2023 FIRST CONSULTATION

1 July – 30 September 2023

Compiled comments for 2023 First Consultation: 2021-002_Revision_DP9_Anastrepha - Discipline lead's response

Summary

Participants

Name	Summary
Australia	Comments completed
Barbados	Barbados supports the adoption of this protocol.
European Union	The comments on the draft standard are submitted by the European Commission on behalf of the European Union and its 27 Member States.
Gabon	annexe validée
Malawi	WE SUPPORT DRAFT REVISION OF DP◦9
Singapore	Singapore is supportive of this draft standard.
South Africa	The draft revision should make provision for the premix on molecular techniques Suggest an additional heading for preservation and Handling

T (Type) - B = Bullet, C = Comment, P = Proposed Change, R = Rating
S (Status) - A = Accepted, C = Closed, O = Open, W = Withdrawn, M = Merged

Para	Text	T	Comment	SC's response
G	(General Comment)	C	*Category : SUBSTANTIVE* **(191) Argentina (1 Oct 2023 4:16 AM)** Argentina supports the COSAVE comments	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(190) Barbados (30 Sep 2023 6:30 PM)** Barbados finds this to be a very comprehensive review of this diagnostic protocol and supports its adoption.	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(189) Costa Rica (30 Sep 2023 1:44 AM)** We have no comments	**Acknowledged**
G	(General Comment)	C	*Category : EDITORIAL* **(188) Paraguay (29 Sep 2023 8:50 PM)** Paraguay de acuerdo con los comentarios de COSAVE.	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(170) European Union (29 Sep 2023 6:18 PM)** In addition to the following comments, we	**Acknowledged**

Para	Text	T	Comment	SC's response
			also support editorial comments submitted by EPPO.	
G	(General Comment)	C	*Category : SUBSTANTIVE* **(169) Russian Federation (29 Sep 2023 4:39 PM)** General Comment: The Russian Federation would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System.	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(168) Belarus (29 Sep 2023 4:12 PM)** General comment: Republic of Belarus, would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System	**Acknowledged**
G	(General Comment)	C	*Category : EDITORIAL* **(167) Switzerland (29 Sep 2023 3:16 PM)** Switzerland would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System.	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(166) Philippines (29 Sep 2023 4:43 AM)** The PH has no further comments on the Revision of DP 09 - Genus Anastrepha	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(165) South Africa (28 Sep 2023 12:54 PM)** The draft revision should make provision for the premix on molecular techniques. We further suggest an additional heading for preservation and handling.	**Modified**. The draft includes sections on molecular methods and includes a subsection in revision on DNA preservation and extraction methods.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(151) Australia (27 Sep 2023 8:46 AM)** Australia has reviewed and is supportive of this current text	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(150) United Kingdom (26 Sep 2023 5:22 PM)** The UK supports the comments the EPPO secretariat have submitted on behalf of those EPPO member countries which are not part of the European Union.	**Acknowledged**
G	(General Comment)	C	*Category : EDITORIAL* **(149) Caribbean Agricultural Health and Food Safety Agency (25 Sep 2023 9:40 PM)**	**Acknowledged**

Para	Text	T	Comment	SC's response
			Guyana supports the revision of this draft annex	
G	(General Comment)	C	*Category : SUBSTANTIVE* **(148) Caribbean Agricultural Health and Food Safety Agency (25 Sep 2023 9:40 PM)** Barbados finds this to be a very comprehensive review of this diagnostic protocol and supports its adoption.	**Acknowledged**
G	(General Comment)	C	*Category : TECHNICAL* **(147) Caribbean Agricultural Health and Food Safety Agency (25 Sep 2023 9:40 PM)** The standard is highly technical and important to the Caribbean region as Anastrepha spp. is most common within the region.	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(144) IPPC Regional Workshop Africa (23 Sep 2023 3:52 PM)** We support the draft revision of DP9	**Acknowledged**
G	(General Comment)	C	*Category : TECHNICAL* **(143) IPPC Regional Workshop Africa (23 Sep 2023 3:52 PM)** Useful revision.	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(142) Malawi (23 Sep 2023 2:48 PM)** We support Draft Revision of DP◦9	**Acknowledged**
G	(General Comment)	C	*Category : TECHNICAL* **(36) Uruguay (18 Sep 2023 6:45 PM)** No comments, we agree with the document as it is	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* Mexico **(34) Mexico (15 Sep 2023 7:12 PM)** Mexico has reviewed and supports the Draft annex to ISPM 27: Revision of DP 09 - Genus Anastrepha Schiner (2021-002) in its current format.	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(27) United States of America (15 Sep 2023 4:49 PM)** The DP9 has well-documented information to identify selected pest species using morphology and molecular data at different life stages. Illustrations, figures, tables, and legends were helpful to follow the keys and	**Acknowledged**

Para	Text	T	Comment	SC's response
			to understand diagnostic descriptions. Both Figure 3 and Figure 4 of the general habitus were great additions, and very informative.	
G	(General Comment)	C	*Category : TECHNICAL* **(26) COSAVE (13 Sep 2023 6:11 PM)** No comments. Cosave agrees with the document as it is	**Acknowledged**
G	(General Comment)	C	*Category : EDITORIAL* **(24) Guyana (3 Sep 2023 11:36 PM)** Guyana supports the review of this draft annex	**Acknowledged**
G	(General Comment)	C	*Category : TECHNICAL* **(15) Congo (23 Aug 2023 9:26 AM)** i agree with this annexe of ISPM 27. Nothing to add,	**Acknowledged**
G	(General Comment)	C	*Category : SUBSTANTIVE* **(1) New Zealand (15 Aug 2023 2:23 AM)** New Zealand supports this DP.	**Acknowledged**
1	**DRAFT REVISION OF DP 9: Genus *Anastrepha* (2021-002)**	C	*Category : TECHNICAL* **(171) European Union (29 Sep 2023 6:19 PM)** EPPO thanks the experts for the revision of the Standard. It is noted that a limited number of species is covered within this DP and one EPPO member country indicated that it may be useful if more Anastrepha species are covered in future versions of the protocol.	**Acknowledged**
1	**DRAFT REVISION OF DP 9: Genus *Anastrepha* (2021-002)**	C	*Category : TECHNICAL* **(87) EPPO (22 Sep 2023 3:24 PM)** EPPO thanks the experts for the revision of the Standard. It is noted that a limited number of species is covered within this DP and one EPPO member country indicated that it may be useful if more Anastrepha species are covered in future versions of the protocol.	**Acknowledged**
46	The revision of this diagnostic protocol was adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in [Month 20--]. [to be completed after adoption]	C	*Category : SUBSTANTIVE* **(146) Caribbean Agricultural Health and Food Safety Agency (25 Sep 2023 9:40 PM)** The Bahamas offers no objections to the adoption of the revision of this diagnostic protocol on Genus Anastrepha.	**Acknowledged**
49	The family Tephritidae comprises over 5 000 species in approximately 500 genera (Norrbom, Carroll and Freidberg, 1999; Norrbom *et al.*, 1999; Norrbom, 2004b,	P	*Category : EDITORIAL* **(153) Colombia (27 Sep 2023 5:12 PM)** It is suggested that ":" be changed to ";"	**Incorporated**

Para	Text	T	Comment	SC's response
	2022). The Tephritidae are distributed worldwide in temperate, tropical and subtropical regions. *Anastrepha* Schiner (Tephritidae: Toxotrypanini) is the largest genus of Tephritidae in the Americas~~.~~; it is represented by more than 300 species, divided into 27 species groups (Norrbom et al. 1999; Mengual et al. 2017; Norrbom et al. 2018; Steck et al. 2019) that occur from the southern United States of America (Texas and Florida) to northern Argentina (Hernández-Ortiz, 1992; Foote, Blanc and Norrbom, 1993; Hernández-Ortiz and Aluja, 1993; Norrbom, 2004b; Norrbom *et al*., 2012, 2015, 2018, 2021). These species include those formerly placed in *Toxotrypana* Gerstaecker, now considered a synonym of *Anastrepha* (Norrbom *et al*., 2018). At least eight species of *Anastrepha* are considered pests of major economic importance because of the commercial value of the cultivated fruits they attack (e.g. mango and citrus) or their wide host range. These eight species are: *A. curvicauda* (Gerstaecker); *A. fraterculus* (Wiedemann); *A. grandis* (Macquart); *A. ludens* (Loew); *A. obliqua* (Macquart); *A. serpentina* (Wiedemann); *A. striata* Schiner; and *A. suspensa* (Loew). *A. fraterculus* has been recognized as a cryptic species complex (Hernández-Ortiz *et al*., 2004, 2012, 2015; Selivon *et al.*, 2004; Selivon, Perondini and Morgante, 2005; Vera *et al*., 2006, Cáceres *et al.*, 2009; Sutton *et al*., 2015). This diagnostic protocol for *Anastrepha* covers identification of the genus and the species of major economic importance. For further general information about species of Tephritidae, see White and Elson-Harris (1992), Aluja and Norrbom (1999) and Norrbom (2010).			
50	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, except for *A. manihoti* Lima which develops in stems. The number of eggs deposited per fruit is variable and depends on both intrinsic and extrinsic factors; some species (e.g. *A. obliqua*) always lay single eggs, others (e.g. *A. bezzii*, *A. grandis*) have large clutch sizes, and others (e.g. *A. ludens*) vary the clutch size based on host fruit size (Aluja *et al.*, 1999). A total of 494 natural host plant species (see ISPM 37 (*Determination of host status of fruit to fruit flies (Tephritidae)*) are known for 148 (43%) of the 328 currently recognized *Anastrepha* species and nine unnamed species (Norrbom, 2022; Rodriguez *et al.*, forthcoming). Published host records for major pests are available at the United States Department of Agriculture Compendium of Fruit Fly Host Information (https://coffhi.cphst.org).	C	*Category : SUBSTANTIVE* **(156) China (28 Sep 2023 8:02 AM)** Adding the related information of life cycle of 8 important species of Anastrepha. The information of life cycle of 8 important species of Anastrepha are necessary to know how many days are needed to rearing eggs or larvae to adults.	**Considered but not incorporated.** The specific details of development are dependent on many factors such as species, climate, and diet.
50	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, except for *A. manihoti* Lima which develops in stems. The	C	*Category : EDITORIAL* **(152) Australia (27 Sep 2023 8:47 AM)** Recommend providing author name at first mention, as for other species listed.	**Incoporated**. Anastrepha bezzi Lima

Para	Text	T	Comment	SC's response
	number of eggs deposited per fruit is variable and depends on both intrinsic and extrinsic factors; some species (e.g. *A. obliqua*) always lay single eggs, others (e.g. *A. bezzii*, *A. grandis*) have large clutch sizes, and others (e.g. *A. ludens*) vary the clutch size based on host fruit size (Aluja *et al.*, 1999). A total of 494 natural host plant species (see ISPM 37 (*Determination of host status of fruit to fruit flies (Tephritidae)*) are known for 148 (43%) of the 328 currently recognized *Anastrepha* species and nine unnamed species (Norrbom, 2022; Rodriguez *et al.*, forthcoming). Published host records for major pests are available at the United States Department of Agriculture Compendium of Fruit Fly Host Information ().			
50	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, except for *A. manihoti* Lima which ~~develops in~~ oviposits on stems, where the larvae develop. The number of eggs deposited per fruit is variable and depends on both intrinsic and extrinsic factors~~;~~: some species (e.g. *A. obliqua*) always lay single eggs, others (e.g. *A. bezzii*, *A. grandis*) have large clutch sizes, and others (e.g. *A. ludens*) vary the clutch size based on host fruit size (Aluja *et al.*, 1999). A total of 494 natural host plant species (see ISPM 37 (*Determination of host status of fruit to fruit flies (Tephritidae)*) are known for 148 (43%) of the 328 currently recognized *Anastrepha* species and nine unnamed species (Norrbom, 2022; Rodriguez *et al.*, forthcoming). Published host records for major pests are available at the United States Department of Agriculture Compendium of Fruit Fly Host Information ().	P	*Category : TECHNICAL* **(37) United States of America (19 Sep 2023 7:52 PM)**	**Incoporated**.
51	The introduction to the American tropics of cultivated exotic species such as *Mangifera indica* and *Citrus* spp. has allowed some pest species of *Anastrepha* to expand their original geographical distribution. However, they still have marked preferences for certain indigenous hosts, which is probably indicative 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 (Norrbom, 2004a).	P	*Category : TECHNICAL* **(38) United States of America (19 Sep 2023 7:52 PM)**	**Considered but not incorporated.** The addition of additional geographic information does not improve clarity. The use of tropics and subtropics of the Americas is relevant for cultivated plants that can be damaged by these flies.
76	*Tephritis mellea* Walker, ~~1836~~ 1837	P	*Category : EDITORIAL* **(3) Thailand (16 Aug 2023 9:30 AM)** correction	**Considered but not incorporated.** The authors confirmed that the date should be 1836 based on original publication. The 1837 date is likely from a book that had an error.
115	*Anastrepha schineri* Hendel, 1914	C	*Category : EDITORIAL* **(88) EPPO (22 Sep 2023 3:24 PM)**	**Incoporated**

Para	Text	T	Comment	SC's response
			Shouldn't the 1914 and 1935 synonyms be listed after the synonym Trypeta grandis, which is from 1873?	
118	*Anastrepha latifasciata* Hering, 1935	C	*Category : EDITORIAL* **(89) EPPO (22 Sep 2023 3:24 PM)** Shouldn't the 1914 and 1935 synonyms be listed after the synonym Trypeta grandis, which is from 1873?	**Incoporated**
127	*Anastrepha lathana* Stone, 1942	C	*Category : EDITORIAL* **(90) EPPO (22 Sep 2023 3:24 PM)** Shouldn't this 1942 synonym be listed after the synonym Acrotoxa ludens, which is from 1873?	**Incoporated.**
136	*Trypeta ludens* Loew, 1873	C	*Category : EDITORIAL* **(25) Canada (11 Sep 2023 6:52 PM)** There are additional column for some species that do not have heading which causes confusion for the reader. Suggest that additional headings be included to provide greater clarity	**Incorporated.** .
166	*Leptoxys serpentina* ~~Macquart~~Wiedemann, 1843	P	*Category : EDITORIAL* **(4) Thailand (16 Aug 2023 9:31 AM)** correction	**Considered but not incorporated.** The authority is Macquart.
169	*Trypeta serpentina* ~~Loew~~Wiedemann, 1873	P	*Category : EDITORIAL* **(5) Thailand (16 Aug 2023 9:33 AM)** correction	**Considered but not incorporated.** The authority is Loew.
172	*Acrotoxa serpentina* ~~Loew~~Wiedemann, 1873	P	*Category : EDITORIAL* **(6) Thailand (16 Aug 2023 9:33 AM)** correction	**Considered but not incorporated.** The authority is Loew.
175	*Urophora vittithorax* Macquart, 1851	C	*Category : EDITORIAL* **(91) EPPO (22 Sep 2023 3:24 PM)** Shouldn't this 1851 synonym be listed before the synonym Trypeta serpentina, which is from 1873?	**Incoporated.**
206	Signs of fruit fly infestation are the presence of soft areas, dark stains, dark pin spots, rot, holes or injuries that might be caused by oviposition or larval feeding activities. To detect punctures made by female flies during oviposition, fruits can be examined under a stereomicroscope by an expert. If larval exit holes are observed, puparia may be detected in the packaging of the fruit. Third instars may not be present when unripe fruit is collected and packed; however, this fruit might host eggs or first or second instars, which are more difficult to detect. On potentially infested fruit showing typical punctures made by ovipositing female flies, eggs and larvae may be seen when ~~cutting~~ the fruit is cut open.	P	*Category : TECHNICAL* **(39) United States of America (19 Sep 2023 7:54 PM)**	**Incoporated.**

