2022 FIRST CONSULTATION

*1 July – 30 September 2022*

Responses to Compiled comments for 2022 First Consultation: DP Genus Ceratitis (2016-001)

Summary

Participants

|  |  |
| --- | --- |
| Name | Summary |
| Cuba | No hay comentarios al protocolo de diagnóstico |
| European Union | The comments are submitted by the European Commission on behalf of the European Union (EU) and its 27 Member States. |
| Singapore | Singapore supports the proposed draft annex to ISPM 27. |
| Syrian Arab Republic | Clarification about, the fruit containers should be inspected for pupae |
| United Kingdom | please ignore |

**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**S** (Status) - A = Accepted, C = Closed, O = Open, W = Withdrawn, M = Merged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 | Comment | TPDP response |
| G | (General Comment) | *Category : SUBSTANTIVE* **(298) Argentina (1 Oct 2022 12:42 AM)** We fully support comments from COSAVE | **NOTED** |
| G | (General Comment) | *Category : SUBSTANTIVE* **(296) Peru (30 Sep 2022 11:13 PM)** The document has been reviewed, there are no comments | **NOTED** |
| G | (General Comment) | *Category : SUBSTANTIVE* **(295) European Union (30 Sep 2022 8:34 PM)** The European Union and its 27 Member States support the comments submitted in the OCS by the European and Mediterranean Plant Protection Organisation (EPPO). | **NOTED** |
| G | (General Comment) | *Category : SUBSTANTIVE* **(294) Antigua and Barbuda (30 Sep 2022 5:20 PM)** This DP is an excellent document which is quite detailed and should provide clear guidance in the identification of Ceratitis species of fruitflies. | **NOTED** |
| G | (General Comment) | *Category : TECHNICAL* **(292) Paraguay (30 Sep 2022 2:08 PM)** Paraguay apoya comentarios de COSAVE. | **NOTED** |
| G | (General Comment) | *Category : EDITORIAL* **(280) Nepal (30 Sep 2022 6:19 AM)** Nepal is okay with the DRAFT ANNEX TO ISPM◦27: Genus Ceratitis (2016-001) and has no comments on it. | **NOTED** |
| G | (General Comment) | *Category : TECHNICAL* **(278) Mozambique (29 Sep 2022 1:20 PM)** The diagnostic protocol plays a very important role in pest identification technics we hope this proposal will help the taxonomist and entomologist to perform their work, we have no comments on this standard we agree with its contents. | **NOTED** |
| G | (General Comment) | *Category : EDITORIAL* **(256) South Africa (28 Sep 2022 7:37 AM)** No further comments | **NOTED** |
| G | (General Comment) | *Category : SUBSTANTIVE* **(252) Belarus (27 Sep 2022 3:41 PM)** Republic of Belarus would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System | **NOTED** |
| G | (General Comment) | *Category : EDITORIAL* **(251) United Kingdom (27 Sep 2022 2:44 PM)** The United Kingdom of Great Britain and Norther Ireland would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System | **NOTED** |
| G | (General Comment) | *Category : TECHNICAL* **(249) Mexico (26 Sep 2022 9:23 PM)** Mexico supports the DRAFT ANNEX TO ISPM◦27: Genus Ceratitis (2016-001). | **NOTED** |
| G | (General Comment) | *Category : SUBSTANTIVE* **(250) Guyana (26 Sep 2022 9:32 PM)** Guyana has no objection at this time. | **NOTED** |
| G | (General Comment) | *Category : EDITORIAL* **(245) Australia (26 Sep 2022 4:01 AM)** It is considered that the 37 instances of the terminology ‘the FAR complex’ should be updated to read ‘the FARQ complex’ throughout this Annex. This will align the text with modern literature, in which ‘FARQ’ is being increasingly used. This will assist in future proofing this Annex. Please see below reference for an example of modern literature using the FARQ complex terminology:   ZHANG, Y., DE MEYER, M., VIRGILIO, M., FENG, S., BADJI, K. and LI, Z. 2021. Phylogenomic resolution of the Ceratitis FARQ complex (Diptera: Tephritidae). Molecular Phylogenetics and Evolution 161: 107160 | **Incorporated** |
| G | (General Comment) | *Category : TECHNICAL* **(180) Uruguay (19 Sep 2022 4:27 PM)** We agree with the document as it is. No comments | **NOTED** |
| G | (General Comment) | *Category : SUBSTANTIVE* **(179) Congo (15 Sep 2022 3:31 PM)** Congo agrees with this DP and has nothing to add | **NOTED** |
| G | (General Comment) | *Category : EDITORIAL* **(174) Malawi (30 Aug 2022 9:50 PM)** We support the draft annex to ISPM 27 | **NOTED** |
| G | (General Comment) | *Category : EDITORIAL* **(161) Barbados (30 Aug 2022 9:09 PM)** This ISPM seems to have been thoroughly researched and well written. Barbados has no objection to this proposed standard. | **NOTED** |
| G | (General Comment) | *Category : EDITORIAL* **(2) Trinidad and Tobago (15 Aug 2022 8:38 PM)** Trinidad and Tobago is in agreement to include a draft to the Annex to ISPM 27 for Ceratitis | **NOTED** |
| 1 | **DRAFT ANNEX TO ISPM 27: Genus*Ceratitis* (2016-001)** | *Category : SUBSTANTIVE* **(279) Russian Federation (29 Sep 2022 4:41 PM)** General Comment: The Russian Federation would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System. | **NOTED** |
| 1 | picturebox.gif**DRAFT ANNEX TO ISPM 27: Genus *Ceratitis* (2016-001)** | *Category : TECHNICAL* **(181) EPPO (20 Sep 2022 5:03 PM)** General comments  Congratulations for the great pictures  We suggest to check the terminology used in this protocol "method" versus "test" | **Incorporated.**  Completed global check of terms |
| 1 | picturebox.gif**DRAFT ANNEX TO ISPM 27: Genus *Ceratitis* (2016-001)** | *Category : SUBSTANTIVE* **(85) Zambia (20 Aug 2022 12:28 PM)** Zambia agrees to the introduction of the standard | **NOTED** |
| 5 | **Document category** | *Category : SUBSTANTIVE* **(297) Cameroon (30 Sep 2022 11:17 PM)** We support the adoption of this draft ISPM on the genus Ceratitis. it brings more clarity | **NOTED** |
| 32 | **CONTENTS** | *Category : TECHNICAL* **(175) Gabon (31 Aug 2022 2:24 PM)** L’annexe ajoutée à la norme est très pertinente dans la mesure où il s’agit du protocole de diagnostic du Genre Ceratitis qui est l’organisme nuisible des fruits. Les fruits faisant l’objet des échanges dans notre région, la maitrise du risque associé à ces fruits est importante.  Toutefois il est nécessaire que les méthodes biochimiques et moléculaires utilisées soient maitrisées par les ONPV. | **NOTED** |
| 37 | 1. Pest information | *Category : TECHNICAL* **(11) United States of America (17 Aug 2022 8:35 PM)** In general, the discussion of subgenera in [38] and [46-53] is not very clear. Since the protocol actually addresses only six species, this taxonomic information is largely unnecessary (also likely to change, as nonmonophyletic subgenera are likely to be revised and monotypic subgenera are pointless) | **Modified**  The inclusion of subgenera classificaiton is helpful with diagnosis because keys and resources are organized using these subgenera and informaiton on phylogenetic status aids in DNA comparison studies. The [38] section is reduced to remove redundant informaiton but the text is retained. |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis*MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : EDITORIAL* **(254) South Africa (28 Sep 2022 7:33 AM)** Suggest replacement of the word: “existence” with “suitable climatic conditions and availability” for Grammatical correction. | **Incorporated.** |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis*MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : EDITORIAL* **(253) South Africa (28 Sep 2022 7:31 AM)** Suggest deletion of the word: " represent" and replace it with :"are regarded as" | **Modified.**  To simplyify text protocol now states Tephritidae is an economically important insect group |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis* MacLeay consists of approximately 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : TECHNICAL* **(183) EPPO (20 Sep 2022 5:03 PM)** 100 is an approximation not an exact number. | **Incorporated.** |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis*MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : TECHNICAL* **(182) EPPO (20 Sep 2022 5:03 PM)** repetition, see taxonomic position | **Incorporated.** |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis* MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : TECHNICAL* **(123) Kenya (29 Aug 2022 8:14 AM)** Although they are commonly referred to as “fruit flies”, larval development does not necessary occur in fruits only but can also take place in other parts of the host plants, including flowers, seeds, leaves and stems (De meyer et al., 2016). | **Modified.**  Text adjusted to be more inclusive. |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis* MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : TECHNICAL* **(122) Kenya (29 Aug 2022 8:13 AM)** Although they are commonly referred to as “fruit flies”, larval development does not necessary occur in fruits only but can also take place in other parts of the host plants, including flowers, seeds, leaves and stems (De meyer et al., 2016). | **Modified.**  Text adjusted to be more inclusive. |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis*MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : EDITORIAL* **(112) United States of America (26 Aug 2022 3:20 PM)** As the detail statement is provided in the bottom of this page.  [paragraph 46; Taxonomic Position], the statement here perhaps could be omitted or shortened by providing reference with paragraph [46] and Secetioc-2 (Taxonomic Information) | **Incorporated.** |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of ~~these~~ tephritid fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis* MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : EDITORIAL* **(111) United States of America (26 Aug 2022 3:18 PM)** for clarity | **Modified.**  Incorporated adjective but also removed fruit |
| 38 | ~~Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution.~~ The biology of these fruit flies is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis* MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : EDITORIAL* **(4) Trinidad and Tobago (15 Aug 2022 8:44 PM)** Misleading and reworded accordingly | **Modified.**  Revised sentence to improve clarity and remove reference to distribtuion of the family. |
| 38 | Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of ~~these fruit flies~~ Tephritidae is dependent on the existence of the host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Ceratitis* MacLeay consists of 100 described species that are predominantly Afrotropical in distribution (De Meyer *et al.*, 2016). The genus consists of six subgenera: *C.*(*Acropteromma*) Bezzi, *C.*(*Ceratalaspis*) Hancock, *C.*(*Ceratitis*) MacLeay, *C.*(*Hoplolophomyia*) Bezzi *C.*(*Pardalaspis*) Bezzi, and *C.*(*Pterandrus*) Bezzi, (Hancock, 1984; De Meyer and Freidberg, 2005). Two of the subgenera are monotypic (i.e. *C*. (*Hoplolophomyia*)and *C.*(*Acropteromma*)) and two are not monophyletic lineages (i.e. *C.*(*Ceratalaspis*) and *C.*(*Pterandrus*)) (De Meyer, 1999; Barr and McPheron, 2006). | *Category : TECHNICAL* **(3) Trinidad and Tobago (15 Aug 2022 8:42 PM)** Remove Fruit flies and put Tephritidae | **Modified.**  Replaced with tephritid flies |
