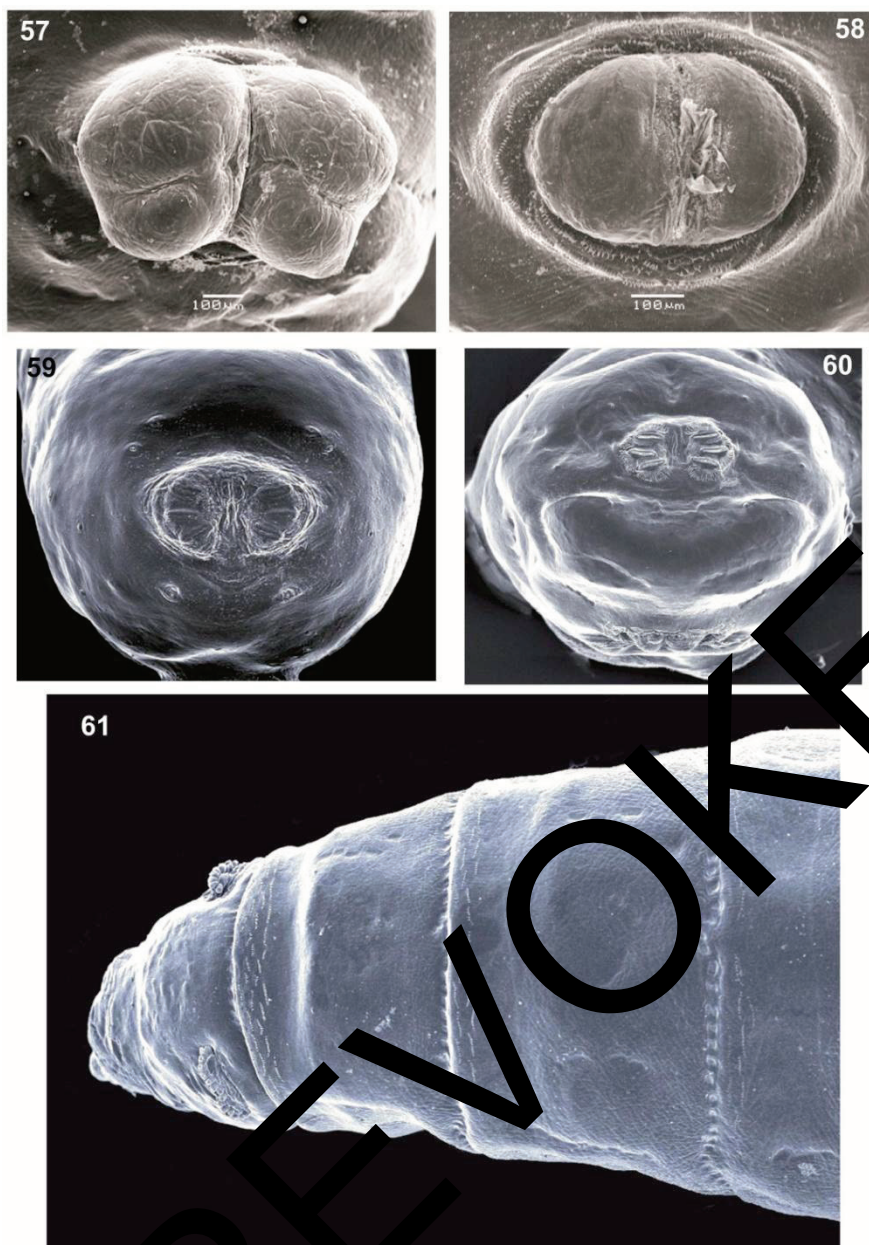


Figures 45–50. (45, 47, 48) Oral segment of third instar larvae. (46, 49, 50) Spiracular plates of caudal segment. (45) *Rhagoletis* sp. (46) *Anastrepha fraterculus*. (47) *Rhagoletis brncici*. (48) *Ceratitidis capitata*. (49) *Toxotrypanid* sp. (50) *Anastrepha obliqua*. Ac, anteno-maxillary complex; 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.
Source: Figures 45 and 47–50 adapted from Frías et al. (2006); Figure 46 adapted from Hernández-Ortiz et al. (2010).



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*; and (56) *Bactrocera cucurbitae*.

Source: Figures 52–55 adapted from Frawley et al. (2006); Figures 51 and 56 adapted from Hernández-Ortiz et al. (2010).



Figures 57-61. (57) anal lobes of *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 the anal lobes showing rows of dorsal spinules. Micrographs courtesy G. Steck.

Publication history

This is not an official part of the standard

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2013-04 Expert consultation

2013-06 Draft presented to TPDP (meeting).

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2015-08 SC adopted DP on behalf of CPM (with no formal objections received).

ISPM 27. Annex 9. Genus *Anastrepha* Schiner (2015). Rome, IPPC, FAO.

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