Para	Text	T	Comment	SC's response
207	Once detected, larvae may be reared to adults (section 4.1.1), which is required to accurately identify a fly to ~~species-level~~ species-level with morphological techniques. The incubation of infested fruits is a common practice to obtain adult flies. As oviposition marks are often difficult to recognize, fruits can be held to check for potential larval development even if there are no signs of fruit fly infestation.	P	*Category : EDITORIAL* **(40) United States of America (19 Sep 2023 7:55 PM)**	**Incoporated.**
209	The taxonomy of the genus *Anastrepha* is based mainly 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; Steck *et al*., 2019) 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; Frías, Selivon and Hernández-Ortiz, 2008; Frías Lasserre, Hernández-Ortiz and López Muñoz, 2009; Dutra *et al.*, 2011a, 2011b, 2012, 2013, 2018a, 2018b; Figueiredo *et al*., 2013; Rodriguez *et al*., 2021). Identification keys for the larvae of the eight species of *Anastrepha* known to be of major economic importance (Table 1) are available (Steck *et al.*, 1990; Carroll *et al.*, 2004) but should be used with consideration of their limitations.	C	*Category : EDITORIAL* **(92) EPPO (22 Sep 2023 3:24 PM)** An overview picture of an adult Anastrepha species is preferred as first figure (i.e. Figure 1) as in the previous version of this DP. It is suggested to cite Figure 3 and Figure 4 here so they become Figure 1 and Figure 2.	**Incorporated**. Figures 3 and 4 are now cited first and are now Figures 1 and 2.
209	The taxonomy of the genus *Anastrepha* is based mainly 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; Steck *et al*., ~~2019) in comparison with adult morphology~~2019). However, some information on egg ~~structures~~ and third-instar ~~larvae~~ larval structures 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; Frías, Selivon and Hernández-Ortiz, 2008; Frías Lasserre, Hernández-Ortiz and López Muñoz, 2009; Dutra *et al.*, 2011a, 2011b, 2012, 2013, 2018a, 2018b; Figueiredo *et al*., 2013; Rodriguez *et al*., 2021). Identification keys for the larvae of the eight species of *Anastrepha* known to be of major economic importance (Table 1) are available (Steck *et al.*, 1990; Carroll *et al.*, 2004) but should be used with consideration of their limitations.	P	*Category : TECHNICAL* **(41) United States of America (19 Sep 2023 7:58 PM)** What limitations? Seems like a key to eight of 300+ species can only tell you that a given larva is not whatever it doesn't key out to, not that it is anything in particular.	**Modified**. Edits incorporated. Text is included to note limitations for the eight species. Larval descriptions are not complete for all species that could use the same fruit hosts as the eight species targeted in the protocol. Furthermore, variation in larval morphology of the eight species is dependent on relatively few studies

Para	Text	T	Comment	SC's response
210	Although the third-instar larvae of some *Anastrepha* species can be discriminated in keys (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, Guillén-Aguilar and López, 2010), the available data are based on very limited sampling for most species. The reliability of these keys cannot, therefore, be guaranteed until further studies are conducted, including studies of additional, closely related species that have not yet been characterized. The most reliable method for identification is rearing larvae to the adult stage. Molecular methods of identification have also been reported (developed?) for some of the major pest species and are included in this diagnostic protocol (section 4.5).	P	*Category : TECHNICAL* **(42) United States of America (19 Sep 2023 7:59 PM)**	**Incoporated.**
211	Several pest species of *Anastrepha* are believed to comprise complex of multiple (yet to be described) cryptic species that are morphologically indistinguishable or require morphometric analysis for their recognition (Hernández-Ortiz *et al.*, 2004, 2012, 2015). The *A. fraterculus* species complex (Table 1) is included in the protocol, but it is identified to the level of complex because revision of its taxonomy and associated molecular diagnosis are not yet fully resolved (Sutton *et al*., 2015, Prezotto *et al*., 2019).	P	*Category : TECHNICAL* **(43) United States of America (19 Sep 2023 8:00 PM)**	**Considered but not incorporated.** The term complex might not apply to all cryptic species.
212	**4.1 Preparation of adults for morphological identification**	P	*Category : EDITORIAL* **(93) EPPO (22 Sep 2023 3:24 PM)** It is suggested to align the title of section 4.1 with the one of section 4.2	**Incoporated.**
213	**4.1.1 Rearing larvae to obtain adults**	C	*Category : EDITORIAL* **(157) China (28 Sep 2023 8:03 AM)** adjust "4.1.1 Rearing larvae to obtain adults "to" 3.3 Rearing larvae to obtain adults"	**Considered but not incorporated.** The decision to rear to an adult is considered to be part of the identification prcoess. Detection of larvae could lead to other decisions.
214	Larvae can be reared to adults by placing infested fruits in containers containing a sterile pupation medium (e.g. damp vermiculite, sand or sawdust) on the bottom. The containers are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium for pupation. It is recommended that each fruit sample is incubated 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 integument and wings acquire the rigidity and characteristic coloration of the species. Adults can be fed with honey and water or a mix of sugar, yeast, wheatgerm and water. The adults are then killed and preserved by placing them in	C	*Category : SUBSTANTIVE* **(158) China (28 Sep 2023 8:04 AM)** Adding the related information of rearing time. The rearing time ise very necessary information	**Considered but not incorporated.** Rearing time varies and greater details based on one facility could be less optimal for other locations rearing adults. The inclusion of general guidance is most appropriate given the available methods for rearing different species and genera of fruit flies. Each laboratory should develop internal protocols. This IPPC docuemtn provides general informaiton needed to initiate that.

Para	Text	T	Comment	SC's response
	70–95% 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).			
214	Larvae can be reared to adults by placing infested fruits in containers containing a sterile pupation medium (e.g. damp vermiculite, sand or sawdust) on the bottom. The containers are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium for pupation. It is recommended that each fruit sample is incubated 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 integument and wings acquire the rigidity and characteristic coloration of the species. Adults can be fed with honey and water or a mix of sugar, yeast, wheatgerm and water. The adults are then killed and preserved by placing them in 70–95% 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).	C	*Category : TECHNICAL* **(44) United States of America (19 Sep 2023 8:01 PM)** Worth mentioning that fly rearing should be conducted in a secure facility from which the adults can't escape?	**Considered but not incorporated.** This is a practical suggesiton but rearing could occur within areas where flies exist. The protocol does not instruct on regulation or quarantine of pests.
214	Larvae can be reared to adults by placing infested fruits in containers containing a sterile pupation medium (e.g. damp vermiculite, sand or sawdust) on the bottom. The containers are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium for pupation. It is recommended that each fruit sample is incubated 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 integument and wings acquire the rigidity and characteristic coloration of the species. Adults can be fed with honey and water or a mix of sugar, yeast, wheatgerm and water. The adults are then killed and preserved by placing them in 70–95% 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).	C	*Category : TECHNICAL* **(28) United States of America (15 Sep 2023 5:10 PM)** Recommend adding Figure 1 here, because it is the first time referring the aculeus	**Incorporated.**

Para	Text	T	Comment	SC's response
214	Larvae can be reared to adults by placing infested fruits in containers containing a sterile pupation medium (e.g. damp vermiculite, sand or sawdust) on the bottom. The containers are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium for pupation. It is recommended that each fruit sample is incubated 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 integument and wings acquire the rigidity and characteristic coloration of the species. Adults can be fed with honey and water or a mix of sugar, yeast, wheatgerm and water. The adults are then killed and preserved by placing them in 70–95% 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).	C	*Category : TECHNICAL* **(18) Kenya (28 Aug 2023 2:49 PM)** Specify the concentration	**Modified.** There is not a single value or range based on data. Prior studies have used 30% (m/v) successfully for Anastrepha fraterculus.
214	Larvae can be reared to adults by placing infested fruits in containers containing a sterile pupation medium (e.g. damp vermiculite, sand or sawdust) on the bottom. The containers are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium for pupation. It is recommended that each fruit sample is incubated 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 integument and wings acquire the rigidity and characteristic coloration of the species. Adults can be fed with honey and water or a mix of sugar, yeast, wheatgerm and water. The adults are then killed and preserved by placing them in 70–95% 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).	C	*Category : TECHNICAL* **(17) Kenya (28 Aug 2023 2:47 PM)** Ethyl acetate is harsh to users and makes the flies very hard and brittle thus breaking wduring pinning .Replace with a milder reagent	**Considered but not incorporated.** This is a commonly used reagent for fruit fly work and it is therefore mentioned. Other agents are acceptable and the protocol does not prescribe ethyl acetate as the only option.
215	**4.1.2 Preparation of ~~adults for microscopic examination~~adults**	P	*Category : EDITORIAL* **(94) EPPO (22 Sep 2023 3:24 PM)** To avoid having the same title for 4.1 and 4.1.2	**Considered but not incorporated.** The title of 4.1 was modified so these are no redundant.

Para	Text	T	Comment	SC's response
216	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.	C	*Category : TECHNICAL* **(29) United States of America (15 Sep 2023 5:11 PM)** Might be helpful to have an image of a sample slide A possible online video link for a dissection? Is ethanol or dry the preferred method of preservation?	**Considered but not incorporated.** The protocol describes the methods as used by an entomologist with experience identifying flies. Additional workshops and training materials are made available but are outside the scope of the protocol.
218	It is preferable to cut off the whole abdomen from a female to dissect the oviscape (syntergosternite 7) (Figure 1), 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) or 10% potassium hydroxide (KOH) 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. This should reveal the aculeus and the eversible membrane. 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 the introductory text of 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.	C	*Category : TECHNICAL* **(173) European Union (29 Sep 2023 6:21 PM)** There is no information in the introductory text of section 4.1.2 on permanent slides. Should this refer to 4.2?	**Modified**. Section 4.2 refers to larvae. Section 4.1.2 is correct but there is no introductory text.
218	It is preferable to cut off the whole abdomen from a female to dissect the oviscape (syntergosternite 7) (Figure 1), 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) or 10% potassium hydroxide (KOH) 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. This should reveal the aculeus and the eversible membrane. 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 the introductory text of 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.	C	*Category : TECHNICAL* **(172) European Union (29 Sep 2023 6:21 PM)** There is no information in the introductory text of section 4.1.2 on permanent slides. Should this refer to 4.2?	**Modified**. Section 4.2 refers to larvae. Section 4.1.2 is correct but there is no introductory text.

Para	Text	T	Comment	SC's response
218	It is preferable to cut off the whole abdomen from a female to dissect the oviscape (syntergosternite 7), the eversible membrane and the aculeus (Figure 1). 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) or 10% potassium hydroxide (KOH) 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. This should reveal the aculeus and the eversible membrane. 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 the introductory text of 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.	C	*Category : TECHNICAL* **(96) EPPO (22 Sep 2023 3:24 PM)** There is no information in the introductory text of section 4.1.2 on permanent slides. Should this refer to 4.2?	**Modified**. Section 4.2 refers to larvae. Section 4.1.2 is correct but there is no introductory text.
218	It is preferable to cut off the whole abdomen from a female to dissect the oviscape (syntergosternite ~~7) (Figure 1)~~7), the eversible membrane and the ~~aculeus~~aculeus (Figure 1). 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) or 10% potassium hydroxide (KOH) 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. This should reveal the aculeus and the eversible membrane. 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 the introductory text of 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.	P	*Category : EDITORIAL* **(95) EPPO (22 Sep 2023 3:24 PM)** The oviscape, the eversible membrane and the aculeus can all be seen on Figure◦1.	**Incorporated.**
218	It is preferable to cut off the whole abdomen from a female to dissect the oviscape (syntergosternite 7) (Figure 1), 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) or 10% potassium hydroxide (KOH) solution and heating it in a boiling water bath for 10–15 min, washing the	C	*Category : TECHNICAL* **(30) United States of America (15 Sep 2023 5:12 PM)** An image example?	**Considered but not incorporated.** The inclusion of images where some characters are difficult to observe would not improve the protocol.

Para	Text	T	Comment	SC's response
	structure with distilled water, and then removing internal contents under a stereomicroscope with the help of dissection forceps. This should reveal the aculeus and the eversible membrane. 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 the introductory text of 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.			
221	**4.2 Preparation of larvae for morphological identification**	C	*Category : EDITORIAL* **(159) China (28 Sep 2023 8:06 AM)** Add "4.2.1 Preparing larvae for stereomicroscope examination" and "4.2.2 Preparing larvae for SEM examination".	**Considered but not incorporated.** The section 4.2 describes information for stereomicrscope, compound microscope, and SEM preapration. The information is not simply divided in section. Current presentation is used to provide comparison and contrast of the three options. Further dividing the section could reduce explanatory value of section.
222	As noted in the introductory text of section 4, observation of adult characters may be necessary to corroborate a morphological identification based on larvae. If immature stages are found, it is recommended that some larvae be preserved for morphological examination by treating them in hot or boiling water, cooling to room temperature, and then storing them in 70% ethanol, and rearing the remaining larvae and pupae to obtain adult specimens for identification (section 4.1.1). Larvae that are to be used for morphological analysis alone can be saved in 70% ethanol after boiling. Larvae that are to be used for both morphological and molecular analysis can have tissue excised (section 4.5.1) and saved in ≥95% ethanol in a freezer (≤–20 °C) until DNA is extracted and the remaining anterior and posterior sections ~~saved~~ saved, (What about adding an explanation, "containing useful morphological characters"?) in 70% ethanol.	P	*Category : TECHNICAL* **(31) United States of America (15 Sep 2023 5:14 PM)** Can you get DNA out of a specimen that has been boiled? Should you not boil specimens intended for DNA work?	**Modified.** Included new text for explaining value of saved larval parts. Boiling insect specimens does not destroy DNA. It has been successfully used for several fly species without loss of DNA in amplification tests. Depending on how insects were killed or collected, not boiling larvae can lead to reduced success of DNA tests.
223	For the hot water treatment, live larvae are killed by placing in water at 65–100 °C for at least two minutes, cooled to room temperature and then preserved in 70% ethanol. If larvae turn partially or completely black after one day in 70% ethanol, the hot water treatment was inadequate, and the water temperature or treatment time should be increased. The larval cuticle may split open on one side near the head, but this is inconsequential for identification purposes. Splitting is minimized if the larvae are run through a graduated alcohol series of 35%–50%–70% ethanol	C	*Category : TECHNICAL* **(19) Kenya (28 Aug 2023 2:50 PM)** with the collection data (i.e., an accession number, locality, host, collector, and collection date)	**Considered but not incorporated.** The comment is correct that these details improve the quality of the specimen. However, the protocol is avoiding being prescriptive in methods and the type of information required will vary for different applications.