| 39 | ~~The genus includes several fruit pests that damage plants used for commercial and subsistence agriculture. The mated females oviposit eggs into fruit using a structure called an ovipositor. After the eggs hatch, direct damage is caused by larval feeding. Secondary damage is caused by the increased susceptibility to opportunistic fruit~~Le genre comprend plusieurs ravageurs des fruits qui endommagent les plantes utilisées pour l'agriculture commerciale et de subsistance. Les femelles accouplées pondent leurs œufs dans les fruits à l'aide d'une structure appelée ovipositeur. Après l'éclosion des œufs, des dommages directs sont causés par l'alimentation des larves. Les dommages secondaires sont causés par la sensibilité accrue aux agents pathogènes opportunistes des fruits résultant des blessures lors de la ponte dans les fruits et des dommages causés par l'alimentation. *Les espèces de Ceratitis*  sont soit des généralistes (polyphages), soit une forme de spécialistes qui se nourrissent d'une espèce particulière (monophages) ou se nourrissent d'une lignée d'espèces végétales (c'est-à-dire sténophages et oligophages). La relation connue entre *Ceratitis* ~~pathogens resulting from injuries during oviposition into the fruit and feeding damage.~~ *~~Ceratitis~~* ~~species are either generalists (polyphagous) or a form of specialist that feeds on a particular species (monophagous) or feeds on a lineage of plant species (i.e. stenophagous and oligophagous). The known relationship between~~ *~~Ceratitis~~* ~~species and their host plants is incomplete for many pests. Some host-use records are from field observations that still require confirmation~~ espèces et leurs plantes hôtes est incomplète pour de nombreux ravageurs. Certains enregistrements d'utilisation d'hôtes proviennent d'observations sur le terrain qui nécessitent encore une confirmation sur les fruits  ~~on infested~~ infestés et certaines espèces de *Ceratitis peuvent* infester une gamme d'hôtes plus large que celle actuellement signalée.  ~~fruits and some~~ *~~Ceratitis~~* ~~species may infest a wider range of hosts than currently reported.~~ | *Category : TECHNICAL* **(291) Mali (30 Sep 2022 11:09 AM)** je n'ai pas d'objection sur le présent projet de document sur le ravageur "Ceratitis" . | **Modified.**  Text simplified to improve clarity. |
| 39 | The genus includes several fruit pests that damage plants used for commercial and subsistence agriculture. The mated females oviposit eggs into fruit using a structure called an ovipositor. After the eggs hatch, larvae cause direct damage ~~is caused~~ to teh fruit by ~~larval feeding~~feeding on it. Secondary damage is caused by the increased susceptibility of the plant to opportunistic fruit pathogens resulting from injuries during oviposition into the fruit and feeding damage. *Ceratitis* species are either ~~generalists~~ generalist feeders (polyphagous) or a form of specialist that feeds on a particular species (monophagous) or feeds on a lineage of plant species (i.e. stenophagous and oligophagous). The known relationship between many species of *Ceratitis* ~~species~~ and their host plants is ~~incomplete for many pests~~incomplete. Some host-use records are from field observations that still require confirmation on infested fruits and some *Ceratitis* species may infest a wider range of hosts than currently reported. | *Category : EDITORIAL* **(257) New Zealand (28 Sep 2022 9:04 AM)** to improve clarity | **Incorporated.**  Incoprorated changes to improve clarity and an additional modificaiton to section on feeding types. |
| 39 | The genus includes several fruit pests that damage ~~plants~~ plant species used for commercial and subsistence agriculture. The mated females oviposit eggs into ~~fruit using a structure called an ovipositor~~fruits. After the eggs hatch, direct damage is caused by larval feeding. Secondary damage is caused by the increased susceptibility to opportunistic fruit pathogens resulting from injuries during oviposition into the fruit and from feeding damage. *Ceratitis* species are either generalists (polyphagous) or ~~a form of specialist~~ specialists that ~~feeds~~ feed on a particular species (monophagous) or ~~feeds~~ on a lineage of plant species (i.e. stenophagous and oligophagous). The ~~known~~ knowledge on the relationship between many *Ceratitis* species and their host plants is ~~incomplete for many pests~~incomplete. Some host-use records are from field observations that still require confirmation on infested fruits (see ISPM 37 (Determination of host status of fruit to fruit flies (Tephritidae)) and some *Ceratitis* species may infest a wider range of hosts than currently reported. | *Category : TECHNICAL* **(184) EPPO (20 Sep 2022 5:03 PM)** is lineage regular terminology? The reference to the ovipositor is not needed We propose a clarification of the sentence starting with 'the known relationship...'. The text was not easily understood.  In the last sentence, "on infested fruits" is not very clear and we therefore suggest to make reference to ISPM 37 that provides useful explanations on this issue. | **Modified.**  Incorporated all changes but did not delete reference to ovipositor. It is useful point and technically correct. |
| 39 | The genus Ceratitis includes several fruit pests that damage plants used for commercial and subsistence agriculture. The mated females oviposit eggs into fruit using a structure called an ovipositor. After the eggs hatch, direct damage is caused by larval feeding. Secondary damage is caused by the increased susceptibility to opportunistic fruit pathogens resulting from injuries during oviposition into the fruit and feeding damage. *Ceratitis* species are either generalists (polyphagous) or a form of specialist that feeds on a particular species (monophagous) or feeds on a lineage of plant species (i.e. stenophagous and oligophagous). The known relationship between *Ceratitis* species and their host plants is incomplete for many pests. Some host-use records are from field observations that still require confirmation on infested fruits and some *Ceratitis* species may infest a wider range of hosts than currently reported. | *Category : TECHNICAL* **(113) United States of America (26 Aug 2022 3:25 PM)** ‘Plant pests’ or ‘fruit tree pest’ may be better ‘term’. Does fruit flay cause significant damage to the host plant itself besides fruits? It may be better to reword ‘that damage fruit crops in commercial and subsistence agriculture. For consistency: either using "primary" damage (and "secondary" as is in the next sentence), or direct damage and then "indirect" damage in the next sentence. | **Incorporated.** |
| 39 | The genus includes several ~~fruit~~ species which are pests of economically important crops that damage plants used for commercial and subsistence agriculture. The mated females oviposit eggs into fruit ~~using a structure called an ovipositor. After the eggs hatch,~~ and direct damage is caused by larval feeding. Secondary damage is caused by ~~the increased susceptibility~~ the to opportunistic fruit pathogens resulting ~~from in~~from~~juries~~ ~~during~~ oviposition ~~into the fruit~~ and feeding damage. *Ceratitis* species are either generalists (polyphagous) or a form of specialist that feeds on a particular species (monophagous) or feeds on a lineage of plant species (i.e. stenophagous and oligophagous). The known relationship between *Ceratitis* species and their host plants is incomplete for many pests. Some host-use records are from field observations that still require confirmation on infested fruits and some *Ceratitis* species may infest a wider range of hosts than currently reported. | *Category : EDITORIAL* **(5) Trinidad and Tobago (15 Aug 2022 8:50 PM)** Sentence structure unclear | **Modified.**  Text adjusted based on multiple requests for improved clarity. |
| 39 | The genus includes several fruit pests that damage plants used for commercial and subsistence agriculture. The mated females oviposit eggs into fruit using a structure called an ovipositor. After the eggs hatch, direct damage is caused by larval feeding. Secondary damage is caused by the increased susceptibility to opportunistic fruit pathogens resulting from injuries during oviposition into the fruit and feeding damage. *Ceratitis* species are either generalists (polyphagous) or a form of specialist that feeds on a particular species (monophagous) or feeds on a lineage of plant species (i.e. stenophagous and oligophagous). The ~~known~~ relationship between *Ceratitis* species ~~and their~~ host plants is incomplete for many pests. Some host-use records are from field observations that still require confirmation on infested fruits and some *Ceratitis* species may infest a wider range of hosts than currently reported. | *Category : TRANSLATION* **(6) Trinidad and Tobago (15 Aug 2022 8:52 PM)** | **Modified.**  Text adjusted based on multiple requests for improved clarity. Concerns addressed. |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their pest distribution and risk they pose. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*)*capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : SUBSTANTIVE* **(258) New Zealand (28 Sep 2022 9:07 AM)** Query: use of the term 'pest' seemingly interchangeably with species (such as in para 101). in some parts of the document the usage is ambiguous e.g. here where “pest distribution” is referred to, it is unclear whether this refers to the total range of the species or just the parts of its range in which it is a pest | **Modified**.  Revised the protocol to remove use of pest when species is more appropriate. |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their pest distribution and risk they pose. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*) *capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can feed and develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : EDITORIAL* **(259) New Zealand (28 Sep 2022 9:08 AM)** | **Incorporated.** |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their pest distribution and risk they pose. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*)*capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : TECHNICAL* **(244) Egypt (24 Sep 2022 11:19 AM)** It is recommended these piece of info. need to be supported with a reference(s): - "This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments.". -"Native to eastern sub-Saharan Africa". | **Modified.**  Citations are included:  White and Elson-Harris 1992; Liquido *et al*., 2016; USDA 2020  The sentence about hosts is simplified because exact numbers are not relevant to the protocol. The fact that the pest is polyphagous is the important point being made. |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their ~~pest~~ distribution ~~and~~ and the pest risk they pose.  The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*) *capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the ~~Mediterranean~~Mediterranean region . This pest can develop on over 400 ~~varieties of plant hosts~~ host plants and survive in tropical, subtropical and temperate environments. | *Category : TECHNICAL* **(185) EPPO (20 Sep 2022 5:03 PM)** Are these really varieties or host plants and what is the reference for 400?  The EPPO Global database has 348 plant species all with references (but one). We propose to split the paragraph for better clarity. | **Modified.**  Incorporated changes. But modified the final sentence based on several sugestions to address concern with clarity. Exact number is not provided for hosts. |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their pest distribution and risk they pose. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*)*capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : SUBSTANTIVE* **(116) China (28 Aug 2022 5:01 PM)** “This pest can develop on over 400 varieties of plant hosts”, is there any reference for the number? Is it correct??  The number needs related reference and to confirm its reliability. | **Modified.**  Provided citations for generalist behavior but removed specific number of hosts as this value will change and the reported hosts can differ from reared hosts under field and lab conditions. |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their pest distribution and risk they pose. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*)*capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : TECHNICAL* **(114) United States of America (26 Aug 2022 3:27 PM)** You may consider providing information on the plant hardiness zone  https://safaris.cipm.info/safarispestmodel/StartupServlet?phz. Plant Hardiness Zones 8-13 are both climatically suitable and contain economically important hosts for C. capitata. | **Modified.**  Removed this statement on survival in environments because it is outside scope of diagnostic protocol and redundant (previous sentence provides examples of spread into regions of world). |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their pest distribution and risk they pose. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*) *capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : TECHNICAL* **(22) United States of America (17 Aug 2022 8:53 PM)** Add citation; Liquido et al. 2020? | **Incorporated.**  Liquido, N., G. McQuate & K. Suiter. 2016. USDA Compendium of fruit fly host information (CoFFHI). Proceedings of the 9th International Symposium on Fruit Flies of Economic Importance 420-434.  USDA. 2020. Compendium of Fruit Fly Host Information (CoFFHI), Edition 5.0. https://coffhi.cphst.org/ |