Para	Text	T	Comment	SC's response
	for two hours each, with an additional change to fresh 70% alcohol. It is advisable to include a label in the storage vial with all sampling information. These samples are ready for examination under a stereomicroscope or subsequent preparation for slide mounting or examining under a scanning electron microscope (SEM).			
224	To prepare specimens for slide mounting, it is necessary to remove (clear) all the internal tissues to allow observation of the cuticle, oral opening, cephaloskeleton, anterior and posterior spiracles, and anal lobes. First, two incisions are made in the larva: one laterally through the thoracic segments, and one between the posterior spiracles and anus. Then the incised larva is immersed in hot 10% NaOH or 10% KOH solution for 10–15 min or until most internal tissues are visibly digested. After digestion, the remaining internal debris is carefully removed using forceps and the specimen flushed with distilled water under a stereomicroscope. The cephaloskeleton is extracted through the lateral incision on the thorax.	C	*Category : TECHNICAL* **(32) United States of America (15 Sep 2023 5:14 PM)** Figure number?	**Incorporated.**
225	Cleared specimens can be placed in glycerine on a glass depression slide with a cover slip for examination or imaging and recording of measurement data under a compound microscope. Afterwards, specimens can be retained as vouchers by returning them to alcohol in a labelled vial, or permanent slide mounts can be made using Canada balsam or Euparal following standard methods. First, the cleared specimen must be dehydrated for 25 min in each of 50%, 75% and 100% ethanol. For mounting with Canada balsam, the specimen should be transferred to lavender oil for 15 min to clear it and then immediately mounted on a slide with one or two drops of Canada balsam. When Euparal is used as the mounting medium, the specimen should be transferred from 100% ethanol to clove oil for about 30 min to clear it before mounting. For permanent mounts, care must be taken to position and stabilize the specimen in the proper orientation before adding the cover slip, otherwise it may be impossible to get realistic images or accurate measurements after the specimen dries in place. Slides must be allowed to dry for several days or weeks (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.	C	*Category : TECHNICAL* **(174) European Union (29 Sep 2023 6:22 PM)** It is not possible to have 100% ethanol so it is proposed to change for '≥95%' ethanol. It will be consistent with line 160. Alternatively absolute ethanol could be used (as in l 214).	**Modified.** Included >99% (absolute) ethanol for the concentration.
225	Cleared specimens can be placed in glycerine on a glass depression slide with a cover slip for examination or imaging and recording of measurement data under a compound microscope. Afterwards, specimens can be retained as vouchers by returning them to alcohol in a labelled vial, or permanent slide mounts can be made using Canada balsam or Euparal following standard methods. First, the cleared specimen must be dehydrated for 25 min in each of 50%, 75% and 100% ethanol. For mounting with Canada balsam, the specimen should be transferred to lavender	C	*Category : TECHNICAL* **(98) EPPO (22 Sep 2023 3:24 PM)** It is not possible to have 100% ethanol so it is proposed to change for '≥95%' ethanol. It will be consistent with line 160. Alternatively absolute ethanol could be used (as in l 214).	**Modified.** Included >99% (absolute) ethanol for the concentration.

Para	Text	T	Comment	SC's response
	oil for 15 min to clear it and then immediately mounted on a slide with one or two drops of Canada balsam. When Euparal is used as the mounting medium, the specimen should be transferred from 100% ethanol to clove oil for about 30 min to clear it before mounting. For permanent mounts, care must be taken to position and stabilize the specimen in the proper orientation before adding the cover slip, otherwise it may be impossible to get realistic images or accurate measurements after the specimen dries in place. Slides must be allowed to dry for several days or weeks (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.			
225	Cleared specimens can be placed in glycerine on a glass depression slide with a cover slip for examination or imaging and recording of measurement data under a compound microscope. Afterwards, specimens can be retained as vouchers by returning them to alcohol in a labelled vial, or permanent slide mounts can be made using Canada balsam or Euparal following standard methods. First, the cleared specimen must be dehydrated for 25 min in each of 50%, 75% and 100% ethanol. For mounting with Canada balsam, the specimen should be transferred to lavender oil for 15 min to clear it and then immediately mounted on a slide with one or two drops of Canada balsam. When Euparal is used as the mounting medium, the specimen should be transferred from 100% ethanol to clove oil for about 30 min to clear it before mounting. For permanent mounts, care must be taken to position and stabilize the specimen in the proper orientation before adding the cover slip, otherwise it may be impossible to get realistic images or accurate measurements after the specimen dries in place. Slides must be allowed to dry for several days or weeks (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.	C	*Category : TECHNICAL* **(97) EPPO (22 Sep 2023 3:24 PM)** It is not possible to have 100% ethanol so it is proposed to change for '≥95%' ethanol. It will be consistent with line 160. Alternatively absolute ethanol could be used (as in l 214).	**Modified.** Included >99% (absolute) ethanol for the concentration.
225	Cleared specimens can be placed in glycerine on a glass depression slide with a cover slip for examination or imaging and recording of measurement data under a compound microscope. Afterwards, specimens can be retained as vouchers by returning them to alcohol in a labelled vial, or permanent slide mounts can be made using Canada balsam or Euparal following standard methods. First, the cleared specimen must be dehydrated for 25 min in each of 50%, 75% and 100% ethanol. For mounting with Canada balsam, the specimen should be transferred to lavender oil for 15 min to clear it and then immediately mounted on a slide with one or two drops of Canada balsam. When Euparal is used as the mounting medium, the specimen should be transferred from 100% ethanol to clove oil for about 30 min to	P	*Category : TECHNICAL* **(45) United States of America (19 Sep 2023 8:05 PM)**	**Incorporated.**

Para	Text	T	Comment	SC's response
	clear it before mounting. For permanent mounts, care must be taken to position and stabilize the specimen in the proper orientation before adding the cover slip, otherwise it may be impossible to get realistic images or accurate measurements after the specimen dries in place. Slides must be allowed to dry for several days or weeks (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~~labelled with unique identifying codes that associate them with the rest of the specimen.			
225	Cleared specimens can be placed in glycerine on a glass depression slide with a cover slip for examination or imaging and recording of measurement data under a compound microscope. Afterwards, specimens can be retained as vouchers by returning them to alcohol in a labelled vial, or permanent slide mounts can be made using Canada balsam or Euparal following standard methods. First, the cleared specimen must be dehydrated for 25 min in each of 50%, 75% and 100% ethanol. For mounting with Canada balsam, the specimen should be transferred to lavender oil for 15 min to clear it and then immediately mounted on a slide with one or two drops of Canada balsam. When Euparal is used as the mounting medium, the specimen should be transferred from 100% ethanol to clove oil for about 30 min to clear it before mounting. For permanent mounts, care must be taken to position and stabilize the specimen in the proper orientation before adding the cover slip, otherwise it may be impossible to get realistic images or accurate measurements after the specimen dries in place. Slides must be allowed to dry for several days or weeks (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.	C	*Category : TECHNICAL* **(20) Kenya (28 Aug 2023 2:51 PM)** specify duration eg 4 weeks , a month etc or put a rider until when the mountant is confirmed dry	**Modified.** Experts commonly use 2-3 weeks. New text is added to explain this range.
226	Morphological examination of larvae can be performed on unmounted larvae (Figures 2A,C) using a stereomicroscope, on slide-mounted larvae (Figure 2B) using a compound microscope, or on critical-point dried larvae using an SEM (Figure 2D).	C	*Category : TECHNICAL* **(21) Kenya (28 Aug 2023 2:52 PM)** scanning Electrom Microscope (SEM)	**Considered but not incorporated.** The acronym was spelled out earlier in this section.
227	With a stereomicroscope it is possible to count oral ridges, accessory plates, and tubules on the anterior spiracles; observe the shape of anterior spiracles and anal lobes, and the presence of dorsal spinules on various body segments; and measure the apical width of anterior spiracles and the length of posterior spiracles. Fine details of the facial mask (preoral lobes, oral ridges and their edges, accessory plates) of an unmounted larva can be observed by using a transmitted-light compound microscope. A clean, dry larva is placed on a piece of facial tissue	C	*Category : TECHNICAL* **(33) United States of America (15 Sep 2023 5:16 PM)** (Figure 2A and 2B, Asp; we suggest including this figure number here instead of including it in the key, 4.4.2. couplet 1)	**Considered but not incorporated.** The Figure is referenced to in the previous and subsequent paragraphs of the same section. The text is not to explain characters that are described in general fly identification resources.

Para	Text	T	Comment	SC's response
	on a glass slide and the head is observed at 100× magnification. Specimens should be re-wetted with alcohol as needed to prevent shrivelling during examination.			
228	On slide-mounted, cleared larvae it is possible to re-examine many of the same external features observed on unmounted specimens and obtain more accurate measurements under a compound microscope. The oral-ridge margins and accessory plates may also be seen, although it may be difficult to prepare a specimen properly to view them. The external posterior spiracles, their hair-like processes and the internal cephaloskeleton are readily visible on cleared specimens under a compound microscope using an objective of 20×, 40× or higher (Figure 2C). Detailed, high-resolution observation of the external morphology of larvae, especially of the facial mask including oral ridges, accessory plates, preoral lobes and sensory organs, is best achieved using an SEM (Figure 2D). The ventral surface of the mouth hook is only visible under SEM. It is therefore recommended that slide-mounting does not include all specimens representing a sample or the only larva available for diagnosis; unmounted larvae should be kept for future analysis.	C	*Category : EDITORIAL* **(99) EPPO (22 Sep 2023 3:24 PM)** It should be refered to Figure 2B.	**Incorporated.**
228	On slide-mounted, cleared larvae it is possible to re-examine many of the same external features observed on unmounted specimens and obtain more accurate measurements under a compound microscope. The oral-ridge margins and accessory plates may also be seen, although it may be difficult to prepare a specimen properly to view them. The external posterior spiracles, their hair-like processes and the internal cephaloskeleton are readily visible on cleared specimens under a compound microscope using an objective of 20×, 40× or higher (Figure 2C). Detailed, high-resolution observation of the external morphology of larvae, especially of the facial mask ~~including~~ (including oral ridges, accessory plates, preoral lobes and sensory ~~organs~~organs), is best achieved using an SEM (Figure 2D). The ventral surface of the mouth hook is only visible under SEM. It is therefore recommended that slide-mounting does not include all specimens representing a sample or the only larva available for diagnosis; unmounted larvae should be kept for future analysis.	P	*Category : EDITORIAL* **(46) United States of America (19 Sep 2023 8:08 PM)**	**Incorporated.**
229	For observation using an SEM, the specimen is 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 before mounting on stubs. Alternatively, specimens can be placed in two additional baths of ethyl acetate, air-dried and mounted on a stub for sputter coating. See Carroll and Wharton (1989), Frías *et al.* (2006), Frías, Selivon and Hernández-Ortiz (2008),	C	*Category : TECHNICAL* **(175) European Union (29 Sep 2023 6:23 PM)** Is it possible to be consistent throughout the protocol and use either '≥95%' or 'absolute ethanol' (see lines 160, 183 and 186)?	**Modified.** Absolute ethnaol and >95% are not always interchangeable. Additional text was added in areas where >99% or absolute ethanol are recommended.

Para	Text	T	Comment	SC's response
	Frías Lasserre, Hernández-Ortiz and López Muñoz (2009) and Rodriguez *et al*. (2021) for further details and variations.			
229	For observation using an SEM, the specimen is 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 before mounting on stubs. Alternatively, specimens can be placed in two additional baths of ethyl acetate, air-dried and mounted on a stub for sputter coating. See Carroll and Wharton (1989), Frías *et al.* (2006), Frías, Selivon and Hernández-Ortiz (2008), Frías Lasserre, Hernández-Ortiz and López Muñoz (2009) and Rodriguez *et al*. (2021) for further details and variations.	C	*Category : TECHNICAL* **(100) EPPO (22 Sep 2023 3:24 PM)** Is it possible to be consistent throughout the protocol and use either '≥95%' or 'absolute ethanol' (see lines 160, 183 and 186)?	**Modified.** Absolute ethnaol and >95% are not always interchangeable. Additionaltext was added in areas where >99% or absolute ethanol are recommended.
232	Adult flies can be diagnosed to genus using a combination of characters.	C	*Category : TECHNICAL* **(47) United States of America (19 Sep 2023 8:09 PM)** What is/are the diagnostic character(s) for the genus? Perhaps, state those first. Tere are too many of "Usually", "sometimes" "often" in this section etc., that are not really helpful, unless you are an expert who recognizes the gestalt. It would be helpful to embed the figures showing the various structures here, rather than putting them at the end.	**Considered but not incorporated.** Section 4.3.1 describes how to identify to genus. The terms "*usually*" and "*often*" are needed to describe states even if not discrete for identification. This is the reality for variation in the flies and removal would alter the meaning of the text and impact the diagnosis. Although not discrete character states or ranges, these trends are helpful in completing an identification
233	~~Body~~Body (Figure 3 and Figure 4): usually predominantly yellow to orange, occasionally mostly ~~brown (Figure 3 and Figure 4)~~brown. Head (Figure 5A): 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 (Figure 5B): 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 white to pale yellow stripes; mesonotum with the following setae, except in the *curvicauda* group, where they are reduced or absent – 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.	P	*Category : EDITORIAL* **(65) United States of America (20 Sep 2023 7:33 PM)**	**Incorporated.**
235	Male terminalia (Figure 6B): epandrium broad in lateral view with lateral surstylus short or elongated; medial surstylus shorter than lateral surstylus with two stout	C	*Category : TECHNICAL* **(22) Kenya (28 Aug 2023 2:53 PM)**	**Modified.**