| 40 | Of the agricultural pests in the genus that exhibit generalist host-use behaviour, six species are included in this diagnostic protocol based on their ~~pest~~ distribution and pest risk they pose. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*) *capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : EDITORIAL* **(21) United States of America (17 Aug 2022 8:53 PM)** For clarity. | **Incorporated.** |
| 40 | Of the agricultural economically important pests in the ~~genus that exhibit generalist host~~genus,~~-use behaviour,~~  six species are included in this diagnostic protocol based on their pest distribution and ~~risk they pose~~risk. The most destructive global pest in the genus is the generalist *C.*(*Ceratitis*) *capitata* (Wiedemann). Native to eastern sub-Saharan Africa, *C*. *capitata* has successfully invaded other regions of Africa, Hawaii, South America, Central America, Australia and countries of the Mediterranean. This pest can develop on over 400 varieties of plant hosts and survive in tropical, subtropical and temperate environments. | *Category : EDITORIAL* **(7) Trinidad and Tobago (15 Aug 2022 8:55 PM)** | **Modified.**  To improve clarity, the sentence is simplified to “Six economically important *Ceratitis* species are included in this diagnostic protocol based on their distributions and statuses as polyphagous pests (White and Elson-Harris 1992, De Meyer et al. 2016).” |
| 41 | The five additional species included in this protocol are found throughout large regions of sub-Saharan Africa. *Ceratitis* (*Ceratalaspis*) *cosyra* (Walker) is a pest of many fruit hosts such as *Annona muricata* (soursop), *Eriobotrya japonica* (loquat), *Mangifera indica* (mango), *Prunus persica* (peach), and *Psidium guajava* (guava). It is found throughout much of sub-Saharan Africa and is reported to be a cryptic species complex (Virgilio *et al.*, 2017). The other four species included in the protocol for species-level identification are *C.*(*Pterandrus*) *fasciventris* (Bezzi), *C.*(*Pterandrus*) *anonae* Graham, *C.*(*Pterandrus*) *rosa* Karsch and *C.*(*Pterandrus*) *quilicii* De Meyer *et al*. These use a wide range and a large number of commercially grown hosts. The distributions for each of these four species include multiple countries across sub-Saharan Africa; although each species has a different distribution range, these ranges can overlap (De Meyer *et al.*, 2015; De Meyer *et al.*, 2016). The four species are included in a taxonomic species complex called the “FAR complex” because of their high morphological and molecular similarity (Barr and McPheron, 2006; Virgilio *et al.*, 2008). | *Category : EDITORIAL* **(260) New Zealand (28 Sep 2022 9:09 AM)** | **Incorporated.** |
| 41 | The five additional species included in this protocol are found throughout large regions of sub-Saharan Africa. *~~Ceratitis~~C.* (*Ceratalaspis*) *cosyra* (Walker) is a pest of many fruit hosts such as *Annona muricata* (soursop), *Eriobotrya japonica* (loquat), *Mangifera indica* (mango), *Prunus persica* (peach), and *Psidium guajava* (guava). It is found throughout much of sub-Saharan Africa and is reported to be a cryptic species complex (Virgilio *et al.*, 2017). The other four species included in the protocol for species-level identification are *C.*(*Pterandrus*) *fasciventris* (Bezzi), *C.*(*Pterandrus*) *anonae* Graham, *C.*(*Pterandrus*) *rosa* Karsch and *C.*(*Pterandrus*) *quilicii* De Meyer *et al*. These use a wide range and a large number of commercially grown hosts. The distributions for each of these four species include multiple countries across sub-Saharan Africa; although each species has a different distribution range, these ranges can overlap (De Meyer *et al.*, 2015; De Meyer *et al.*, 2016). The four species are included in a taxonomic species complex called the “FAR complex” because of high morphological and molecular similarity (Barr and McPheron, 2006; Virgilio *et al.*, 2008). | *Category : EDITORIAL* **(237) Colombia (21 Sep 2022 5:12 AM)** No es necesario colocar toda la palabra | **Incorporated.** |
| 41 | The five additional species included in this protocol are found throughout large regions of sub-Saharan Africa.  *Ceratitis* (*Ceratalaspis*) *cosyra* (Walker) is a pest of many fruit hosts such as *Annona muricata* (soursop), *Eriobotrya japonica* (loquat), *Mangifera indica* (mango), *Prunus persica* (peach), and *Psidium guajava* (guava). It is found throughout much of sub-Saharan Africa and is reported to be a cryptic species complex (Virgilio *et al.*, 2017).  The other four species included in the ~~protocol for species-level identification~~ protocol are *C.*(*Pterandrus*) *fasciventris* (Bezzi), *C.*(*Pterandrus*) *anonae* Graham, *C.*(*Pterandrus*) *rosa* Karsch and *C.*(*Pterandrus*) *quilicii* De Meyer *et al*. These ~~use~~ infest a wide range and a large number of commercially grown hosts. The distributions for each of these four species include multiple countries across sub-Saharan Africa; although each species has a different distribution range, these ranges can overlap (De Meyer *et al.*, 2015; De Meyer *et al.*, 2016). The four species are included in a taxonomic species complex called the “FAR complex” because of high morphological and molecular similarity (Barr and McPheron, 2006; Virgilio *et al.*, 2008). | *Category : TECHNICAL* **(186) EPPO (20 Sep 2022 5:03 PM)** 1 For the first sentence: we suggest the deletion of "are found throughout large regions of sub-Saharan Africa" except if this information is there to specifically stressed here the contrast with C. capitata. The information on the sub-saharan Africa is given for the different species below. IF the deletion is accepted a redrafting should be considered Along the following lines "Five additional species are included in this protocol" 2 "for species-level identification" is not needed.  3 For better clarity, we suggest to begin a new paragraph for each species or complex of species. 4 Infest is a better word than use 5 Should "FAR complex" not be called FARQ, this should be checked throughout. | **Incorporated**. |
| 41 | The five additional species included in this protocol are found throughout large regions of sub-Saharan Africa. *Ceratitis* (*Ceratalaspis*) *cosyra* (Walker) is a pest of many fruit hosts such as *Annona muricata* (soursop), *Eriobotrya japonica* (loquat), *Mangifera indica* (mango), *Prunus persica* (peach), and *Psidium guajava* (guava). It is found throughout much of sub-Saharan Africa and is reported to be a cryptic species complex (Virgilio *et al.*, 2017). The other four species included in the protocol for species-level identification are *C.*(*Pterandrus*) *fasciventris* (Bezzi), *C.*(*Pterandrus*) *anonae* Graham, *C.*(*Pterandrus*) *rosa* Karsch and *C.*(*Pterandrus*) *quilicii* De Meyer *et al*. These use a wide range and a large number of commercially grown hosts. The distributions for each of these four species include multiple countries across sub-Saharan Africa; although each species has a different distribution range, these ranges can overlap (De Meyer *et al.*, 2015; De Meyer *et al.*, 2016). The four species are included in a taxonomic species complex called the ~~“FAR~~ “FARQ complex” because of high morphological and molecular similarity (Barr and McPheron, 2006; Virgilio *et al.*, 2008). | *Category : SUBSTANTIVE* **(117) China (28 Aug 2022 5:02 PM)** According to Zhang et al., 2021, “FARQ complex” is more suitable than “FAR complex”. The reference is cited as “Zhang Y., Meyer M.D., Virgilio M, Feng S.Q, Badji K, Li Z.H.. Phylogenomic resolution of the Ceratitis FARQ complex (Diptera: Tephritidae). Molecular phylogenetics and Evolution, 2021, 161:107160.” | **Incorporated.** |
| 41 | The five additional species included in this protocol are found throughout large regions of sub-Saharan Africa. *Ceratitis* (*Ceratalaspis*) *cosyra* (Walker) is a pest of many fruit hosts such as *Annona muricata* (soursop), *Eriobotrya japonica* (loquat), *Mangifera indica* (mango), *Prunus persica* (peach), and *Psidium guajava* (guava). It is found throughout much of sub-Saharan Africa and is reported to be a cryptic species complex (Virgilio *et al.*, 2017). The other four species included in the protocol for species-level identification are *C.*(*Pterandrus*) *fasciventris* (Bezzi), *C.*(*Pterandrus*) *anonae* Graham, *C.*(*Pterandrus*) *rosa* Karsch and *C.*(*Pterandrus*) *quilicii* De Meyer *et al*. These use a wide range and a large number of commercially grown hosts. The distributions for each of these four species include multiple countries across sub-Saharan Africa; although each species has a different distribution range, these ranges can overlap (De Meyer *et al.*, 2015; De Meyer *et al.*, 2016). ~~The~~ These four species are included in a taxonomic species complex called the “FAR complex” because of high morphological and molecular similarity (Barr and McPheron, 2006; Virgilio *et al.*, 2008). | *Category : EDITORIAL* **(115) United States of America (26 Aug 2022 3:28 PM)** clarity | **Incorporated.** |
| 41 | The five additional species included in this protocol are found throughout large regions of sub-Saharan Africa. *Ceratitis* (*Ceratalaspis*) *cosyra* (Walker) is a pest of many fruit hosts such as *Annona muricata* (soursop), *Eriobotrya japonica* (loquat), *Mangifera indica* (mango), *Prunus persica* (peach), and *Psidium guajava* (guava). It is found throughout much of sub-Saharan Africa and is reported to be a cryptic species complex (Virgilio *et al.*, 2017). The other four species included in the protocol for species-level identification are *~~C.~~*(*Pterandrus*) *fasciventris* (Bezzi), *C.*(*Pterandrus*) *anonae* Graham, *C.*(*Pterandrus*) *rosa* Karsch and *C.*(*Pterandrus*) *quilicii* De Meyer *et al*. These use a wide range and a large number of commercially grown hosts. The distributions for each of these four species include multiple countries across sub-Saharan Africa; although each species has a different distribution range, these ranges can overlap (De Meyer *et al.*, 2015; De Meyer *et al.*, 2016). The four species are included in a taxonomic species complex called the “FAR complex” because of high morphological and molecular similarity (Barr and McPheron, 2006; Virgilio *et al.*, 2008). | *Category : TRANSLATION* **(8) Trinidad and Tobago (15 Aug 2022 9:04 PM)** Please explain C.◦ | **Considered but not incorporated.**  The C. is for *Ceratitis* and inclusion is needed to provide full taxonomic name. |
| 46 | The genus consists of six subgenera as proposed by Hancock (1984) and revised in several ~~publications~~ studies (De Meyer, 1996, 1998, 2000; De Meyer and Copeland, 2001; De Meyer and Freidberg, 2005): | *Category : TECHNICAL* **(131) United States of America (29 Aug 2022 7:21 PM)** preferred word | **Incorporated.** |
| 48 | *Ceratitis* (*Ceratalaspis*) Hancock, ~~198~~1984 | *Category : EDITORIAL* **(187) EPPO (20 Sep 2022 5:03 PM)** Date is not complete | **Incorporated.** |
| 54 | Common names and synonyms of the fruit fly species included in this protocol are ~~listed~~ in Table 1. | *Category : EDITORIAL* **(261) New Zealand (28 Sep 2022 9:10 AM)** | **Incorporated.** |
| 54 | Common names and synonyms of the *Ceratitis* fruit fly species included in this protocol are listed in Table 1. | *Category : EDITORIAL* **(132) United States of America (29 Aug 2022 7:31 PM)** clarity | **Incorporated.** |
| 55 | **Table 1.**Common names and synonyms of fruit fly species of major economic importance belonging to the genus *Ceratitis*and included in this diagnostic protocol | *Category : EDITORIAL* **(133) United States of America (29 Aug 2022 7:32 PM)** The font and text size in all parts of Table 1 and other tables (Table 2 and 3) differ from the documents' other sections. Is there the standard format for this document? | **Incorporated.** |
| 55 | **Table 1.** Common names and synonyms of fruit fly species of major economic importance belonging to the genus *Ceratitis* and included in this diagnostic protocol | *Category : TECHNICAL* **(12) United States of America (17 Aug 2022 8:37 PM)** In zoology, an alternative generic (or subgeneric) combination is not a synonym. A synonym is a name published with a separate type that is subsequently considered to refer to the same entity as another published name at the same rank. Moving a species from one genus (or subgenus) to another is a taxonomic choice that does not bear on typification A more general way to refer to both synonyms and alternative generic combinations is as “other names”. | **Incorporated.**  To address comment, protocol now includes “other names” in column’s header and in the table’s caption |
| 59 | *Ceratitis* (*Pterandrus*) *anonae* Graham, 1908 | *Category : TECHNICAL* **(13) United States of America (17 Aug 2022 8:39 PM)** problems with the indication of authorship in this table, as follows: [62] “Pterandrus anonae Bezzi 1918” is an alternative generic combination made by Bezzi in 1918, but that does not change the authorship of the species name, which should be Pterandrus anonae (Graham, 1908). [73] “Pardalaspis giffardi var. sarcocephali Bezzi, 1924” should be Ceratitis giffardi var. sarcocephali Bezzi, 1924. [79] “Pterandrus rosa Munro, 1956” is not an available name, and therefore not a synonym of Ceratitis fasciventris – perhaps a misidentification? [80] “Ceratitis (Pterandrus) rosa Hancock, 1984” is also not an available name, like the previous. [83] “Ceratitis rosa R2, “highland” is not a name, period. The footnote [88] should say “prior to de Meyer et al. 2016, Ceratitis quilicii and C. rosa were considered to be conspecific.” [86] “Pterandrus rosa Bezzi, 1918” is an alternative combination made by Bezzi in 1918, but that does not change the authorship of the species name, which should be Pterandrus rosa (Karsch, 1887). [87] see 83. (nomenclature checked in Crosskey et al. 1980. Catalogue of the Diptera of the Afrotropical Region, BM(NH), London. | **Modified.**  Updated the foot note as recommended.  Include R1 and R2 names because these are used in literature about species. |
| 60 | add unknown in cell | *Category : EDITORIAL* **(238) Colombia (21 Sep 2022 5:13 AM)** empty cell in common name | **Modified**  Unknown would mean a common name exists. Inserted a hyphen to each cell that is empty. |
| 64 | Mediterranean fruit fly, | *Category : TECHNICAL* **(188) EPPO (20 Sep 2022 5:03 PM)** | **Incorporated** |
| 76 | add unknown in cell | *Category : EDITORIAL* **(239) Colombia (21 Sep 2022 5:14 AM)** empty cell in common name | **Modified**  Unknown would mean a common name exists. Inserted a hyphen to each cell that is empty. |
| 82 | add unknown in cell | *Category : EDITORIAL* **(240) Colombia (21 Sep 2022 5:14 AM)** empty cell in common name | **Modified**  Unknown would mean a common name exists. Inserted a hyphen to each cell that is empty. |
| 88 | *Note:* \* ~~Prior to formal description of~~ Before *Ceratitis ~~quilicii~~quilicii* was formally described, *C. quilicii* and *C. rosa sensu stricto* were referred to under these informal names. See De Meyer *et al.* (2015). | *Category : EDITORIAL* **(262) New Zealand (28 Sep 2022 9:12 AM)** | **Modified**  Based on similar comments this has been updated to simplify text (see comment for para 59) |
| 89 | **3.** **~~Detection~~Field detection** | *Category : TECHNICAL* **(134) United States of America (29 Aug 2022 7:33 PM)** better term | **Considered but not incorporated**  The heading of Detection is part of format for protocols |
| 90 | Fruit flies of the genus *Ceratitis* are detected mainly by trap for adults or in ~~fruits~~fruits for eggs and larvae. Male attractant lures are commonly used for *C. capitata* adults (Tan *et al.*, 2014) and may be useful for pest species in the subgenera *Ceratitis* and *Pterandrus* but are known to be ~~not effective~~ ineffective for all species in the genus (De Meyer, 1999). The most commonly used lures are trimedlure (for *Ceratitis capitata* and representatives of the *Ceratitis* FAR complex), terpinyl-acetate (for *C. cosyra*) and enriched ginger oil lure (Mwatawala , 2013; Manrakhan *et al.*, 2017). Other male attractants have been examined, such as methyl eugenol for species in the subgenus *Paradalaspis* (De Meyer, 1999). In addition, food-based attractants have been reported ~~as being~~ to be effective for many adult flies (Epsky~~, Kendra and Schnell~~ *et al*, 2014; Manrakhan , 2017). Immature stages of flies, such as eggs and larvae (first, second and third instars), can be found during ~~an~~ inspection of fruits. Larvae usually exit the fruit after feeding, and the immobile pupal stage develops elsewhere (e.g., in leaf litter, soil, or shipping containers). | *Category : EDITORIAL* **(271) New Zealand (29 Sep 2022 12:02 AM)** | **Modified**  Incorporated all edits but also adjusted text to include comments of other reviewers. |
| 90 | Fruit flies of the genus *Ceratitis* are detected mainly by ~~trap for~~ trapping adults or by finding larvae in fruits. Male attractant lures are commonly used for *C. capitata* adults (Tan *et al.*, 2014) and may be useful for pest species in the subgenera *Ceratitis* and *Pterandrus* but are ~~known to be~~ not effective for all species in the genus (De Meyer, 1999). The most commonly used lures are trimedlure (for *Ceratitis capitata* and representatives of the *Ceratitis* FAR complex), terpinyl-acetate (for *C. cosyra*) and enriched ginger oil lure (Mwatawala , 2013; Manrakhan *et al.*, 2017). Other male attractants have been ~~examined~~evaluated, such as methyl eugenol for species in the subgenus *Paradalaspis* (De Meyer, 1999). In addition, food-based attractants have been reported as being effective for many adult flies (Epsky, Kendra and Schnell, 2014; Manrakhan , 2017)~~.~~ .  Immature stages ~~of flies, such as eggs~~ (eggs and ~~larvae (first~~larvae, first, second and third instars), can be found during an inspection of the fruits. ~~Larvae usually~~ After completing their development, larvae exit the ~~fruit after feeding,~~ fruits and the immobile pupal stage develops elsewhere (e.g., in leaf litter, soil, or ~~shipping containers)~~packaging). | *Category : TECHNICAL* **(189) EPPO (20 Sep 2022 5:03 PM)** Suggestion of clearer technical terminology and straightforward text. For better clarity, we suggest to begin a new paragraph for traps (male attractant lures) and for fruits (immature stages of flies). | **Incorporated** |
| 90 | Fruit flies of the genus *Ceratitis* are detected mainly by ~~trap for~~ trap adults or in fruits. Male attractant lures are commonly used for *C. capitata* adults (Tan *et al.*, 2014) and may be useful for pest species in the subgenera *Ceratitis* and *Pterandrus* but are known to be not effective for all species in the genus (De Meyer, 1999). The most commonly used lures are trimedlure (for *Ceratitis capitata* and representatives of the *Ceratitis* FAR complex), terpinyl-acetate (for *C. cosyra*) and enriched ginger oil lure (Mwatawala , 2013; Manrakhan *et al.*, 2017). Other male attractants have been examined, such as methyl eugenol for species in the subgenus *Paradalaspis* (De Meyer, 1999). In addition, food-based attractants have been reported as being effective for many adult flies (Epsky, Kendra and Schnell, 2014; Manrakhan , 2017). Immature stages of flies, such as eggs and larvae (first, second and third instars), can be found during an inspection of fruits. Larvae usually exit the fruit after feeding, and the immobile pupal stage develops elsewhere (e.g., in leaf litter, soil, or shipping containers). | *Category : TECHNICAL* **(125) Kenya (29 Aug 2022 8:19 AM)** Fruit flies of the genus Ceratitis are detected mainly by using of a pheromone trap for to capture adults or inin fruits where the eggs and larval stages are found. | **Modified**  Text updated to address concerns of multiple commentators for improved clarity |
| 90 | Fruit flies of the genus *Ceratitis* are detected mainly by trap for adults or in fruits. Male attractant lures are commonly used for *C. capitata* adults (Tan *et al.*, 2014) and may be useful for pest species in the subgenera *Ceratitis* and *Pterandrus* but are known to be not effective for all species in the genus (De Meyer, 1999). The most commonly used lures are trimedlure (for *Ceratitis capitata* and representatives of the *Ceratitis* FAR complex), terpinyl-acetate (for *C. cosyra*) and enriched ginger oil lure (Mwatawala , 2013; Manrakhan *et al.*, 2017). Other male attractants have been examined, such as methyl eugenol for species in the subgenus *Paradalaspis* (De Meyer, 1999). In addition, food-based attractants have been reported as being effective for many adult flies (Epsky, Kendra and Schnell, 2014; Manrakhan , 2017). Immature stages of flies, such as eggs and larvae (first, second and third instars), can be found during an inspection of fruits. Larvae usually exit the fruit after feeding, and the immobile pupal stage develops elsewhere (e.g., in leaf litter, soil, or shipping containers). | *Category : TECHNICAL* **(124) Kenya (29 Aug 2022 8:17 AM)** by using of a pheromone | **Considered but not incorporated**  The term lures includes pheromones and is more inclusive as the attractants could change. The protocol is not providing instructions on how to trap flies. It only provides a general description of detetction. |
| 90 | Fruit flies of the genus *Ceratitis* are detected mainly by trap for adults or ~~in~~ from emergence from fruits. Male attractant lures are commonly used for *C. capitata* adults (Tan *et al.*, 2014) and may be useful for pest species in the subgenera *Ceratitis* and *Pterandrus* but are known to be not effective for all species in the genus (De Meyer, 1999). The most commonly used lures are trimedlure (for *Ceratitis capitata* and representatives of the *Ceratitis* FAR complex), terpinyl-acetate (for *C. cosyra*) and enriched ginger oil lure (Mwatawala , 2013; Manrakhan *et al.*, 2017). Other male attractants have been examined, such as methyl eugenol for species in the subgenus *Paradalaspis* (De Meyer, 1999). In addition, food-based attractants have been reported as being effective for many adult flies (Epsky, Kendra and Schnell, 2014; Manrakhan , 2017). Immature stages of flies, such as eggs and larvae (first, second and third instars), can be found during an inspection of fruits. Larvae usually exit the fruit after feeding, and the immobile pupal stage develops elsewhere (e.g., in leaf litter, soil, or shipping containers). | *Category : EDITORIAL* **(9) Trinidad and Tobago (15 Aug 2022 9:07 PM)** | **Modified**  The comment was addressed in revised text. |
| 93 | **3.2** **~~Inspection of~~ Inspecting fruits** | *Category : EDITORIAL* **(272) New Zealand (29 Sep 2022 12:06 AM)** | **Incorporated** |