Para	Text	T	Comment	SC's response
	blackish prensisetae apically; proctiger membranous, weakly sclerotized at least 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.		shorter is relative ,provide specific measurements	Changed "with lateral surstylus short or elongated" to " with lateral surstylus short or moderately elongate, part distal to prensisetae no more than 5.5 times as long as prensiseta, without anterior or posterior lobes apically"
236	Female terminalia (Figure 1 and Figure ~~6C)~~6C, Figure 8): oviscape tube-like, variable in length, basally with flange-like lateral lobes; eversible membrane (usually inverted inside oviscape) basally with dorsal group of hook-like sclerotized denticles (sometimes referred to as the rasper); aculeus (usually inverted inside eversible membrane and oviscape) well sclerotized, tip (Figure 8) sometimes serrated on lateral margins.	P	*Category : TECHNICAL* **(66) United States of America (20 Sep 2023 7:34 PM)**	**Incorporated.**
239	1. Wing (Figure 7A) with only broad, uninterrupted costal band filling all of wing anterior to vein R_{4+5}, and more diffuse band covering cell *cua* and base of cell m_4; most setae, including postpronotal, presutural supra-alar, dorsocentral, intra-alar and scutellar setae, absent or small and weak, much shorter than scutellum length; abdomen petiolate; body predominantly yellow with conspicuous brown markings (Figure 3A and Figure 3B); anatergite at most with dark dorsal and ventrolateral spots; scutellum with at most base and lateral third of apical margin brown; scutum with dark posterior mark broader than long and separate from dark submedial stripes and dark sublateral stripes, the latter strongly laterally curved posteriorly (Figure 3B); oviscape elongate, usually longer than thorax and abdomen combined, and strongly curved (Figure 3A). (Larvae infest papaya, other Caricaceae, and Apocynaceae.) ***Anastrepha curvicauda*** **(Gerstaecker)**	C	*Category : TECHNICAL* **(48) United States of America (19 Sep 2023 8:13 PM)** "usually" for characters in a key are not helpful.	**Considered but not incorporated.** The terms "*usually*" and "*often*" are needed to describe states even if not discrete for identification. This is the reality for variation in the flies and removal would alter the meaning of the text and impact the diagnosis. Although not discrete character states or ranges, these trends are helpful in completing an identification.
252	– Both mediotergite and subscutellum (Figure 5C) with broad, dark-brown to black, lateral markings; scuto-scutellar suture usually with medial brown spot (as in Figure 4B and Figure 4D); 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 8H); wing pattern variable (Figure 7H). (Generalist pest.) ***Anastrepha fraterculus*** **(Wiedemann) species complex**	C	*Category : SUBSTANTIVE* **(160) China (28 Sep 2023 8:08 AM)** Adding the figure of whole adult of Anastrepha fraterculus	**Considered but not incorporated.** This species is a cryptic species complex not well characterized by a single exemplar. The presentation of character states is more diagnostically informative.
252	– ~~Both mediotergite~~ Subscutellum (Figure 5C) and ~~subscutellum~~ mediotergite ~~(Figure 5C)~~ with broad, dark-brown to black, lateral markings; scuto-scutellar suture usually with medial brown spot (as in Figure 4B and Figure 4D); 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 8H); wing pattern variable (Figure 7H). (Generalist pest.) ***Anastrepha fraterculus*** **(Wiedemann) species complex**	P	*Category : TECHNICAL* **(67) United States of America (20 Sep 2023 7:38 PM)** if you choose to this couplet to start with subscutellum	**Incorporated.**

Para	Text	T	Comment	SC's response
254	When a larva is detected in fruit, identification of the instar stage is not always certain. A newly molted third instar may be smaller than some fully developed second instars and less than half its potential fully developed size (Steck *et al.*, 2022). Typical relative sizes of the egg and three larval instars are shown in Figure 9A. The best characters to separate instars in all species are absolute sizes of the cephaloskeleton and spiracles: they never overlap between instars. However, these data are not published for second or first instars of most species. Another differentiating feature between second and third instars of *Anastrepha* is the presence or absence of a subapical tooth on the mouthhook: it is present in the second instar and subequal in size to the apical tooth (Figure 9B) but absent in the third instar (Figure 9C). Third instars of many Dacinae also have a subapical tooth, but usually it is much smaller than the apical tooth and not subequal in size (Figure 9D).	C	*Category : TECHNICAL* **(176) European Union (29 Sep 2023 6:24 PM)** No egg is visible in Figure 9A.	**Modified**. Removed the egg reference.
254	When a larva is detected in fruit, identification of the instar stage is not always certain. A newly molted third instar may be smaller than some fully developed second instars and less than half its potential fully developed size (Steck *et al.*, 2022). Typical relative sizes of the egg and three larval instars are shown in Figure 9A. The best characters to separate instars in all species are absolute sizes of the cephaloskeleton and spiracles: they never overlap between instars. However, these data are not published for second or first instars of most species. Another differentiating feature between second and third instars of *Anastrepha* is the presence or absence of a subapical tooth on the mouthhook: it is present in the second instar and subequal in size to the apical tooth (Figure 9B) but absent in the third instar (Figure 9C). Third instars of many Dacinae also have a subapical tooth, but usually it is much smaller than the apical tooth and not subequal in size (Figure 9D).	C	*Category : TECHNICAL* **(101) EPPO (22 Sep 2023 3:24 PM)** No egg is visible in Figure 9A	**Modified**. Removed the egg reference.
254	When a larva is detected in fruit, identification of the instar stage is not always certain. A newly molted third instar may be smaller than some fully developed second ~~instars~~ instars, and less than half its potential fully developed size (Steck *et al.*, 2022). Typical relative sizes of the egg and three larval instars are shown in Figure 9A. The best characters to separate instars in all species are absolute sizes of the cephaloskeleton and spiracles: they never overlap between instars. However, these data are not published for second or first instars of most species. Another differentiating feature between second and third instars of *Anastrepha* is the presence or absence of a subapical tooth on the mouthhook: it is present ~~in the second instar~~ and subequal in size to the apical tooth in the second instar (Figure 9B) but absent in the third instar (Figure 9C). Third instars of many	P	*Category : TECHNICAL* **(49) United States of America (19 Sep 2023 8:15 PM)**	**Incorporated.**

Para	Text	T	Comment	SC's response
	Dacinae also have a subapical tooth, but usually it is much smaller than the apical tooth and not subequal in size (Figure 9D).			
254	When a larva is detected in fruit, identification of the instar stage is not always certain. A newly molted third instar may be smaller than some fully developed second instars and less than half its potential fully developed size (Steck *et al.*, 2022). Typical relative sizes of the egg and three larval instars are shown in Figure 9A. The best characters to separate instars in all species are absolute sizes of the cephaloskeleton and spiracles: they never overlap between instars. However, these data are not published for second or first instars of most species. Another differentiating feature between second and third instars of *Anastrepha* is the presence or absence of a subapical tooth on the mouthhook: it is present in the second instar and subequal in size to the apical tooth (Figure 9B) but absent in the third instar (Figure 9C). Third instars of many Dacinae also have a subapical tooth, but usually it is much smaller than the apical tooth and not subequal in size (Figure 9D).	C	*Category : TECHNICAL* **(23) Kenya (28 Aug 2023 2:55 PM)** specs in size eg 1mm,2mm would be better when comparing	**Considered but not incorporated.** These structures are not always measured in exact lengths. The comparison of subapical to apical tooth is relative. Subequal is for when simialr but slightly smaller. If smaller then it will be very notable.
256	The following key is adapted from White and Elson-Harris (1992), Carroll *et al*. (2004), Frías *et al*. (2006) and Frias, Selivon and Hernández-Ortiz (2008).	C	*Category : EDITORIAL* **(102) EPPO (22 Sep 2023 3:24 PM)** Frías instead of Frias	**Incorporated.**
258	– Posterior spiracles nearly flush with body surface; tubercles, if present, on caudal segment only; with three posterior spiracular slits, elongate or oval, usually subparallel (Figure 2A and Figure 10A). (Tephritidae) **2**	C	*Category : TECHNICAL* **(50) United States of America (19 Sep 2023 8:16 PM)** It would be helpful toswitch all the couplets in this section around - it is easier to identify the target characters than the non-target characters.	**Considered but not incorporated.** These need to be compared at same time in key, and order would not impact use of the key.
265	1. Anterior spiracle ~~(Figure 2B and Figure 14)~~ () with ≥22 tubules (Figure 16A and Figure 16C). **2**	P	*Category : TECHNICAL* **(68) United States of America (20 Sep 2023 7:40 PM)** may not be necessary	**Considered but not incorporated.** It is true that this might not be necessary as states are simialr at couplet. But it helps draw attention to example of morphology in figures.
266	– Anterior spiracle ~~(Figure 2B and Figure 14)~~ with ≤22 tubules (Figure 16B, Figure 16D to Figure 16H). **3**	P	*Category : TECHNICAL* **(69) United States of America (20 Sep 2023 7:41 PM)** may not be necessary	**Considered but not incorporated.** It is true that this might not be necessary as states are simialr at couplet. But it helps draw attention to example of morphology in figures.
267	2. Anterior spiracle with 22–30 ~~tubules~~tubules (Figure 16A); caudal tubercles strongly reduced; posterior spiracular processes reduced (SP-I and SP-IV with 2–7 trunks, basal width ca. one-tenth the length of spiracular slits, and processes short). (Main hosts: papaya (*Carica papaya*); distribution: tropical Americas and United	P	*Category : TECHNICAL* **(70) United States of America (20 Sep 2023 7:42 PM)** Figure 21 may need labels of SP-I and SP-IV	**Modified**. Updated text.

Para	Text	T	Comment	SC's response
	States of America (Florida).) (Figure ~~16A and Figure~~ 21A.) ***Anastrepha curvicauda***			Updated Figure 21 with the location of SP-I, SP-II, SP-III, and SP-IV to image.
268	– Anterior spiracles with 28–37 ~~tubules~~tubules (Figure 16C); caudal sensilla normally developed; posterior spiracular processes normally developed (SP-I and SP-IV with 11–22 trunks, basal width ca. a quarter to a third the length of spiracular slits, and processes long). (Main hosts: Cucurbitaceae; distribution: Panama to Argentina.) (Figure ~~16C and Figure~~ 21C) ***Anastrepha grandis***	P	*Category : TECHNICAL* **(71) United States of America (20 Sep 2023 7:44 PM)**	**Incorporated.**
271	4. Oral ridges 6–10; preoral organ with four or more sensilla; dorsal posterior spiracular processes (SP-I) 13–22 with medium to wide bases. (Main hosts: fruits of Myrtaceae; distribution: tropical Americas.) ~~(Figure~~ (Figure 16G, Figure 17G, Figure 18G, Figure 19G, Figure ~~20G, Figure 16G~~ 20G and Figure 21G.) ***Anastrepha striata***	P	*Category : EDITORIAL* **(154) Colombia (27 Sep 2023 5:21 PM)** It is suggested to list figures in alphanumeric order.	**Incorporated.**
271	4. Oral ridges 6–10; preoral organ with four or more sensilla; dorsal posterior spiracular processes (SP-I) 13–22 with medium to wide bases. (Main hosts: fruits of Myrtaceae; distribution: tropical Americas.) (Figure 17G, Figure 18G, Figure 19G, Figure 20G, Figure 16G and Figure 21G.) ***Anastrepha striata***	C	*Category : TECHNICAL* **(72) United States of America (20 Sep 2023 7:44 PM)** May need a label in Figure 17G	**Modified.** A label would not be helpful in Fig 17G, because the preoral organ and sensilla are too small to see. A new figure (Figure 24) is added to the protocol that shows an example of a preoral organ and its sensilla clearly.
272	– Oral ridges 11–17; preoral organ with three sensilla; dorsal posterior spiracular processes (SP-I) 5–15 with narrow bases. (Main hosts: *Citrus* spp. (Rutaceae) or *Mangifera indica*; distribution: United States of America (southern Texas) to Panama.) (Figure 11B, Figure 12A, Figure 12B, Figure 14C, Figure 14D, Figure ~~23B, Figure~~ 16D, Figure 17D, Figure 18D, Figure 19D, Figure ~~20D~~ 20D, Figure 21D and Figure ~~21D~~23B.) ***Anastrepha ludens*** (some)	P	*Category : EDITORIAL* **(155) Colombia (27 Sep 2023 5:23 PM)** It is suggested to list figures in alphanumeric order.	**Incorporated.**
276	– Accessory plates ≤6. (Polyphagous pests; widely distributed but not Greater Antilles (*A. fraterculus* complex) or Greater Antilles and United States of America (Florida) (*A. suspensa*).) ~~(A, Figure~~ (Figure 9C, Figure 16H, Figure 17H, Figure 18H, Figure 19H, Figure 20H, Figure 21H and Figure 23A.) ***Anastrepha fraterculus* complex** (some), ***Anastrepha suspensa***	P	*Category : EDITORIAL* **(103) EPPO (22 Sep 2023 3:24 PM)** Typo	**Incorporated.**
284	Perpendicular to or at oblique angle to maxillary ~~palpus~~palpus (Figure 12)	P	*Category : TECHNICAL* **(73) United States of America (20 Sep 2023 7:53 PM)**	**Incorporated.**

Para	Text	T	Comment	SC's response
287	Absent (Figure 13C)	P	*Category : TECHNICAL* **(74) United States of America (20 Sep 2023 7:54 PM)**	**Incorporated.**
290	Numerous, elongate; accessory plates ~~present~~present (Figure 18)	P	*Category : TECHNICAL* **(75) United States of America (20 Sep 2023 7:54 PM)**	**Incorporated.**
293	Posterior region truncate, without distinct neck; preapical tooth absent; dental sclerite ~~absent~~ absent (Figure 20)	P	*Category : TECHNICAL* **(76) United States of America (20 Sep 2023 7:55 PM)**	**Incorporated.**
296	Usually bilobed, tubules in a single or double ~~row~~row (Figure 14)	P	*Category : TECHNICAL* **(77) United States of America (20 Sep 2023 7:56 PM)**	**Incorporated.**
299	~~Absent~~Absent (Figure 10B)	P	*Category : TECHNICAL* **(78) United States of America (20 Sep 2023 7:56 PM)**	**Incorporated.**
302	Spiracular slits elongate, dorsal and medial slits parallel, posterior slit at oblique ~~angle~~angle (Figure 21)	P	*Category : TECHNICAL* **(79) United States of America (20 Sep 2023 7:56 PM)**	**Incorporated.**
305	Two to eight frontal and one or two orbital setae; ocellar setae very weak or indistinct; postocular setae unicolorous (Figure 5A)	P	*Category : TECHNICAL* **(80) United States of America (20 Sep 2023 7:57 PM)**	**Incorporated.**
308	One postpronotal, two notopleural, one presutural supra-alar, one postsutural supra-alar, one postalar, one intra-alar, one dorsocentral, one acrostichal (rarely absent) and two scutellar setae (except in *curvicauda* group, where these setae are small and some may be absent) (Figure 5B)	P	*Category : TECHNICAL* **(81) United States of America (20 Sep 2023 7:58 PM)**	**Incorporated.**
311	Veins: Vein M_1 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*); anterior cubital cell (*cua*) with well-developed posteroapical ~~extension~~ extension (Figure 6A)	P	*Category : TECHNICAL* **(82) United States of America (20 Sep 2023 7:58 PM)**	**Incorporated.**
317	Lateral surstylus short or moderately 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 (Figure 6B)	P	*Category : TECHNICAL* **(83) United States of America (20 Sep 2023 7:59 PM)**	**Incorporated.**
320	Oviscape tube-like, variable in length; eversible membrane basally with dorsal hook-like sclerotized teeth usually in triangular or suboval pattern; aculeus well sclerotized, length variable, tip sometimes serrated on lateral ~~margins~~margins (Figure 1)	P	*Category : TECHNICAL* **(84) United States of America (20 Sep 2023 7:59 PM)**	**Incorporated.**
322	**Table 3.** Diagnostic morphological characters of adults of *Anastrepha* species	C	*Category : EDITORIAL* **(104) EPPO (22 Sep 2023 3:24 PM)**	**Considered but not incorporated.**