| 94 | Fruits with soft areas, dark stains, dark pin spots, rot, orifices or injuries that might have originated from female oviposition or larval-feeding activities should be targeted for inspection. In order to detect punctures made by female flies during oviposition, fruits should be examined under a microscope by an expert. If larval exit holes are observed, the fruit containers should be inspected for pupae. Third instars may not be present when unripe fruits are collected and packed; however, these fruits might host eggs and first or second instars, which are more difficult to detect. Potentially infested fruits that show typical punctures made by ovipositing female flies should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits. | *Category : TECHNICAL* **(293) Antigua and Barbuda (30 Sep 2022 5:00 PM)** Is there any available information that points to instances when oviposition has not resulted in puncture or other marks left on the fruit? | **Considered but not incorporated**  The statement is that fruit should be inspected if there is evidence of puncture wounds. It is possible that larvae are not in those fruits.  Lack of evidence of a puncture or mark could be because there is not a puncture or the puncture was not detectable for that fruit. The protocol cannot provide methods for that distinction at this time.. |
| 94 | Fruits with soft areas, dark stains, dark pin spots, rot, orifices or injuries that might ~~have originated from female~~ be caused oviposition or ~~larval-feeding~~ larval feeding activities should be targeted for inspection. ~~In order to~~ To detect punctures made by female flies during oviposition, fruits should be examined under a microscope by an expert. If larval exit holes are observed, the fruit containers should be inspected for pupae. Third instars may not be present when unripe ~~fruits are~~ fruit is collected and packed; however, ~~these fruits~~ this fruit might host eggs and first or second instars, which are more difficult to detect. Potentially infested ~~fruits~~ fruit that ~~show~~ shows typical punctures made by ovipositing female flies should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits. | *Category : EDITORIAL* **(273) New Zealand (29 Sep 2022 12:09 AM)** oviposition is by definition by females. remove hyphen between larvae and feeding. This term is used above non-hyphenated. | **Modified**  The editorial changes were included along with other suggested text changes from other comments to improve text clarity. |
| 94 | ~~Fruits with~~ Signs of fruit flies infestation on fruits are the presence of soft areas, dark stains, dark pin spots, rot, ~~orifices~~ holes or injuries that might have originated from female oviposition or larval-feeding activities ~~should be targeted for inspection~~. In order to detect punctures made by female flies during oviposition, fruits ~~should be~~ are examined under a ~~microscope~~ stereo-microscope by an expert. If larval exit holes are observed, ~~the fruit containers should~~ puparium may be ~~inspected for pupae~~detected in the packaging. Third instars may not be present when unripe fruits are collected and packed; however, these fruits might host eggs and first or second instars, which are more difficult to detect. ~~Potentially~~ On potentially infested fruits ~~that show~~ showing typical punctures made by ovipositing female flies ~~should be cut open to search for~~ eggs ~~or larvae inside. The success of detection depends on careful sampling~~ and ~~examination of fruits~~larvae may be seen when cutting open . | *Category : SUBSTANTIVE* **(190) EPPO (20 Sep 2022 5:03 PM)** We believe that this paragrph should be reworded as suggested to be less prescriptive thus being more in line with the instructions to authors of IPPC protocols (page 11) .  Holes is a better term than orifice.  Microscope should be replaced by stereo-microscope In recent EPPO protocols we have been asked to use puparium and not pupae (sentence pupae may be detected)  The last sentence is obvious and can be deleted (we have also included some editorial in this paragraph) | **Incorporated** |
| 94 | Fruits with soft areas, dark stains, dark pin spots, rot, orifices or injuries that might have originated from female oviposition or larval-feeding activities should be targeted for inspection. In order to detect punctures made by female flies during oviposition, fruits should be examined under a microscope by an expert. If larval exit holes are observed, the fruit containers should be inspected for pupae. Third instars may not be present when unripe fruits are collected and packed; however, these fruits might host eggs and first or second instars, which are more difficult to detect. Potentially infested fruits that show typical punctures made by ovipositing female flies should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits. | *Category : TECHNICAL* **(126) Kenya (29 Aug 2022 8:21 AM)** . If larval exit holes are observed, the fruit containers should be inspected for pupae. Third instar larvaes may not be present whenbe unlikely to be detected when unripe fruits are collected and packed; however, these fruits might host eggs and first or second instars, which are more difficult to detect. | **Modified**  The revised text addresses these comments about difficulty to detect. |
| 94 | Fruits with soft areas, dark stains, dark pin spots, rot, orifices or injuries that might have originated from female oviposition or larval-feeding activities should be targeted for inspection. In order to detect punctures made by female flies during oviposition, fruits should be examined under a microscope by an expert. If larval exit holes are observed, the fruit containers should be inspected for pupae. Third instars may not be present when unripe fruits are collected and packed; however, these fruits might host eggs and first or second instars, which are more difficult to detect. Potentially infested fruits that show typical punctures made by ovipositing female flies should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits. | *Category : TECHNICAL* **(1) Syrian Arab Republic (30 Jul 2022 2:03 PM)** Clarification about, the fruit containers should be inspected for pupae | **Modified**  Revised text addresses the general comment about puparium. The decision to inspect all containers or use different sampling strategy is not within the scope of the diagnositc protocol. When holes are observed, then detection of puparium in packages is recommended to increase likelihood of detection. |
| 95 | Once detected, larvae may be reared to adults for identification (section 3.3). Rearing of adults is required to accurately identify a fly to species level using morphological techniques. The incubation of infested fruits is a common practice to obtain adult flies, which is necessary to identify species in this protocol. Even if there are no signs of fruit fly infestation, an incubation could be conducted as an oviposition mark is often difficult to recognize. | *Category : TECHNICAL* **(274) New Zealand (29 Sep 2022 12:22 AM)** It would be useful to indicate incubation conditions (e.g. temperature, humidity) and rear any larvae as per section 3.3. | **Modified**  Conditions vary for laboratories and general resources like White & Elson-Harris (1992) do not provide these estimates or ranges. Using method from Copeland et al.(2002) for medfly, temperature values are now included as an option. Lab reared *Ceratitis* in Africa were from fruit kept at approximately 24oC in winter months and that open containers of water were at lab to support humid environment (but no values of humidity provided). Duyck et al. (2006) reported on effects of humidity of survival to adult stage.Citations are now included for information. |
| 95 | Once detected, larvae may be reared to adults for identification (section 3.3). Rearing of adults is required to accurately identify a fly to species level ~~using~~ with morphological techniques. The incubation of infested fruits is a common practice to obtain adult flies, which is necessary to identify species in this protocol. Even if there are no signs of fruit fly infestation, an incubation ~~could~~ can be conducted as an oviposition mark is often difficult to recognize. | *Category : TECHNICAL* **(191) EPPO (20 Sep 2022 5:03 PM)** Is incubation recomnended?  1 With seems clearer. 2) Can seems a more appropriate tense? | **Incorporated** |
| 95 | Once detected, larvae may be reared to adults for identification (section 3.3). Rearing of adults is required to accurately identify a fly to species level using morphological techniques. The incubation of infested fruits is a common practice to obtain adult flies, which is necessary to identify species in this protocol. Even if there are no signs of fruit fly infestation, an incubation could be conducted as an oviposition mark is often difficult to recognize. | *Category : SUBSTANTIVE* **(118) China (28 Aug 2022 5:03 PM)** Add molecular identification measures. “Once detected, larvae may be reared to adults for identification (section 3.3)” should be changed into “Once detected, larvae may be reared to adults for morphological identification (section 3.3) or be directly identified by molecular analysis.” | **Considered but not incorporated.**  The section indicates that rearing may be performed but does not indciate it must be performed. However, species level determinations for all species in the protocol require adult morphology. If molecular methods are to be used, the protocol provides instrucitons for that in current version.” |
| 95 | Once detected, larvae may be reared to adults for identification (section 3.3). Rearing of adults is required to accurately identify a fly to species level using morphological techniques. ~~The incubation of infested fruits is a common practice to obtain adult flies, which is necessary to identify species in this protocol. Even if there are no signs of fruit fly infestation, an incubation could be conducted as an oviposition mark is often difficult to recognize.~~ | *Category : EDITORIAL* **(10) Trinidad and Tobago (15 Aug 2022 9:12 PM)** | **Modified.**  The removal of the first sentence in edited text is incorporated. It is redundant with other text.  The final sentence of the paragraph is about incubation of fruit without signs of puncture. It is not prescriptive and is included to alert those using the protocol of the difficulty with detecting marks or puncture. Evidence other than signs on fruit puncture could be reason to attempt to detect larvae through rearing. |
| 96 | 3.3 Rearing larvae to obtain adults | *Category : TECHNICAL* **(14) United States of America (17 Aug 2022 8:41 PM)** 3.3 Rearing larvae to obtain adults [97] – might want to say something here about ensuring that quarantine insects are properly contained to ensure that they don’t escape | **Considered but not incorporated.**  This is correct but the protocol only provides guidance on methdos to complete accurate identification. Other concerns with quarantine are at discretion of PPO institutions and their protection requirements. |
| 97 | Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g., damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should 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 several days to ensure that the integument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and ~~water.~~ water or a mix of sugar, yeast, wheat germ and water The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. | *Category : TECHNICAL* **(299) Brazil (1 Oct 2022 12:52 AM)** An other option | **Incorporated** |
| 97 | Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g., damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should 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 several days to ensure that the integument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. | *Category : EDITORIAL* **(275) New Zealand (29 Sep 2022 12:25 AM)** Does this mean honey and sugar, or honey or sugar? | **Modified**  Text clarified and honey is one type of sugar to use |
| 97 | Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g., damp vermiculite, sand or sawdust) ~~at~~ on the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should 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 several days to ensure that the integument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. | *Category : EDITORIAL* **(192) EPPO (20 Sep 2022 5:03 PM)** | **Incorporated** |
| 97 | Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g., damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should be observed, and pupae ~~gathered~~ are collected daily. The collected 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 several days to ensure that the integument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. | *Category : EDITORIAL* **(178) Myanmar (5 Sep 2022 1:47 PM)** | **Incorporated** |
| 97 | Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g., damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should 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 several days to ensure that the integument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. | *Category : TECHNICAL* **(135) United States of America (29 Aug 2022 7:35 PM)** Is for preserving/storing samples for morphological identifications? Is there any guideline for preserving/storing samples for molecular identification at this stage? | **Modified**  A sentence has been added at end of paragraph to direct reader to section 4.3.1 for information on molecular analysis. |
| 97 | Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g., damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should 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 several days to ensure that the integument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) yeast (protein) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. | *Category : SUBSTANTIVE* **(96) Thailand (25 Aug 2022 11:54 AM)** We would like to add "yeast (protein)" as one of component in feed for fruit flies. | **Modified**  Sentence was modified based on previous comment to be inclusive of other options and to the mention of yeast. This protocol provides examples of how to complete this but many alternative food components would be acceptable. |
| 98 | Prior to mounting (before they harden), it is useful to gently squeeze the apical part of the preabdomen with forceps, then squeeze the base of the oviscape to expose the aculeus tip for females. Alternatively, this will need to be dissected later in flies. The aedeagus is not commonly used for ~~examination~~ identification of *Ceratitis* males. | *Category : EDITORIAL* **(276) New Zealand (29 Sep 2022 12:25 AM)** | **Incorporated** |
| 100 | Identification at the level of species or species complex requires morphological examination of adult flies or molecular analysis. For some species, accurate identification can only be completed for male specimens because the female form has not been described or females lack diagnostic features. In addition to keys developed for species in each subgenus (De Meyer, 1996, 1998, 2000; De Meyer and Freidberg, 2005), an online multi-entry Lucid key to frugivorous flies of Africa is available that can be used to identify *Ceratitis* species (Virgilio, ~~White and De Meyer~~et al., 2014). | *Category : EDITORIAL* **(23) United States of America (17 Aug 2022 8:55 PM)** citation | **Incorporated** |
| 101 | It is not reliable to morphologically identify eggs, most larvae or pupae to the species level. There are descriptions of third instars for some species but not all pests in the family. These descriptions of the third instar can be used to discriminate among the described species (White and Elson-Harris, 1992; Steck and Ekesi, 2015) but not to distinguish with reliability one pest from all other pests. This is true of all *Ceratitis* pests. The descriptions of third instar *Ceratitis* are usually based on laboratory colony material and might not accurately represent the true diversity of the species (Steck and Ekesi, 2015). The most reliable method for identifying species is rearing larvae to the adult stage or molecular analysis. | *Category : SUBSTANTIVE* **(281) New Zealand (30 Sep 2022 7:33 AM)** Query: use of the term pest seemingly interchangeably with species. Also in some parts of the document the usage is ambiguous e.g. in Section 40 where “pest distribution” is referred to, it is unclear whether this refers to the total range of the species or just the parts of its range in which it is a pest | **Modified**  The use of the word pest is being replaced by species where appropriate. In this case, most species lack descriptions and even economically important species lack descriptions. |
| 101 | It is not reliable to morphologically identify eggs, most larvae or pupae to ~~the~~ species level. There are descriptions of third instars for some species but not all pests in the family. These descriptions of the third instar can be used to discriminate among the described species (White and Elson-Harris, 1992; Steck and Ekesi, 2015) but not to distinguish with reliability one pest from all other pests. This is true of all *Ceratitis* pests. The descriptions of third instar *Ceratitis* are usually based on laboratory colony material and might not accurately represent the true diversity of the species (Steck and Ekesi, 2015). The most reliable method for identifying species is rearing larvae to the adult stage or molecular analysis. | *Category : EDITORIAL* **(263) Canada (28 Sep 2022 9:38 PM)** | **Incorporated** |
| 101 | It is not reliable to morphologically identify eggs, most larvae or pupae to the species level. There are descriptions of third instars for some species but not all pests in the family. These descriptions of the third instar can be used to discriminate among the described pest species (White and Elson-Harris, 1992; Steck and Ekesi, 2015) but not to distinguish with reliability one pest from all other pests. This is true of all *Ceratitis* pests. The descriptions of third instar *Ceratitis* are usually based on laboratory colony material and might not accurately represent the true diversity of the species (Steck and Ekesi, 2015). The most reliable method for identifying species is rearing larvae to the adult stage or molecular analysis. | *Category : EDITORIAL* **(247) Australia (26 Sep 2022 4:04 AM)** This statement requires rewording as pests are also described as species. It is suggested that the word pest is added to link species to the word pest as used throughout the remainder of the sentence. | **Modified**  Incoproated change and updated other uses of pest to have term used consistently throughout |
| 101 | It is not reliable to morphologically identify eggs, most larvae or pupae to the species level. There are descriptions of third instars for some species but not all pests in the ~~family~~genus. These descriptions of the third instar can be used to discriminate among the described species (White and Elson-Harris, 1992; Steck and Ekesi, 2015) but not to distinguish with reliability one pest from all other pests. This is true of all *Ceratitis* pests. The descriptions of third instar *Ceratitis* are usually based on laboratory colony material and might not accurately represent the true diversity of the species (Steck and Ekesi, 2015). The most reliable method for identifying species is rearing larvae to the adult stage or molecular analysis. | *Category : EDITORIAL* **(246) Australia (26 Sep 2022 4:03 AM)** Consider updating to genus is discussing all of genus Ceratitis. If it is intended to discuss pests at the family level additional text should be added to clarify this intention. | **Modified**.  Family level is correct. A sentence has been added to clarify. |
| 102 | A key to identifying economically important genera based on third instars has been published (White and Elson-Harris, 1992), and an online identification tool that includes 81 economically important species of 13 genera is available (Carroll *et al*. 2004). *Ceratitis* is the only economically important genus from the tribe Ceratitidini included in the key and the diversity of each genus in the key is based on examination of a limited number of species with larval descriptions available. Steck and Ekesi (2015) reported that a character previously used to distinguish *Ceratitis* and *Bactrocera* larvae was based on limited taxon sampling. Morphological examination of a third instar can provide diagnostic information but may not allow an identification to be completed without additional molecular diagnostic information.  Host and geographical distribution records are not included in the current protocol as diagnostic features of *Ceratitis* species because the values are incomplete for many species and subject to change over time. The scope of the protocol is therefore limited to morphological (sections 4.1 and 4.2) and molecular (section 4.3) characters. | *Category : EDITORIAL* **(193) EPPO (20 Sep 2022 5:03 PM)** 1) For more clarity: creation of a new paragraph for host and geographical distribution records because this is a different issue. 2) Addition of a logical link. 3) and 4) Precisions given. | **Incorporated**. |
| 102 | A key to identifying economically important genera based on third instars has been published (White and Elson-Harris, 1992), and an online identification tool that includes 81 economically important species of 13 genera is available (Carroll *et al*. 2004). *Ceratitis* is the only economically important genus from the tribe Ceratitidini included in the key and the diversity of each genus in the key is based on examination of a limited number of species with larval descriptions available. Steck and Ekesi (2015) reported that a character previously used to distinguish *Ceratitis* and *Bactrocera* larvae was based on limited taxon sampling. Morphological examination of a third instar can provide diagnostic information but may not allow an identification to be completed without additional molecular diagnostic information. Host and geographical distribution records are not included in the current protocol as diagnostic features of *Ceratitis* species because the values are incomplete for many species and subject to change over time. The scope of the protocol is limited to morphological and molecular characters. | *Category : TECHNICAL* **(15) United States of America (17 Aug 2022 8:42 PM)** It sounds like the 3rd instar key not reliable, and should not be used. It is rather surprising to see such attention paid to identifying 3rd instar larvae in sections 219-350. | **Considered but not incorporated**  Larval morphology has challenges but is incldued because some methods are available and common to examine flies for screening prior to final identification. |
| 103 | Molecular methods for *Ceratitis* species identification have been reported for several of the most destructive, polyphagous pests: *C. capitata* (Barr *et al.*, 2006; Huang *et al.*, 2009; Barr et al., 2012; Dhami *et al.*, 2016), *C*. *cosyra* (Barr *et al.*, 2006; Virgilio *et al.*, 2017), and the four members of the FAR complex – *C*. *fasciventris*, *C*. *anonae*, *C*. *rosa* and *C*. *quilicii* (Virgilio *et al.*, 2019). These studies have considered the molecular phylogeny of the genus (Barr and McPheron, 2006; Barr and Wiegmann, 2009; Erbout *et al.*, 2011) to include species that would have a greater probability of ~~cross-reacting~~ a false positive with a target pest or lead to incorrect interpretation of a diagnostic result. Only methods that have the taxonomic sampling needed to demonstrate reliable species identification are included in this diagnostic protocol. These include a real-time polymerase chain reaction (PCR) method for *C*. *capitata* (Dhami *et al.*, 2016) and DNA barcoding methods for the identification of *C. capitata*, *C*. *cosyra* and the FAR complex using DNA sequencing of part of the cytochrome c oxidase I (*COI*) gene (section 4.3). | *Category : EDITORIAL* **(248) Australia (26 Sep 2022 4:05 AM)** The use of the term cross-reacting is confusing. It is suggested to replace with an alternate term such as false positive. | **Incorporated** |