Para	Text	T	Comment	SC's response
			The position of the table could be horizontal: in such way, the species names could be entered horizontally, while the different characters' description would go vertically for better comparison.	Either format presentation will provide adequate identification information.
590	Hernández-Ortiz, V., Barradas-Juanz, N. and Díaz-Castelazo, C. 2019. A review of the natural host plants of the *Anastrepha fraterculus* complex in the Americas. *Area-wide management of fruit fly pests*, pp.~~89-122~~89–122. Martinez Alava, J.O. 2022. *Morfología y taxonomía de las formas inmaduras del género* Anastrepha *Schiner (DipteraTephritidae) para Colombia*. Bogotá D.C., Colombia, Universidad Nacional de Colombia. PhD dissertation.	P	*Category : EDITORIAL* **(105) EPPO (22 Sep 2023 3:24 PM)** Typo: long dash. Typo; new line	**Incorporated.**
592	*Steck, G.J., Carroll, L.E., Celedonio, H. & Guillen, J.C. 1990. Methods for identification of* Anastrepha *larvae (DipteraXTephritidae), and key to 13 species. Proceedings of the Entomological Society of Washington 92, 333–346.* **4.5 Molecular identification of economically important species of *Anastrepha***	C	*Category : EDITORIAL* **(106) EPPO (22 Sep 2023 3:24 PM)** No italics for authors	**Incorporated.**
593	Molecular diagnostic methods allow for the identification of the *Anastrepha* pest species *A. grandis*, *A. ludens*, *A. obliqua*, *A. serpentina*, *A. striata* and *A. suspensa*. The procedures described in Barr *et al*. (2017, 2018) target DNA using the polymerase chain reaction (PCR) and conventional sequencing. Guidance for sequencing the cytochrome oxidase subunit I (*COI*) gene is provided in Folmer *et al*. (1994) and Barr *et al*. (2018) and for the internal transcribed spacer 2 (ITS2) region in Ji, Zhang and He (2003) and Barr *et al*. (2017). These methods yield sequences that will allow diagnosticians to make accurate identifications. The analytical specificity of the sequence datasets was supported by sampling of the pests across a broad geographical and taxonomic range. Specificity is also supported by several molecular studies of pest genetic diversity in the genus *Anastrepha* (Boykin *et al*., 2010; Ruiz-Arce *et al*., 2012, 2015, 2019; Barr *et al*., 2017, 2018; Bartolini *et al*., 2020) and systematic relationships (McPheron *et al*., 1999; Smith-Caldas *et al*., 2001; Barr *et al*., 2005; Silva and Barr 2008; Mengual *et al*. 2017).	C	*Category : TECHNICAL* **(177) European Union (29 Sep 2023 6:26 PM)** It should be mentioned in this introductory paragraph why some species are excluded (fraterculus complex because being a complex; curvicauda because of insufficient data to diagnose).	**Modified**. New text was added to explain that A. curvicauda can be identified (based on newer datasets that are in preparation for publication) and that A. frateruclus species complex cannot be identified using these methods.
593	Molecular diagnostic methods allow for the identification of the *Anastrepha* pest species *A. grandis*, *A. ludens*, *A. obliqua*, *A. serpentina*, *A. striata* and *A. suspensa*. The procedures described in Barr *et al*. (2017, 2018) target DNA using the polymerase chain reaction (PCR) and conventional sequencing. Guidance for sequencing the cytochrome oxidase subunit I (*COI*) gene is provided in Folmer *et al*. (1994) and Barr *et al*. (2018) and for the internal transcribed spacer 2 (ITS2) region in Ji, Zhang and He (2003) and Barr *et al*. (2017). These methods yield sequences that will allow diagnosticians to make accurate identifications. The analytical specificity of the sequence datasets was supported by sampling of the pests across a broad geographical and taxonomic range. Specificity is also	C	*Category : SUBSTANTIVE* **(161) China (28 Sep 2023 8:09 AM)** Adding the related information of the high-throughput detection method for Anastrepha ludens and Anastrepha obliqua based on microfluidic dynamic array, including Primer/Probe sequences. This method published in Molecular Ecology Resources in 2016 by Chinese team and international collaborations (Jiang F., Fu W., Clarke A. R., Schutze M., K., Susanto A., Zhu S.F.*, Li Z.H. *. A high-throughput	**Considered but not incorporated.** The suggested publication is an excellent study but does not report a diagnostic that compares diversity among Anastrepha species. It is not focused on this genera and not appropriate for this section of the protocol text.

Para	Text	T	Comment	SC's response
	supported by several molecular studies of pest genetic diversity in the genus *Anastrepha* (Boykin *et al*., 2010; Ruiz-Arce *et al*., 2012, 2015, 2019; Barr *et al*., 2017, 2018; Bartolini *et al*., 2020) and systematic relationships (McPheron *et al*., 1999; Smith-Caldas *et al*., 2001; Barr *et al*., 2005; Silva and Barr 2008; Mengual *et al*. 2017).		detection method for invasive fruit fly (Diptera: Tephritidae) species based on microfluidic dynamic array. Molecular Ecology Resources, 2016, 16: 1378–1388	
593	Molecular diagnostic methods allow for the identification of the *Anastrepha* pest species *A. grandis*, *A. ludens*, *A. obliqua*, *A. serpentina*, *A. striata* and *A. suspensa*. The procedures described in Barr *et al*. (2017, 2018) target DNA using the polymerase chain reaction (PCR) and conventional sequencing. Guidance for sequencing the cytochrome oxidase subunit I (*COI*) gene is provided in Folmer *et al*. (1994) and Barr *et al*. (2018) and for the internal transcribed spacer 2 (ITS2) region in Ji, Zhang and He (2003) and Barr *et al*. (2017). These methods yield sequences that will allow diagnosticians to make accurate identifications. The analytical specificity of the sequence datasets was supported by sampling of the pests across a broad geographical and taxonomic range. Specificity is also supported by several molecular studies of pest genetic diversity in the genus *Anastrepha* (Boykin *et al*., 2010; Ruiz-Arce *et al*., 2012, 2015, 2019; Barr *et al*., 2017, 2018; Bartolini *et al*., 2020) and systematic relationships (McPheron *et al*., 1999; Smith-Caldas *et al*., 2001; Barr *et al*., 2005; Silva and Barr 2008; Mengual *et al*. 2017).	C	*Category : EDITORIAL* **(108) EPPO (22 Sep 2023 3:24 PM)** The citation of references with 3 authors in the text is not consistent - here an example.	**Incorporated.**
593	Molecular diagnostic methods allow for the identification of the *Anastrepha* pest species *A. grandis*, *A. ludens*, *A. obliqua*, *A. serpentina*, *A. striata* and *A. suspensa*. The procedures described in Barr *et al*. (2017, 2018) target DNA using the polymerase chain reaction (PCR) and conventional sequencing. Guidance for sequencing the cytochrome oxidase subunit I (*COI*) gene is provided in Folmer *et al*. (1994) and Barr *et al*. (2018) and for the internal transcribed spacer 2 (ITS2) region in Ji, Zhang and He (2003) and Barr *et al*. (2017). These methods yield sequences that will allow diagnosticians to make accurate identifications. The analytical specificity of the sequence datasets was supported by sampling of the pests across a broad geographical and taxonomic range. Specificity is also supported by several molecular studies of pest genetic diversity in the genus *Anastrepha* (Boykin *et al*., 2010; Ruiz-Arce *et al*., 2012, 2015, 2019; Barr *et al*., 2017, 2018; Bartolini *et al*., 2020) and systematic relationships (McPheron *et al*., 1999; Smith-Caldas *et al*., 2001; Barr *et al*., 2005; Silva and Barr 2008; Mengual *et al*. 2017).	C	*Category : TECHNICAL* **(107) EPPO (22 Sep 2023 3:24 PM)** It should be mentioned in this introductory paragraph why some species are excluded (fraterculus complex because being a complex; curvicauda because of insufficient data to diagnose)	**Incorporated.**
594	**4.5.1 DNA extraction methods**	C	*Category : TECHNICAL* **(51) United States of America (19 Sep 2023 8:18 PM)**	**Modified**.

Para	Text	T	Comment	SC's response
			This section is not so much about DNA extraction as preservation of specimens for DNA extraction. It would be better to move most of it to section 4.2.	The majority of the section is focused on extraction. It is helpful to have the informaiton of preservation and extraction together. To better describe the section it is renamed as DNA preservation and extraction methods. In addition, text is located in sections 4.1.2 (adult) and 4.2 (immature) to refer reader to 4.5.1 for more information.
595	Specimens should be stored in ~~70–100%~~ minimum 70% ethanol (Vink *et al*., 2005) immediately after collecting and then either maintained in ≥95% ethanol or kept at −20 °C or lower temperatures with or without ≥95% ethanol to minimize the degradation of nucleic acids. Commercial kits are effective for isolating DNA. In addition, Armstrong and Ball (2005) and Boykin *et al.* (2014) provide procedures that have been shown to successfully isolate sufficient quantities of DNA from a single leg for PCR.	P	*Category : TECHNICAL* **(179) European Union (29 Sep 2023 6:28 PM)** It is not possible to have 100% ethanol so it is proposed to change the text for 'minimum 70%'.	**Incorporated.**
595	Specimens should be stored in 70–100% ethanol (Vink *et al*., 2005) immediately after collecting and then either maintained in ≥95% ethanol or kept at −20 °C or lower temperatures with or without ≥95% ethanol to minimize the degradation of nucleic acids. Commercial kits are effective for isolating DNA. In addition, Armstrong and Ball (2005) and Boykin *et al.* (2014) provide procedures that have been shown to successfully isolate sufficient quantities of DNA from a single leg for PCR.	C	*Category : TECHNICAL* **(178) European Union (29 Sep 2023 6:27 PM)** It is not clear if the samples should be dried before being transferred at -20°C (if stored without >95% ethanol)? Also, it should be indicated that the ethanol has to be removed prior DNA extraction.	**Modified**. If stored in ethanol it is recommended to maintain in ethanol at cold temperature. The text is modified to simplify recommendation. Storage as dry specimens (post removal of ethanol or freeze immediately after killing) is suitable but should be kept at very cold temperatures. There is no benefit in describing this less common practice. Those working in insect molecular labs will know this common practice.
595	Specimens should be stored in minimum 70% ethanol (Vink *et al*., 2005) immediately after collecting and then either maintained in ≥95% ethanol or kept at −20 °C or lower temperatures with or without ≥95% ethanol to minimize the degradation of nucleic acids. Commercial kits are effective for isolating DNA. In addition, Armstrong and Ball (2005) and Boykin *et al.* (2014) provide procedures that have been shown to successfully isolate sufficient quantities of DNA from a single leg for PCR.	C	*Category : TECHNICAL* **(110) EPPO (22 Sep 2023 3:24 PM)** It is not clear if the samples should be dried before being transferred at -20°C (if stored without >95% ethanol)? Also, it should be indicated that the ethanol has to be removed prior DNA extraction.	**Modified**. If stored in ethanol it is recommended to maintain in ethanol at cold temperature. The text is modified to simplify recommendation. Storage as dry specimens (post removal of ethanol or freeze immediately after killing) is suitable but should be kept at very cold temperatures. There is no benefit in

Para	Text	T	Comment	SC's response
				describing this less common practice. Those working in insect molecular labs will know this common practice
595	Specimens should be stored in ~~70–100%~~ minimum 70% ethanol (Vink *et al*., 2005) immediately after collecting and then either maintained in ≥95% ethanol or kept at −20 °C or lower temperatures with or without ≥95% ethanol to minimize the degradation of nucleic acids. Commercial kits are effective for isolating DNA. In addition, Armstrong and Ball (2005) and Boykin *et al.* (2014) provide procedures that have been shown to successfully isolate sufficient quantities of DNA from a single leg for PCR.	P	*Category : TECHNICAL* **(109) EPPO (22 Sep 2023 3:24 PM)** It is not possible to have 100% ethanol so it is proposed to change the text for 'minimum 70%'	**Incorporated.**
596	In cases where molecular and morphological methods are to be used, it is recommended that a portion of the larva (such as abdominal segment 4 or 5) be excised, or a hind leg be removed, and stored in ethanol for DNA extraction. The remaining specimen can be prepared for morphological work.	C	*Category : TECHNICAL* **(180) European Union (29 Sep 2023 6:29 PM)** This part is not consistent with lines 466/467. The two sentences should be merged so it is clear which part of the body should be removed for DNA extraction.	**Modified.** These terms are consistent. A4 and A5 are indicated in Fiugure 2A. Reference has been added for clarity.
596	In cases where molecular and morphological methods are to be used, it is recommended that a portion of the larva (such as abdominal segment 4 or 5) be excised, or a hind leg be removed, and stored in ethanol for DNA extraction. The remaining specimen can be prepared for morphological work.	C	*Category : TECHNICAL* **(111) EPPO (22 Sep 2023 3:24 PM)** This part is not consistent with lines 466/467. The two sentences should be merged so it is clear which part of the body should be removed for DNA extraction.	**Modified.** These terms are consistent. A4 and A5 are indicated in Fiugure 2A. Reference has been added for clarity.
597	For larvae (prepared according to section 4.2), the mid-section of the body can be removed, leaving the head and caudal areas intact. This approach is minimally invasive and recommended because the remaining specimen can be used for future studies including morphological identifications (Barr and McPheron, 2006). Preparation of larvae for morphological examination includes a boiling step before storage. This boiling step is compatible with molecular study but not required to process larvae in molecular analyses. The boiling step is recommended if a voucher of the specimen is to be retained for morphological examination. It is possible to soak larvae in DNA extraction lysis buffers overnight to isolate nucleic acids from specimens, and then use the larvae in slide mounting. These buffer-soaked larvae, however, are not appropriate for SEM examination.	C	*Category : TECHNICAL* **(182) European Union (29 Sep 2023 6:30 PM)** Inconsistent with line 162 where it is indicated that larvae should be placed in water at 60-100°C. A boiling step suggests that the water should be boiling (100°C).	**Modified** Line 223 of section 4.2 states to kill larva in water at 65-100 oC using hot water treatment. The text has been updated to indicate hot water treatment or boiling can be used to avoid inconsistency in sections.
597	For larvae (prepared according to section 4.2), the mid-section of the body can be removed, leaving the head and caudal areas intact. This approach is minimally invasive and recommended because the remaining specimen can be used for future studies including morphological identifications (Barr and McPheron, 2006). Preparation of larvae for morphological examination includes a boiling step before storage. This boiling step is compatible with molecular study	C	*Category : TECHNICAL* **(181) European Union (29 Sep 2023 6:29 PM)** It is unclear if the boiling step is needed or not in this case.	**Modified** It can be done on larvae with or without boiling. New text added: "It is possible to soak larvae (that were hot water treated or not) in DNA extraction lysis buffers overnight to isolate nucleic acids from specimens,

Para	Text	T	Comment	SC's response
	but not required to process larvae in molecular analyses. The boiling step is recommended if a voucher of the specimen is to be retained for morphological examination. It is possible to soak larvae in DNA extraction lysis buffers overnight to isolate nucleic acids from specimens, and then use the larvae in slide mounting. These buffer-soaked larvae, however, are not appropriate for SEM examination.			and then use the larvae in slide mounting."
597	For larvae (prepared according to section 4.2), the mid-section of the body can be removed, leaving the head and caudal areas intact. This approach is minimally invasive and recommended because the remaining specimen can be used for future studies including morphological identifications (Barr and McPheron, 2006). Preparation of larvae for morphological examination includes a boiling step before storage. This boiling step is compatible with molecular study but not required to process larvae in molecular analyses. The boiling step is recommended if a voucher of the specimen is to be retained for morphological examination. It is possible to soak larvae in DNA extraction lysis buffers overnight to isolate nucleic acids from specimens, and then use the larvae in slide mounting. These buffer-soaked larvae, however, are not appropriate for SEM examination.	C	*Category : TECHNICAL* **(113) EPPO (22 Sep 2023 3:25 PM)** It is unclear if the boiling step is needed or not in this case.	**Modified** It can be done on larvae with or without boiling. New text added: "It is possible to soak larvae (that were hot water treated or not) in DNA extraction lysis buffers overnight to isolate nucleic acids from specimens, and then use the larvae in slide mounting."
597	For larvae (prepared according to section 4.2), the mid-section of the body can be removed, leaving the head and caudal areas intact. This approach is minimally invasive and recommended because the remaining specimen can be used for future studies including morphological identifications (Barr and McPheron, 2006). Preparation of larvae for morphological examination includes a boiling step before storage. This boiling step is compatible with molecular study but not required to process larvae in molecular analyses. The boiling step is recommended if a voucher of the specimen is to be retained for morphological examination. It is possible to soak larvae in DNA extraction lysis buffers overnight to isolate nucleic acids from specimens, and then use the larvae in slide mounting. These buffer-soaked larvae, however, are not appropriate for SEM examination.	C	*Category : TECHNICAL* **(112) EPPO (22 Sep 2023 3:25 PM)** Inconsistent with line 162 where it is indicated that larvae should be placed in water at 60-100°C. A boiling step suggests that the water should be boiling (100°C).	**Modified** Line 223 of section 4.2 states to kill larva in water at 65-100 oC using hot water treatment. The text has been updated to indicate hot water treatment or boiling can be used to avoid inconsistency in sections.
644	Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: ~~294–9~~294–299.	P	*Category : EDITORIAL* **(114) EPPO (22 Sep 2023 3:25 PM)** For consistency with the other references.	**Incorporated.**
646	A method for amplifying ITS2 in *Anastrepha* DNA was reported in Ji et al. (2003) and Barr *et al*. (2017). The primer set used in the study results in PCR products of variable length (230–290 bp). The fragment size of the amplicons is not used to diagnose the species. Fixed differences between species caused by nucleotide substitutions and insertions were used to diagnose three species in the study: *A. ludens*, *A. obliqua* and *A. suspensa*. Table 6 provides a version of the Barr *et al*.	P	*Category : SUBSTANTIVE* **(162) China (28 Sep 2023 8:10 AM)** Ji et al (2003) also reported a method for amplifying ITS2 in Anastrepha DNA, and the forward primer being from Ji et al. (2003). So add Ji et al. (2003) here.	**Incorporated.**

Para	Text	T	Comment	SC's response
	(2017) PCR master mix composition and the primers used, with cycling parameters modified for PCR amplification.			
646	A method for amplifying ITS2 in *Anastrepha* DNA was reported in Barr *et al*. (2017). The primer set used in ~~the study~~ Author (year) results in PCR products of variable length (230–290 bp). The fragment size of the amplicons is not used to diagnose the species. Fixed differences between species caused by nucleotide substitutions and insertions were used to diagnose three species in the study: *A. ludens*, *A. obliqua* and *A. suspensa*. Table 6 provides a version of the Barr *et al*. (2017) PCR master mix composition and the primers used, with cycling parameters modified for PCR amplification.	P	*Category : TECHNICAL* **(64) Japan (20 Sep 2023 7:45 AM)** The length of PCR products (230-290bp) is not indicated in Barr et al. 2017. Reference on the primer set should be indicated.	**Modified**. The author was added. However, the details on the range of products were not explicitly stated in the publication. It was available from the method and data records. These values were confirmed and reported in this protocol based on that data. There is no paper to cite with range reported in text.
647	The ITS2 oligonucleotide primers used are as follows, the forward primer being from Ji *et al*. (2003) and the reverse primer from Barr *et al*. (2017):	C	*Category : EDITORIAL* **(115) EPPO (22 Sep 2023 3:25 PM)** The citation of references with 3 authors in the text is not consistent - here an example.	**Incorporated.**
697	The use of a bidirectional sequencing approach to PCR products will yield two DNA sequence reads for the same DNA target but in opposite directions. The instrument output will provide the user with sequence data reported as text, the instrument trace signal (chromatogram) and quality scores (Phred). This information will help in the determination of nucleotide base calls (the assignment of bases from the chromatogram) that will provide a more accurate read during the editing process. Using software or manual alignment methods, the forward and reverse sequences for the same DNA sample should then be aligned to create a consensus sequence. The consensus sequence must be visually inspected for accurate calls. Sites that are not corroborated by data in both sequences should not be considered as accurate and should be assigned as an ambiguous base (i.e. N = A, C, T or G). If multiple peaks are observed at a nucleotide site in both the forward-primed and reverse-primed sequences, or both show high quality scores (>30) but are conflicting calls, then the site should be assigned as an ambiguous base (i.e. N) in the consensus sequence. Diagnosis should only be performed on edited sequences having less than 0.5% ambiguous bases. The final length of the query sequence should be approximately ~~600~~ 709 base pairs (bp) for *COI*. The final length of the edited ITS2 sequence after primers are removed ranges from 179 to 239 bp. Additional information on data editing processes is available in EPPO (2021).	P	*Category : SUBSTANTIVE* **(163) China (28 Sep 2023 8:11 AM)** "The final length of the query sequence should be approximately 600 base pairs (bp) for COI". add "cutting of the primers" to "The final length of the query sequence should be approximately 600 base pairs (bp) for COI cutting of the primers."	**Modiifed.** The final length of the amplified product in Table 5 includes primer sequences. After removal of primers and removal of poor base calls, the researcher should expect a edited sequence of 600-650 bases. It is not exact but should not be 709 bp. New text is added: "The final length of the query sequence should be approximately 600-650 base pairs (bp) for *COI* (after removal of primers and poor-quality data at ends)."
698	Once a consensus sequence is generated, the query for either *COI* or ITS2 can be performed using the default setting of the Basic Local Alignment Search Tool for nucleotides (BLASTN) of National Center for Biology Information: . The best	C	*Category : TECHNICAL* **(184) European Union (29 Sep 2023 6:32 PM)**	**Modiifed.**

Para	Text	T	Comment	SC's response
	sequence match between the consensus and the database as measured with the highest Max Score is considered to be a species in the genus *Anastrepha*. If the consensus is a best match to an *Anastrepha* record, then the consensus sequence is appropriate for further comparison and interpretation in this diagnostic protocol for each species (section 4.5.6 and section 4.5.7). If the consensus is a best match to DNA other than a *Anastrepha* record, then the consensus sequence probably represents DNA from a contaminant or an *Anastrepha* species not previously reported. No pseudogenes or intra-individual copies were found to occur in the species examined with *COI* by Barr *et al*. (2018) and with ITS2 by Barr *et al*. (2017). Nevertheless, the consensus sequence of the *COI* gene should be translated into an amino acid sequence and compared to the amino acid translation of *Anastrepha* records to detect evidence of premature stop codons and reading-frame shifts that suggest a pseudogene has been amplified and sequenced. The taxonomic representation in the ITS2 data is not as comprehensive as the *COI* sequence resource. Consequently, although use of *COI* is recommended for molecular confirmation of *A. ludens*, the *COI* sequence data are insufficient to diagnose *A. obliqua* and *A. suspensa*. For complete identification of *A. obliqua* and *A. suspensa*, both ITS2 data and *COI* data are used.		For COI, could additional databases (e.g. BOLD, EPPO-Q-bank) be used?	Other resources could be used. The statement indicatres that NCBI can be used to accomplish this. New text is added to explain that other databases could be used. " Other databases with comparable species representation to NCBI could be used instead, but performance should be verified by laboratory."
698	Once a consensus sequence is generated, the query for either *COI* or ITS2 can be performed using the default setting of the Basic Local Alignment Search Tool for nucleotides (BLASTN) of National Center for Biology Information: . The best sequence match between the consensus and the database as measured with the highest Max Score is considered to be a species in the genus *Anastrepha*. If the consensus is a best match to an *Anastrepha* record, then the consensus sequence is appropriate for further comparison and interpretation in this diagnostic protocol for each species (section 4.5.6 and section 4.5.7). If the consensus is a best match to DNA other than a *Anastrepha* record, then the consensus sequence probably represents DNA from a contaminant or an *Anastrepha* species not previously reported. No pseudogenes or intra-individual copies were found to occur in the species examined with *COI* by Barr *et al*. (2018) and with ITS2 by Barr *et al*. (2017). Nevertheless, the consensus sequence of the *COI* gene should be translated into an amino acid sequence and compared to the amino acid translation of *Anastrepha* records to detect evidence of premature stop codons and reading-frame shifts that suggest a pseudogene has been amplified and sequenced. The taxonomic representation in the ITS2 data is not as comprehensive as the *COI* sequence resource. Consequently, although use of *COI* is recommended for molecular confirmation of *A. ludens*, the *COI* sequence data are insufficient to	C	*Category : TECHNICAL* **(183) European Union (29 Sep 2023 6:31 PM)** Why does the sentence only refers to A. ludens here and not the other species? The 8 species should be referred to. It is suggested to move this information in introduction and to make it clear for each species if barcoding can or not be used for identification and, if it can be used, with which genes.	**Modified.** *Removed old text.* *New text to 4.5 para 593: "Identification of the A. fraterculus species complex is not supported using the currently available molecular methods. Identification of A. curvicauda, A. grandis, A. ludens, A. serpentina, and A. striata can be completed using COI sequences. The COI sequence data, however, are insufficient to diagnose A. obliqua and A. suspensa. For complete identification of A. obliqua and A. suspensa, both ITS2 data and COI data are used."*

Para	Text	T	Comment	SC's response
	diagnose *A. obliqua* and *A. suspensa*. For complete identification of *A. obliqua* and *A. suspensa*, both ITS2 data and *COI* data are used.			
698	Once a consensus sequence is generated, the query for either *COI* or ITS2 can be performed using the default setting of the Basic Local Alignment Search Tool for nucleotides (BLASTN) of National Center for Biology Information: . The best sequence match between the consensus and the database as measured with the highest Max Score is considered to be a species in the genus *Anastrepha*. If the consensus is a best match to an *Anastrepha* record, then the consensus sequence is appropriate for further comparison and interpretation in this diagnostic protocol for each species (section 4.5.6 and section 4.5.7). If the consensus is a best match to DNA other than a *Anastrepha* record, then the consensus sequence probably represents DNA from a contaminant or an *Anastrepha* species not previously reported. No pseudogenes or intra-individual copies were found to occur in the species examined with *COI* by Barr *et al*. (2018) and with ITS2 by Barr *et al*. (2017). Nevertheless, the consensus sequence of the *COI* gene should be translated into an amino acid sequence and compared to the amino acid translation of *Anastrepha* records to detect evidence of premature stop codons and reading-frame shifts that suggest a pseudogene has been amplified and sequenced. The taxonomic representation in the ITS2 data is not as comprehensive as the *COI* sequence resource. Consequently, although use of *COI* is recommended for molecular confirmation of *A. ludens*, the *COI* sequence data are insufficient to diagnose *A. obliqua* and *A. suspensa*. For complete identification of *A. obliqua* and *A. suspensa*, both ITS2 data and *COI* data are used.	C	*Category : TECHNICAL* **(117) EPPO (22 Sep 2023 3:25 PM)** Why does the sentence only refers to A. ludens here and not the other species? The 8 species should be referred to. It is suggested to move this information in introduction and to make it clear for each species if barcoding can or not be used for identification and, if it can be used, with which genes.	**Modified.** *Removed old text.* *New text to 4.5 para 593: "Identification of the A. fraterculus species complex is not supported using the currently available molecular methods. Identification of A. curvicauda, A. grandis, A. ludens, A. serpentina, and A. striata can be completed using COI sequences. The COI sequence data, however, are insufficient to diagnose A. obliqua and A. suspensa. For complete identification of A. obliqua and A. suspensa, both ITS2 data and COI data are used."*
698	Once a consensus sequence is generated, the query for either *COI* or ITS2 can be performed using the default setting of the Basic Local Alignment Search Tool for nucleotides (BLASTN) of National Center for Biology Information: . The best sequence match between the consensus and the database as measured with the highest Max Score is considered to be a species in the genus *Anastrepha*. If the consensus is a best match to an *Anastrepha* record, then the consensus sequence is appropriate for further comparison and interpretation in this diagnostic protocol for each species (section 4.5.6 and section 4.5.7). If the consensus is a best match to DNA other than a *Anastrepha* record, then the consensus sequence probably represents DNA from a contaminant or an *Anastrepha* species not previously reported. No pseudogenes or intra-individual copies were found to occur in the species examined with *COI* by Barr *et al*. (2018) and with ITS2 by Barr *et al*. (2017). Nevertheless, the consensus sequence of the *COI* gene should be translated into an amino acid sequence and compared to the amino acid translation	C	*Category : TECHNICAL* **(116) EPPO (22 Sep 2023 3:25 PM)** For COI, could additional databases (e.g. BOLD, EPPO-Q-bank) be used?	**Modiifed.** Other resources could be used. The statement indicates that NCBI can be used to accomplish this. New text is added to explain that other databases could be used. " Other databases with comparable species representation to NCBI could be used instead, but performance should be verified by laboratory."