| 103 | Molecular methods for *Ceratitis* species identification have been ~~reported~~ published for several of the most destructive, polyphagous pests: *C. capitata* (Barr *et al.*, 2006; Huang *et al.*, 2009; Barr et al., 2012; Dhami *et al.*, 2016), *C*. *cosyra* (Barr *et al.*, 2006; Virgilio *et al.*, 2017), and the four members of the FAR complex – *C*. *fasciventris*, *C*. *anonae*, *C*. *rosa* and *C*. *quilicii* (Virgilio *et al.*, 2019). These studies have considered the molecular phylogeny of the genus (Barr and McPheron, 2006; Barr and Wiegmann, 2009; Erbout *et al.*, 2011) to include species that would have a greater probability of cross-reacting with a target pest or lead to incorrect interpretation of a diagnostic result. Only methods that have the taxonomic sampling needed to demonstrate reliable species identification are included in this diagnostic protocol. These include a real-time polymerase chain reaction (PCR) method for *C*. *capitata* (Dhami *et al.*, 2016) and DNA barcoding methods for the identification of *C. capitata*, *C*. *cosyra* and the FAR complex using DNA sequencing of part of the cytochrome c oxidase I (*COI*) gene (section 4.3). | *Category : TECHNICAL* **(194) EPPO (20 Sep 2022 5:03 PM)** Published seems better than reported We do not understand what is meant by "the taxonomic sampling needed" is this about analytical specificity (inclusivity and exclusivity)   With regards to reliable species identification: were the FARQ complex larvae also identified to the species level? | **Modified**.  Incorporated edit for “published” instead of “reported” in revised text.  The text about reliabiity and analytical sensitivity is further clarified in revised text.  FARQ species have molecular studies demonstrating identification but are not appropriate for routine identificaiton given expense, instrumentation, and time required for genomic analysis of the species. These are valid methods but not included in the protocol. This is consistent with recommendaiton of the co-authors of the original genomic study. |
| 103 | Molecular methods for *Ceratitis* species identification have been reported for several of the most destructive, polyphagous pests: *C. capitata* (Barr *et al.*, 2006; Huang *et al.*, 2009; Barr et al., 2012; Dhami *et al.*, 2016), *C*. *cosyra* (Barr *et al.*, 2006; Virgilio *et al.*, 2017), and the four members of the FAR complex – *C*. *fasciventris*, *C*. *anonae*, *C*. *rosa* and *C*. *quilicii* (Virgilio *et al.*, 2019). These studies have considered the molecular phylogeny of the genus (Barr and McPheron, 2006; Barr and Wiegmann, 2009; Erbout *et al.*, 2011) to include species that would have a greater probability of cross-reacting with a target pest or lead to incorrect interpretation of a diagnostic result. Only methods that have the taxonomic sampling needed to demonstrate reliable species identification are included in this diagnostic protocol. These include a real-time polymerase chain reaction (PCR) method for *C*. *capitata* (Dhami *et al.*, 2016) and DNA barcoding methods for the identification of *C. capitata*, *C*. *cosyra* and the FAR complex using DNA sequencing of part of the mitochondrial cytochrome c oxidase I (*COI*) gene ~~(section~~as described in section 4.~~3)~~3. | *Category : TECHNICAL* **(137) United States of America (29 Aug 2022 7:38 PM)** It may be good to add references here even though they are provided in section 4.3 | **Modified**  Incorporated change.The reference to section 4.3 was not modified because it is consistent with other documents. |
| 103 | Molecular methods for *Ceratitis* species identification have been reported for several of the most destructive, polyphagous pests: *C. capitata* (Barr *et al.*, 2006; Huang *et al.*, 2009; Barr et al., 2012; Dhami *et al.*, 2016), *C*. *cosyra* (Barr *et al.*, 2006; Virgilio *et al.*, 2017), and the four members of the FAR complex – *C*. *fasciventris*, *C*. *anonae*, *C*. *rosa* and *C*. *quilicii* (Virgilio *et al.*, 2019). These studies have considered the molecular phylogeny of the genus (Barr and McPheron, 2006; Barr and Wiegmann, 2009; Erbout *et al.*, 2011) to include species that would have a greater probability of cross-reacting with a target pest or lead to incorrect interpretation of a diagnostic result. Only methods that have the taxonomic sampling needed to demonstrate reliable species identification are included in this diagnostic protocol. These include a real-time polymerase chain reaction (PCR) method for *C*. *capitata* (Dhami *et al.*, 2016) and DNA barcoding methods for the identification of *C. capitata*, *C*. *cosyra* and the FAR complex using DNA sequencing of part of the cytochrome c oxidase I (*COI*) gene (section 4.3). | *Category : TECHNICAL* **(136) United States of America (29 Aug 2022 7:36 PM)** redundant - was already explained earlier | **Incorporated** |
| 103 | Molecular methods for *Ceratitis* species identification have been reported for several of the most destructive, polyphagous pests: *C. capitata* (Barr *et al.*, 2006; Huang *et al.*, 2009; Barr et al., 2012; Dhami *et al.*, 2016), *C*. *cosyra* (Barr *et al.*, 2006; Virgilio *et al.*, 2017), and the four members of the FAR complex – *C*. *fasciventris*, *C*. *anonae*, *C*. *rosa* and *C*. *quilicii* (Virgilio *et al.*, 2019). These studies have considered the molecular phylogeny of the genus (Barr and McPheron, 2006; Barr and Wiegmann, 2009; Erbout *et al.*, 2011) to include species that would have a greater probability of cross-reacting with a target pest or lead to incorrect interpretation of a diagnostic result. Only methods that have the taxonomic sampling needed to demonstrate reliable species identification are included in this diagnostic protocol. These include a real-time polymerase chain reaction (PCR) method for *C*. *capitata* (Dhami *et al.*, 2016) and DNA barcoding methods for the identification of *C. capitata*, *C*. *cosyra* and the FAR complex using DNA sequencing of part of the cytochrome c oxidase I (*COI*) gene (section 4.3). | *Category : SUBSTANTIVE* **(119) China (28 Aug 2022 5:04 PM)** Add ”Zhang et al., 2021”as reference. Add related information as “FARQ complex and other similar species can be identified based on re-sequencing. | **Incorporated** |
| 103 | Molecular methods for *Ceratitis* species identification have been reported for several of the most destructive, polyphagous pests: *C. capitata* (Barr *et al.*, 2006; Huang *et al.*, 2009; Barr ~~et al~~*et al*., 2012; Dhami *et al.*, 2016), *C*. *cosyra* (Barr *et al.*, 2006; Virgilio *et al.*, 2017), and the four members of the FAR complex – *C*. *fasciventris*, *C*. *anonae*, *C*. *rosa* and *C*. *quilicii* (Virgilio *et al.*, 2019). These studies have considered the molecular phylogeny of the genus (Barr and McPheron, 2006; Barr and Wiegmann, 2009; Erbout *et al.*, 2011) to include species that would have a greater probability of cross-reacting with a target pest or lead to incorrect interpretation of a diagnostic result. Only methods that have the taxonomic sampling needed to demonstrate reliable species identification are included in this diagnostic protocol. These include a real-time polymerase chain reaction (PCR) method for *C*. *capitata* (Dhami *et al.*, 2016) and DNA barcoding methods for the identification of *C. capitata*, *C*. *cosyra* and the FAR complex using DNA sequencing of part of the cytochrome c oxidase I (*COI*) gene (section 4.3). | *Category : EDITORIAL* **(103) Thailand (26 Aug 2022 4:27 AM)** | **Incorporated** |
| 104 | DNA barcode records for other *Ceratitis* species are reported in the literature (Barr *et al.*, 2012; Virgilio *et al.*, 2012) and can be accessed using DNA databases. Formal examination of reference data specificity has not been reported for the other ~~pests~~ species not included in this protocol. The restriction fragment length polymorphism method of Barr *et al.* (2006) is also not included in this protocol as it lacks profiles for several important ~~pests~~ species in the genus that are represented in DNA barcode studies. Methods to identify insects to the level of genus *Ceratitis* based on DNA barcodes have not been formally described or published; consequently, methods to identify the genus are not included in this protocol. | *Category : EDITORIAL* **(195) EPPO (20 Sep 2022 5:03 PM)** Revised change by bouhot-delduc on 19 Aug 2022 14:50 | **Incorporated** |
| 104 | DNA barcode records for other *Ceratitis* species are reported in the literature (Barr *et al.*, 2012; Virgilio *et al.*, 2012) and can be accessed using DNA databases. Formal examination of reference data specificity has not been reported for the other pests not included in this protocol. The restriction fragment length polymorphism method of Barr *et al.* (2006) is also not included in this protocol as it lacks profiles for several important pests in the genus that are represented in DNA barcode studies. Methods to identify insects to the level of genus *Ceratitis* based on DNA barcodes have not been formally described or published; consequently, methods to identify the genus are not included in this protocol. | *Category : TECHNICAL* **(138) United States of America (29 Aug 2022 7:40 PM)** this statement may not be necessary consistent with the previous paragraph [103]: ‘Only methods that have the taxonomic sampling needed to demonstrate reliable species identification are included in this diagnostic protocol.’ | **Incorporated** |
| 106 | The destruction of insect tissue for DNA-based identification can preclude morphological examination unless care is taken to retain body parts needed for such examination. The use of a fly leg for DNA extraction is recommended for some species when molecular data are to be collected, but the specimen should be saved for morphological analysis. The presence of characters on fore and mid legs are diagnostically informative in the genus, and at least one ~~row~~ leg of ~~legs~~ each pair should be retained for morphological examination. When a larva is needed for morphological examination, excision of tissue from the midsection should be performed to collect molecular data. For guidance on preparing a specimen for molecular study, see section 4.3.1. | *Category : EDITORIAL* **(196) EPPO (20 Sep 2022 5:03 PM)** Clearer | **Incorporated** |
| 106 | The destruction of insect tissue for DNA-based identification can preclude morphological examination unless care is taken to retain the remaining body parts needed for such examination. The use of a fly leg for DNA extraction is recommended for some species when molecular data are to be collected, but the remaining body parts of the specimen should be saved for morphological analysis. The presence of characters on fore and mid legs are diagnostically informative in the genus, and at least one row of legs should be retained for morphological examination. When a larva is needed for morphological examination, excision of tissue from the midsection should be performed to collect molecular data. For guidance on preparing a specimen for molecular study, see section 4.3.1. | *Category : TECHNICAL* **(139) United States of America (29 Aug 2022 7:41 PM)** clarity | **Incorporated** |
| 107 | Molecular methods can be used for all life stages. Morphological identification methods are not available for eggs and pupae~~, and if these life stages are included in molecular analyses, they do not need to be heat treated~~. | *Category : EDITORIAL* **(197) EPPO (20 Sep 2022 5:03 PM)** Unnecessary? | **Incorporated** |
| 107 | Molecular methods can be used for all life stages. Morphological identification methods are not available for eggs and pupae, and if these life stages are included in molecular analyses, they do not need to be heat treated. | *Category : TECHNICAL* **(140) United States of America (29 Aug 2022 7:43 PM)** Nothing was mentioned about ‘heat treatment’ before this section. It may be better to provide a brief description of ‘heat treatment’ and why/ when it is done or not. | **Modified**.  Text was removed to avoid confusion. |
| 109 | The diagnostic characters required to complete identification to the ~~pest~~ species covered by this protocol and to the genus are provided below. Additional resources on general characters for tephritid fruit fly identification are provided in White and Elson-Harris (1992). | *Category : EDITORIAL* **(282) New Zealand (30 Sep 2022 7:35 AM)** see comments for para 40 and para 101 | **Incorporated** |