Para	Text	T	Comment	SC's response
	of *Anastrepha* records to detect evidence of premature stop codons and reading-frame shifts that suggest a pseudogene has been amplified and sequenced. The taxonomic representation in the ITS2 data is not as comprehensive as the *COI* sequence resource. Consequently, although use of *COI* is recommended for molecular confirmation of *A. ludens*, the *COI* sequence data are insufficient to diagnose *A. obliqua* and *A. suspensa*. For complete identification of *A. obliqua* and *A. suspensa*, both ITS2 data and *COI* data are used.			
698	Once a consensus sequence is ~~generated~~generated from an unknown sample, the query for either *COI* or ITS2 can be performed using the default setting of the Basic Local Alignment Search Tool for nucleotides (BLASTN) of National Center for Biology Information: . The best sequence match between the consensus and the database as measured with the highest Max Score is considered to be a species in the genus *Anastrepha*. If the ~~consensus~~ unknown is a best match to an *Anastrepha* record, then the ~~consensus~~ unknown sequence is appropriate for further comparison and interpretation in this diagnostic protocol for each species (section 4.5.6 and section 4.5.7). If the ~~consensus~~ unknown is a best match to DNA other than ~~a~~ an *Anastrepha* record, then the ~~consensus~~ sequence probably represents DNA from a contaminant or an *Anastrepha* species not previously reported. No pseudogenes or intra-individual copies were found to occur in the species examined with *COI* by Barr *et al*. (2018) and with ITS2 by Barr *et al*. (2017). Nevertheless, the ~~consensus~~ sequence of the *COI* gene should be translated into an amino acid sequence and compared to the amino acid translation of *Anastrepha* records to detect evidence of premature stop codons and reading-frame shifts that suggest a pseudogene ~~has~~ may have been amplified and sequenced. The taxonomic representation ~~in the~~ of ITS2 data is not as comprehensive as the *COI* sequence resource. ~~Consequently, although use of~~ *COI* ~~is recommended for molecular confirmation~~ on its own can confirm an identification of *A. ludens*, but the *COI* sequence data are insufficient to diagnose *A. obliqua* and *A. suspensa*. For complete identification of *A. obliqua* and *A. suspensa*, both ITS2 data and *COI* data are used.	P	*Category : TECHNICAL* **(52) United States of America (19 Sep 2023 8:32 PM)**	**Modified.** Text incoproated but removed final sentences in response to other comment.
700	To identify a specimen using *COI*, the consensus *COI* sequence can be compared to reference sequences reported in Barr *et al*. (2017): GenBank KU511143–KU511157, MF695132–MF695457, MF695459–MF695586 and MF838771–MF838840. The edited consensus sequence and GenBank reference sequences can be aligned using CLUSTAL W (Thompson, Gibson and Higgins, 2003) and the alignment used to calculate uncorrected, pairwise *p*-distance estimates in Molecular Evolutionary Genetic Analysis (MEGA) software (Kumar, Stecher and Tamura, 2016). Barr *et al*. (2017) demonstrated that a barcode gap exists for the	C	*Category : TECHNICAL* **(185) European Union (29 Sep 2023 6:33 PM)** More explanation is needed to explain why there is insufficient data available. There are COI data available in BOLD (originating from three countries) and they form a single BIN.	**Modified.** This species was not included in prior study because it was in another genus. The authors have data generated for this species and confimred that methods used by Barr et al. (2017) are appropriate for this pest as well. The protocol is being

Para	Text	T	Comment	SC's response
	species *A. grandis*, *A. ludens*, *A. serpentina* and *A. striata*, and that *p*-distance estimates can be used to diagnose these species. Phylogenetic analysis of *COI* can also be used to diagnose these four species. The *COI* data are not sufficient to diagnose *A. fraterculus*, *A. obliqua* or *A. suspensa* and there are insufficient data to diagnose *A. curvicauda*.			revised with new informaiton to complete ID of A. curvicauda.
700	To identify a specimen using *COI*, the consensus *COI* sequence can be compared to reference sequences reported in Barr *et al*. (2017): GenBank KU511143–KU511157, MF695132–MF695457, MF695459–MF695586 and MF838771–MF838840. The edited consensus sequence and GenBank reference sequences can be aligned using CLUSTAL W (Thompson, Gibson and Higgins, 2003) and the alignment used to calculate uncorrected, pairwise *p*-distance estimates in Molecular Evolutionary Genetic Analysis (MEGA) software (Kumar, Stecher and Tamura, 2016). Barr *et al*. (2017) demonstrated that a barcode gap exists for the species *A. grandis*, *A. ludens*, *A. serpentina* and *A. striata*, and that *p*-distance estimates can be used to diagnose these species. Phylogenetic analysis of *COI* can also be used to diagnose these four species. The *COI* data are not sufficient to diagnose *A. fraterculus*, *A. obliqua* or *A. suspensa* and there are insufficient data to diagnose *A. curvicauda*.	C	*Category : TECHNICAL* **(118) EPPO (22 Sep 2023 3:25 PM)** More explanation is needed to explain why there is insufficient data available. There are COI data available in BOLD (originating from three countries) and they form a single BIN.	**Modified.** This species was not included in prior study because it was in another genus. The authors have data generated for this species and confimred that methods used by Barr et al. (2017) are appropriate for this pest as well. The protocol is being revised with new informaiton to complete ID of A. curvicauda.
700	To identify a specimen using *COI*, ~~the consensus~~ its *COI* sequence can be compared to reference sequences reported in Barr *et al*. (2017): GenBank KU511143–KU511157, MF695132–MF695457, MF695459–MF695586 and MF838771–MF838840. The edited consensus sequence and GenBank reference sequences can be aligned using CLUSTAL W (Thompson, Gibson and Higgins, 2003) and the alignment used to calculate uncorrected, pairwise *p*-distance estimates in Molecular Evolutionary Genetic Analysis (MEGA) software (Kumar, Stecher and Tamura, 2016). Barr *et al*. (2017) demonstrated that a barcode gap exists for the species *A. grandis*, *A. ludens*, *A. serpentina* and *A. striata*, and that *p*-distance estimates can be used to diagnose these species. Phylogenetic analysis of *COI* can also be used to diagnose these four species. The *COI* data are not sufficient to diagnose *A. fraterculus*, *A. obliqua* or *A. suspensa* and there are insufficient data to diagnose *A. curvicauda*.	P	*Category : TECHNICAL* **(53) United States of America (19 Sep 2023 9:26 PM)** Isn't this pretty much what a BLAST search does?	**Considered but not incorporated.** The BLAST search is a local optimized alignment approach and the methods described here use global alignment approach. These can result in different ourcomes especially in presence of pseudocopies or poor sequence data.
706	Alternatively, to diagnose *A. grandis*, *A. ludens*, *A. serpentina* or *A. striata* using *COI* ~~phylogenetic tree~~ phylogenetic analysis, the alignment including all the reference sequences of Barr *et al*. (2017) and the ~~edited consensus~~ unknown sequence can be analysed in a character-based tree search (e.g. maximum likelihood or maximum parsimony). Identification as one of the four species	P	*Category : TECHNICAL* **(54) United States of America (19 Sep 2023 9:27 PM)**	**Incorporated.**

Para	Text	T	Comment	SC's response
	requires two conditions to be observed in the tree topology using the revised criteria for tree-based identification of Meier *et al*. (2006):			
708	Condition 2: The consensus sequence is ~~at least one node into~~ nested within the clade in condition 1 (i.e. the consensus sequence is not the sister taxon to all of the conspecific reference sequences in the clade).	P	*Category : TECHNICAL* **(55) United States of America (19 Sep 2023 9:29 PM)**	**Incorporated.**
711	To identify a specimen as *A. obliqua* or *A. suspensa*, both the *COI* and ITS2 sequences must be analysed. First, a phylogenetic ~~tree-based~~ analysis as described in section 4.5.6 using the *COI* consensus sequence should be completed. In addition, the edited ITS2 consensus sequence should be compared to each of the ITS2 reference sequences found in GenBank (KU510999–KU511142; PopSet 1046760793) using *p*-distance pairwise comparisons.	P	*Category : TECHNICAL* **(56) United States of America (19 Sep 2023 9:31 PM)**	**Incorporated.**
714	Condition 2: The consensus ITS2 sequence is identical to an ITS2 record of *A. obliqua* (i.e. there are no base-substitution differences and no ~~inserts~~ insertions or deletions between the two sequences).	P	*Category : EDITORIAL* **(57) United States of America (19 Sep 2023 9:32 PM)**	**Incorporated.**
717	Condition 2: The consensus ITS2 sequence is identical to an ITS2 record of *A. suspensa* (i.e. there are no base-substitution differences and no ~~inserts~~ insertions or deletions between the two sequences).	P	*Category : EDITORIAL* **(58) United States of America (19 Sep 2023 9:33 PM)**	**Incorporated.**
733	Ministry of Agriculture and Rural Development (MARD), Plant Protection Department (PPD), Plant Quarantine Diagnostic Centre (PQDC), Viet Nam; Hoang Kim Thoa; email: or).	C	*Category : EDITORIAL* **(120) EPPO (22 Sep 2023 3:25 PM)** Consistency to ensure with section 7 (Acknowledgments).	**Acknowledged**
733	Ministry of Agriculture and Rural Development (MARD), Plant Protection Department (PPD), Plant Quarantine Diagnostic Centre (PQDC), Viet ~~Nam; Hoang~~ Nam (Hoang Kim Thoa; email: or).	P	*Category : EDITORIAL* **(119) EPPO (22 Sep 2023 3:25 PM)** Typo	**Acknowledged**
736	This protocol was revised by Ignacio Dumois (SENASA, Argentina (see preceding section)), Thoa Kim-Hoang (PQDC, MARD, Viet Nam (see preceding section)) Allen Norrbom (Systematic Entomology Laboratory, USDA, United States of America (see preceding section)), Raul Ruiz-Arce (APHIS, USDA, United States of America), and Gary Steck (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, United States of America (see preceding section)). The first adopted version of the protocol was written by ~~A.~~ Alicia Basso (Universidad de Buenos Aires, Facultad de Agronomía, Argentina (see preceding section)), and ~~V.~~ Vicente Hernández-Ortiz (Instituto de Ecología A.C., Red de Interacciones Multitróficas, México (see preceding section)) with the collaboration of ~~N.~~ Norma Vaccaro (Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Concordia, Argentina).	P	*Category : EDITORIAL* **(122) EPPO (22 Sep 2023 3:25 PM)** For consistency within the paragraph and with section 6 (Contact points for further information).	**Incorporated.**

Para	Text	T	Comment	SC's response
736	This protocol was revised by Ignacio Dumois (SENASA, Argentina (see preceding section)), Thoa Kim-Hoang (PQDC, MARD, Viet Nam (see preceding section)) Allen Norrbom (Systematic Entomology Laboratory, USDA, United States of America (see preceding section)), Raul Ruiz-Arce (APHIS, USDA, United States of America (see preceding section)), and Gary Steck (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, United States of America (see preceding section)). The first adopted version of the protocol was written by A. Basso (Universidad de Buenos Aires, Facultad de Agronomía, Argentina (see preceding section)), and V. Hernández-Ortiz (Instituto de Ecología A.C., Red de Interacciones Multitróficas, México (see preceding section)) with the collaboration of N. Vaccaro (Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Concordia, Argentina).	C	*Category : EDITORIAL* **(121) EPPO (22 Sep 2023 3:25 PM)** Consistency to ensure with section 6 (Contact points for further information).	**Acknowledged**
737	In addition, the following experts were significantly involved in the development of this protocol: ~~V.~~ Valérie Balmès (Anses, Laboratoire de la santé des végétaux, Unité entomologie et plantes invasives, France). ~~N.~~Norman B. Barr (APHIS, USDA, United States of America (see preceding section)), ~~D.~~ Daniel Frías (Universidad Metropolitana de Ciencias de la Educación, Chile (see preceding section)), A. Listre (Ministerio de Ganadería, Agricultura y Pesca, Dirección General de Servicios Agrícolas, Uruguay), M. Malipatil (La Trobe University, Bioprotection, Biosciences Research Division, Department of Environment and Primary Industries (Victoria), Australia)~~,~~ , A.L. Terra (Ministerio de Ganadería, Agricultura y Pesca, Dirección General de Servicios Agrícolas, Uruguay), O. Volonterio (Ministerio de Ganadería, Agricultura y Pesca, Dirección General de Servicios Agrícolas, Uruguay), and ~~R.~~Roberto A. Zucchi (Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Brazil (see preceding section)).	P	*Category : EDITORIAL* **(123) EPPO (22 Sep 2023 3:25 PM)** For consistency with section 6 (Contact points for further information). Please note it remains to put the full first names of A. Listre, M. Malipatil, A.L. Terra and O. Volonterio, which I don't know. Typo, space missing.	**Incorporated.**
743	~~**Barr, N.B. & McPheron, B.A.** 2006. Molecular phylogenetics of the genus *Ceratitis* (Diptera: Tephritidae). *Molecular Phylogenetics and Evolution*, 38(1), 216–230.~~	P	*Category : EDITORIAL* **(124) EPPO (22 Sep 2023 3:25 PM)** To be put after Barr, Cui... (alphabetical order).	**Incorporated.**
744	**Barr, N.B., Cui, L. & McPheron, B.A.** 2005. Molecular systematics of nuclear *gene* period in genus *Anastrepha* (Tephritidae). *Annals of the Entomological Society of America*, 98(2): 173–180.	P	*Category : EDITORIAL* **(125) EPPO (22 Sep 2023 3:25 PM)** Alphabetical order.	**Incorporated.**
753	**Carroll, L.E. & Wharton, R.A.** 1989. Morphology of the immature stages of *Anastrepha ludens* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, 82: 201–214.	P	*Category : TECHNICAL* **(59) United States of America (19 Sep 2023 9:34 PM)**	**Considered but not incorporated.**

Para	Text	T	Comment	SC's response
	Diaz & Steck??			There is not a publication for Diaz & Steck. These are referenced as sources of images.
762	**Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R.** 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: ~~294–9~~294–299.	P	*Category : EDITORIAL* **(126) EPPO (22 Sep 2023 3:25 PM)** For consistency with the other references.	**Incorporated.**
766	~~**Frias**~~**Frías, D., Selivon, D. & Hernández-Ortiz, V.** 2008. Taxonomy of immature stages: new morphological characters for Tephritidae larvae identification. In: A. Malavasi, R. Sugayama, R. Zucchi & J. Sivinski, eds. *Fruit flies of economic importance – From basic to applied knowledge.* Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance, 10–15 September 2006, Salvador, Brazil, pp. 29–44.	P	*Category : EDITORIAL* **(127) EPPO (22 Sep 2023 3:25 PM)** Typo	**Incorporated.**
790	**Norrbom, A.L., Zucchi, R.A. & Hernández-Ortiz, V.** ~~1999c~~1999. Phylogeny of the genera *Anastrepha* and *Toxotrypana* (Trypetinae: Toxotrypanini) based on morphology. In: M. Aluja & A.L. Norrbom, eds. *Fruit flies (Tephritidae) – Phylogeny and evolution of behavior*, pp. 299–342. Boca Raton, USA, CRC Press.	P	*Category : EDITORIAL* **(128) EPPO (22 Sep 2023 3:25 PM)** Delete the letter c. It is not needed.	**Incorporated.**
813	**9. Figures**	C	*Category : EDITORIAL* **(129) EPPO (22 Sep 2023 3:25 PM)** It is suggested to start with an overview picture of an adult Anastrepha species as Figure 1, not with a figure that is a detail. See also the previous version of this DP. A proposal was made in the text to have Figure 3 and Figure 4 as the first figures of the DP.	**Incorporated.**
816	**Figure 1.** Ovipositor of adult female of *Anastrepha striata* in ventral view: (A) aculeus and eversible membrane retracted inside oviscape; (B) aculeus and eversible membrane partially everted; (C) aculeus and eversible membrane completely everted.	C	*Category : TECHNICAL* **(60) United States of America (19 Sep 2023 9:36 PM)** We like the style of this figure, with the fully spelled out names of the structures and the lines pointing to the structures, MUCH better than the style in Figs. 2, 5 and 6.	**Considered but not incorporated.** We agree that this image is high quality and use of spelling out names is ideal. But also acknowledge that other images are appropriate even if needing to use caption and notes to explain names.
817	*Source:* Adapted from~~:~~: Norrbom et al. (2012)	P	*Category : TECHNICAL* **(61) United States of America (19 Sep 2023 9:37 PM)**	**Incorporated**
818	**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.** 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and*	C	*Category : TECHNICAL* **(62) United States of America (19 Sep 2023 9:38 PM)**	**Considered but not incorporated.**

Para	Text	T	Comment	SC's response
	interactive keys. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm		Don't include references in the figure legends.	The format for IPPC protocols uses this placement.
818	~~**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.**~~Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J. 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and interactive keys*. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm	P	*Category : EDITORIAL* **(12) Thailand (16 Aug 2023 9:38 AM)**	**Incorporated.**
822	**Figure 2.** Third instars: (A) habitus showing location of major anatomical features; (B) pseudocephalon, ventrolateral view; (C) slide-mounted larva, cleared cuticle with cephaloskeleton removed; (D) pseudocephalon, ventral view, scanning electron micrograph.	C	*Category : EDITORIAL* **(130) EPPO (22 Sep 2023 3:25 PM)** Aren't the legend of (B) and (C) inverted, i.e. shouldn't it be: (B) slide-mounted larva, cleared cuticle with cephaloskeleton removed; (C) pseudocephalon, ventrolateral view; ?	**Incorporated.**
823	*Notes: A1–A8*, first to eighth abdominal segments; *ANT*, antenna; *AP*, accessory plates; *ASp*, anterior spiracle; *CS*, cephaloskeleton; LB, llabium; LL, lateral lips; *MH*, mouthhook; *MP*, maxillary palp; *PC*, pseudocephalon; *OR*, oral ridges; *POL*, preoral lobes; *PSp*, posterior spiracles; *sp*, spinules; *T1–T3*, first to third thoracic segments.	C	*Category : TECHNICAL* **(187) European Union (29 Sep 2023 6:36 PM)** Spinules are not visible in any of these figures. Adding a figure for spinules might be useful (Fig. 23 is not very clear) or remove "sp" from the notes.	**Modified.** Remove spinules from note. The Figure 23 provides example of spinules (and show how SEM is better method at visualizing structure).
823	*Notes: A1–A8*, first to eighth abdominal segments; *ANT*, antenna; *AP*, accessory plates; *ASp*, anterior spiracle; *CS*, cephaloskeleton; *LB*, labium; *LL*, lateral lips; *MH*, mouthhook; *MP*, maxillary palp; *PC*, pseudocephalon; *OR*, oral ridges; *POL*, preoral lobes; *PSp*, posterior spiracles; *sp*, spinules; *T1–T3*, first to third thoracic segments.	C	*Category : TECHNICAL* **(134) EPPO (22 Sep 2023 3:25 PM)** Spinules are not visible in any of these figures. Adding a figure for spinules might be useful (Fig. 23 is not very clear) or remove "sp" from the notes.	**Modified.** Remove spinules from note. The Fugure 23 provides exaple of spinules (and show how SEM is better method at visualizing structure).
823	*Notes: A1–A8*, first to eighth abdominal segments; *ANT*, antenna; *AP*, accessory plates; *ASp*, anterior spiracle; *CS*, cephaloskeleton; *LB*, llabium; *LL*, lateral lips; *MH*, mouthhook; *MP*, maxillary palp; *PC*, pseudocephalon; *OR*, oral ridges; *POL*, preoral lobes; *PSp*, posterior spiracles; *sp*, spinules; *T1–T3*, first to third thoracic segments.	C	*Category : EDITORIAL* **(133) EPPO (22 Sep 2023 3:25 PM)** "OR, oral ridges" should be before "PC, pseudocephalon"	**Incorporated.**
823	*Notes: A1–A8*, first to eighth abdominal segments; *ANT*, antenna; *AP*, accessory plates; *ASp*, anterior spiracle; *CS*, cephaloskeleton; ~~LB~~*LB*, ~~llabium~~labium; ~~LL~~*LL*, lateral lips; *MH*, mouthhook; *MP*, maxillary palp; *PC*, pseudocephalon; *OR*, oral ridges; *POL*, preoral lobes; *PSp*, posterior spiracles; *sp*, spinules; *T1–T3*, first to third thoracic segments.	P	*Category : EDITORIAL* **(132) EPPO (22 Sep 2023 3:25 PM)** Italics for "LB" and "LL". Typo	**Incorporated.**
823	*Notes: A1–A8*, first to eighth abdominal segments; *ANT*, antenna; *AP*, accessory plates; *ASp*, anterior spiracle; *CS*, cephaloskeleton; LB, llabium; LL, lateral lips; *MH*, mouthhook; *MP*, maxillary palp; *PC*, pseudocephalon; *OR*, oral ridges; *POL*, preoral lobes; *PSp*, posterior spiracles; *sp*, spinules; *T1–T3*, first to third thoracic segments.	C	*Category : EDITORIAL* **(131) EPPO (22 Sep 2023 3:25 PM)** The legend of "MOL" which appears in Figures 2C and 2D is missing.	**Incorporated** Median oral lobe
830	**Figure 3.** (A) Habitus of adult female of *Anastrepha curvicauda* (papaya fruit fly) in lateral view. (B) Thorax of adult female of *Anastrepha curvicauda* in dorsal view. (C) Habitus of adult female of *Anastrepha serpentina* (sapote fruit fly) in dorsal view. (D) Habitus of adult female of *Anastrepha striata* (American guava fruit fly) in dorsal view. *Note: scale bar 1,~~0mm~~0 mm.*	P	*Category : EDITORIAL* **(135) EPPO (22 Sep 2023 3:25 PM)** Typo: a space missing.	**Incorporated.**

Para	Text	T	Comment	SC's response
832	~~**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.**~~Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J. 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and interactive keys*. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm	P	*Category : EDITORIAL* **(11) Thailand (16 Aug 2023 9:37 AM)**	**Incorporated.**
837	*Sources:* (A, C, D) adapted from Norrbom ***et al.*** (2012); (B) V. Hernández-Ortiz.	C	*Category : EDITORIAL* **(136) EPPO (22 Sep 2023 3:25 PM)** Please check. If this refers to Hernández-Ortiz, V. 1992 add the reference in the sources. If it is just Hernández-Ortiz that provided the picture it is ok as it is.	**Considered but not incorporated.** The image is courtesy of Hernández-Ortiz (and used in prior verison of adopted protocol). No change to source needed.
838	**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.** 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and interactive keys*. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm	C	*Category : EDITORIAL* **(137) EPPO (22 Sep 2023 3:25 PM)** Police too big and the authors shouldn't be in bold for consistency with the other figures.	**Incorporated.**
838	~~**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.**~~Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J. 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and interactive keys*. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm	P	*Category : EDITORIAL* **(10) Thailand (16 Aug 2023 9:37 AM)**	**Incorporated.**
851	**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.** 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and interactive keys*. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm	C	*Category : EDITORIAL* **(138) EPPO (22 Sep 2023 3:25 PM)** Police too big and the authors shouldn't be in bold for consistency with the other figures.	**Incorporated.**
851	Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J~~**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.**~~ 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and interactive keys*. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm	P	*Category : EDITORIAL* **(9) Thailand (16 Aug 2023 9:36 AM)**	**Incorporated.**
853	**Figure 7.** Wing pattern of ~~Anastrepha~~ *Anastrepha* species: (A) *A. curvicauda*; (B) *A. grandis*; (C) *A. serpentina*; (D) *A. striata*; (E) *A. suspensa*; (F) *A. ludens*; (G) *A. obliqua*; (H) *A. fraterculus* ~~(Brazil)~~.	P	*Category : EDITORIAL* **(7) Thailand (16 Aug 2023 9:35 AM)**	**Incorporated.**
856	~~**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.**~~Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J. 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and interactive keys*. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm	P	*Category : EDITORIAL* **(139) EPPO (22 Sep 2023 3:25 PM)** The authors shouldn't be in bold for consistency with the other figures.	**Incorporated.**
856	~~**Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J.**~~Norrbom, A.L., Korytkowski, C.A., Zucchi, R.A., Uramoto, K., Venable, G.L., McCormick, J. & Dallwitz, M.J. 2012 onwards. Anastrepha *and* Toxotrypana – *Descriptions, illustrations, and*	P	*Category : EDITORIAL* **(8) Thailand (16 Aug 2023 9:36 AM)**	**Incorporated.**

Para	Text	T	Comment	SC's response
	interactive keys. Version: 9th April 2019. Delta-intkey.com. Available at https://www.delta-intkey.com/anatox/index.htm			
860	**Figure 8.** Morphology of the aculeus tip in ventral view of *Anastrepha* species of major economic importance: (A) *A. curvicauda*; (B) *A. grandis*; (C) *A. serpentina*; (D) *A. striata*; (E) *A. suspensa*; (F) *A. ludens*; (G) *A. obliqua*; (H) *A. ~~fraterculus~~ fraterculus~~(Brazil)~~*.	P	*Category : EDITORIAL* **(13) Thailand (16 Aug 2023 9:38 AM)**	**Incorporated.**
865	**Figure 9.** (A) Lateral habitus: (A-A), instar; (A-B), instar; (A-C), instar; of *Anastrepha suspensa*, showing differences in sizes. (B) Cephaloskeleton of *Anastrepha suspensa*, second instar; arrow indicates subapical tooth on mouthhook that is subequal in size to apical tooth. (C) Cephaloskeleton of *Anastrepha suspensa*, third instar; arrow indicates lack of subapical tooth on mouthhook. (D) Cephaloskeleton of *Ceratitis fasciventris*, third instar; arrow indicates subapical tooth on mouthhook that is much smaller than apical tooth.	C	*Category : SUBSTANTIVE* **(164) China (28 Sep 2023 8:12 AM)** Amend legend of larvaae A"A B C" to "a b c"	**Modified.** Additional borders were added to image to improve clairty. The use of capital lettering is retained.
865	**Figure 9.** (A) Lateral habitus: (A-A), first instar; (A-B), second instar; (A-C), third instar; of *Anastrepha suspensa*, showing differences in sizes. (B) Cephaloskeleton of *Anastrepha suspensa*, second instar; arrow indicates subapical tooth on mouthhook that is subequal in size to apical tooth. (C) Cephaloskeleton of *Anastrepha suspensa*, third instar; arrow indicates lack of subapical tooth on mouthhook. (D) Cephaloskeleton of *Ceratitis fasciventris*, third instar; arrow indicates subapical tooth on mouthhook that is much smaller than apical tooth.	P	*Category : EDITORIAL* **(140) EPPO (22 Sep 2023 3:25 PM)** ? (Please see paragraph 254 i.e. the first paragraph of section 4.4.)	**Incorporated.**
865	**Figure 9.** (A) Lateral habitus: (A-A), instar; (A-B), instar; (A-C), instar; of *Anastrepha suspensa*, showing differences in sizes. (B) Cephaloskeleton of *Anastrepha suspensa*, second instar; arrow indicates subapical tooth on mouthhook that is subequal in size to apical tooth. (C) Cephaloskeleton of *Anastrepha suspensa*, third instar; arrow indicates lack of subapical tooth on mouthhook. (D) Cephaloskeleton of *Ceratitis fasciventris*, third instar; arrow indicates subapical tooth on mouthhook that is much smaller than apical tooth.	C	*Category : TECHNICAL* **(63) United States of America (19 Sep 2023 9:40 PM)** Why not instar 1-3, as in text?	**Incorporated.**
865	**Figure 9.** (A) Lateral habitus: (A-A), first instar; (A-B)~~,~~, second instar; (A-C), third instar; of *Anastrepha suspensa*, showing differences in sizes. (B) Cephaloskeleton of *Anastrepha suspensa*, second instar; arrow indicates subapical tooth on mouthhook that is subequal in size to apical tooth. (C) Cephaloskeleton of *Anastrepha suspensa*, third instar; arrow indicates lack of subapical tooth on mouthhook. (D) Cephaloskeleton of *Ceratitis fasciventris*, third instar; arrow indicates subapical tooth on mouthhook that is much smaller than apical tooth.	P	*Category : SUBSTANTIVE* **(14) Thailand (16 Aug 2023 9:40 AM)**	**Incorporated.**
917	**Figure 21.** Posterior spiracle: (A) *Anastrepha curvicauda*; (B) *Anastrepha fraterculus*; (C) *Anastrepha grandis*; (D) *Anastrepha ludens*; (E) *Anastrepha obliqua*; (F) *Anastrepha serpentina*; ~~50V~~(G) *Anastrepha striata*; (H) *Anastrepha suspensa*.	P	*Category : EDITORIAL* **(141) EPPO (22 Sep 2023 3:25 PM)** Typo	**Incorporated.**
917	**Figure 21.** Posterior spiracle: (A) *Anastrepha curvicauda*; (B) *Anastrepha fraterculus*; (C) *Anastrepha grandis*; (D) *Anastrepha ludens*; (E) *Anastrepha obliqua*; (F) *Anastrepha serpentina*;~~-~~ (G) ~~50V~~*Anastrepha striata*; (H) *Anastrepha suspensa*.	P	*Category : TECHNICAL* **(86) United States of America (20 Sep 2023 8:06 PM)**	**Incorporated.**