| 109 | The diagnostic characters required to complete identification to the pest species covered by this protocol and to the genus are provided below. Additional resources on general characters for ~~tephritid~~ Tephritid fruit fly identification are provided in White and Elson-Harris (1992). | *Category : TECHNICAL* **(127) Kenya (29 Aug 2022 8:24 AM)** Additional resources on general characters for Ttephritid fruit fly identification are provided in White and Elson-Harris (1992). | **Considered but not incorporated**  As an adjective family is not captialized. |
| 111 | Proper preparation of specimens is essential for accurate morphological identification. General instructions on the preparation of adult fruit fly specimens are given by White and Elson-Harris (1992). | *Category : SUBSTANTIVE* **(120) China (28 Aug 2022 5:04 PM)** Add identification of the genus Ceratitis. Add the key to larvae of major economically important species of the genus Ceratis  Describe the detailed morphological characteristics of the adults of genus Ceratitis ensure that the fruit fly belong to genus Ceratitis. | **Considered but not incoporated**  Section 4.1.2 provides the adult characters to identify to the genus. Experts determined that a key would be less clear for accurate identification. This is because there is no single (or small set of) unique character to differentiate members of the genus from those of other genera. Therefore a key is not included. Users of the protocol should be familiar with fruit fly identification.  Section 4.2.2 provides the 3rd instar characters to identify to the genus. This requires expert knowledge to successfully perform and reliability is not high with this method. The description of characters was determined to be a superior approach to keys for diagnosing these larvae. |
| 114 | Wing characters can usually be observed without mounting, so mounting is not recommended as a general practice. It may be necessary for morphometric studies, but it is not necessary to observe the characters used in section 4.1.3. If permanent mounts are made, it is recommended that one of the wings be cut off from its base (the right wing is preferred because it facilitates comparison with images reported in the literature and this diagnostic protocol). | *Category : TECHNICAL* **(283) New Zealand (30 Sep 2022 7:36 AM)** Suggest specifying slide-mounting if that is the intent, since pinned specimens are also commonly referred to as mounted | **Incorporated** |
| 114 | Wing characters can usually be observed without slide mounting, so mounting is not recommended as a general practice. It may be necessary for morphometric studies, but it is not necessary to observe the characters used in section 4.1.3. If permanent slide mounts are made, it is recommended that one of the wings be cut off from its base (the right wing is preferred because it facilitates comparison with images reported in the literature and this diagnostic protocol). | *Category : EDITORIAL* **(264) Canada (28 Sep 2022 9:39 PM)** | **Incorporated** |
| 117 | There is no unambiguous character that differentiates all representatives of the genus *Ceratitis* from any of the other closely related genera within the Dacinae. The combination of the presence of prescutellar setae (Figure 1), presence of basal scutellar setae (Figure 2) and the short appendix of the wing cell bcu (the posterior cubital cell or cup) with a constriction at the base (Figure 3) excludes other dacine genera that consist of pest species (such as *Bactrocera* Macquart, *Dacus* Fabricius and *Zeugodacus* Hendel) as well as any other non-dacine genera. | *Category : TECHNICAL* **(265) Canada (28 Sep 2022 9:40 PM)** Whose terms are being followed for : wing cell bcu. Perhaps term should be updated to follow Cumming & Wood (2017), especially since this is what is used for the Tephritidae chapter of the Manual of Afrotropical Diptera published in 2021. | **Incorporated**  Terminology used as given in the glossary of White et al. (1999) . That glossary is for Tephritidae and accepted by the vast majority of fruit fly researchers. Cell bcu is indeed the accepted term (also as in Cumming & Wood) but we mentioned cup as this is found in the (older) literature referring to this character. The text is rephrased accordingly |
| 117 | There is no unambiguous character that differentiates all representatives of the genus *Ceratitis* from any of the other closely related genera within the Dacinae. The combination of the presence of prescutellar setae (Figure 1), presence of basal scutellar setae (Figure 2) and the short appendix of the wing cell bcu (the posterior cubital cell or cup) with a constriction at the base (Figure 3) excludes other dacine genera that consist of pest species (such as *Bactrocera*Macquart, *Dacus* Fabricius and *Zeugodacus*Hendel) as well as any other non-dacine genera. | *Category : TECHNICAL* **(198) EPPO (20 Sep 2022 5:03 PM)** 'prescutellar': should it be acrostichal?  We may be wrong, but the reference for terminology used in this document is not mentioned while it would be very useful to avoid confusion | **Incorporated**  These are indeed the acrostical setae. However, as in fruit flies there is at most only one pair of these and situated anterior of the scutellum. It is referred to by some researchers as ‘prescutellar’. We have rephrased the text, using acrostichal throughout but indicating the term ‘prescutellar’ as a synonymous term, under 4.1.2. |
| 117 | There is no unambiguous character that differentiates all representatives of the genus *Ceratitis* from any of the other closely related genera within the Dacinae. The combination of the presence of prescutellar acrostichal setae (Figure 1), presence of basal scutellar setae (Figure 2) and the short appendix of the wing cell ~~bcu (the~~ cua ((= cell bcu, the posterior cubital ~~cell~~ cell, or cell cup) with a constriction at the base (Figure 3) excludes other dacine genera ~~that consist~~ that contain of pest species (such as *Bactrocera* Macquart, *Dacus* Fabricius and *Zeugodacus* Hendel) as well as any other non-dacine genera. | *Category : TECHNICAL* **(24) United States of America (17 Aug 2022 8:59 PM)** They “consist” of more than just pest species. | **Incorporated**. |
| 118 | The following combination of characters differentiates representatives of the genus *Ceratitis* from other dacine genera with a similar appearance. | *Category : SUBSTANTIVE* **(284) New Zealand (30 Sep 2022 7:37 AM)** it’s unclear exactly which combination of characters is referred to- is it the two scutellar characters or does it include the wing band characters (for unambiguous differentiation of the genus)? It would help to bullet point the relevant characters | **Modified**.  All are requird to confirm status. Bullet points added |
| 119 | Scutellum roundish and swollen (Figure 2) (excludes representatives of the genera *Carpophthoromyia*Austen and *Perilampsis* Bezzi, which have a flattened and less rounded scutellum, see Figure 4). | *Category : EDITORIAL* **(285) New Zealand (30 Sep 2022 7:39 AM)** This is not a full sentence. Suggest making the two scutellum paragraphs more similar to the wing banding paragraph - start them with "The scutellum of most Ceratitis species is roundish and swollen. Carpophthoromyia and Perilampsis have flattened and less rounded scutellum." | **Incorporated** |
| 119 | Scutellum ~~roundish~~ rounded and swollen (Figure 2) (excludes representatives of the genera *Carpophthoromyia* Austen and *Perilampsis* Bezzi, which have a flattened and less rounded scutellum, see Figure 4). | *Category : EDITORIAL* **(25) United States of America (17 Aug 2022 9:00 PM)** correct word | **Incorporated** |
| 120 | Scutellum with three dark apical markings. These markings can be clearly separated (Figure 2) or partially fused (Figure 5). In some cases, they cover most of the apical and central part of the scutellum (Figure 6), while in some other cases they are reduced to small dark spots (Figure 7). This excludes representatives of the genus *Capparimyia* Bezzi, which have only two dark apical markings (Figure 8), and several representatives of the genus *Trirhithrum* Bezzi that have a completely black scutellum (Figure 9). It also excludes some representatives of the genus *Neoceratitis* Hendel that have a single dark apical marking (Figure 10). | *Category : TECHNICAL* **(199) EPPO (20 Sep 2022 5:03 PM)** In the picture the apex appears roundish as seen from above. Is it swollen as seen from the side? Specify Dorsally or laterally swollen. | **Modified**  It is swollen when viewed from the side (laterally) but can also be swollen dorsally. Text now poroivides direction of how to observe. |
| 120 | Scutellum with three dark apical ~~markings. These~~ markings which can be clearly separated (Figure 2) or partially fused (Figure 5). In some cases, they cover most of the apical and central part of the scutellum (Figure 6), while in some other cases they are reduced to small dark spots (Figure 7). This character excludes ~~representatives~~ species of the genus *Capparimyia* Bezzi, which have only two dark apical markings (Figure 8), and several representatives of the genus *Trirhithrum* Bezzi that have a completely black scutellum (Figure 9). It also excludes some representatives of the genus *Neoceratitis* Hendel that have a single dark apical marking (Figure 10). | *Category : EDITORIAL* **(26) United States of America (17 Aug 2022 9:02 PM)** clarity | **Incorporated** |
| 121 | The majority of *Ceratitis* species have a typical wing banding pattern consisting of an anterior apical band, a discal band, and a subapical band (Figure 3). In some cases, an additional posterior apical band is present (Figure 11). A few *Ceratitis* species have wing banding that deviates from the normal pattern (i.e. *C. divaricata* (Munro, 1933), *C. flexuosa* (Walker, 1853), *C. munroanum* (Bezzi, 1926), *C. taitaensis* De Meyer and Copeland, 2016, *C. whartoni* De Meyer and Copeland, 2009) but none of them is of economic significance. The typical wing banding is also shared by some *Trirhithrum* and *Neoceratitis* species. The latter two groups can be separated from *Ceratitis* by the banding being dark black to black-brown combined with the presence of a posterior apical band (Figure 12) or at least a triangular extension “tooth” attached to the anterior apical band (Figure 13). *Ceratitis* species usually have a yellow to brown wing banding (Figure 2, Figure 11). | *Category : TECHNICAL* **(202) EPPO (20 Sep 2022 5:03 PM)** Is this groups or genera? (sentence the latter two groups) | **Modified**  Text changed to genera |
| 121 | The majority of *Ceratitis* species have a typical wing banding pattern consisting of an anterior apical band, a discal band, and a subapical band (Figure 3). In some cases, an additional posterior apical band is present (Figure 11). A few *Ceratitis* species have wing banding that deviates from the normal pattern (i.e. *C. divaricata* (Munro, 1933), *C. flexuosa* (Walker, 1853), *C. munroanum* (Bezzi, 1926), *C. taitaensis* De Meyer and Copeland, 2016, *C. whartoni* De Meyer and Copeland, 2009) but none of them is of economic significance. The typical wing banding is also shared by some *Trirhithrum* and *Neoceratitis* species. The latter two groups can be separated from *Ceratitis* by the banding being dark black to black-brown combined with the presence of a posterior apical band (Figure 12) or at least a triangular extension “tooth” attached to the anterior apical band (Figure 13). *Ceratitis* species usually have a yellow to brown wing banding (Figure ~~2~~3, Figure 11). | *Category : EDITORIAL* **(201) EPPO (20 Sep 2022 5:03 PM)** Error in the figure number. | **Incorporated** |
| 121 | The majority of *Ceratitis* species have a typical wing banding pattern consisting of an anterior apical band, a discal band, and a subapical band (Figure 3). In some cases, an additional posterior apical band is present (Figure 11). A few *Ceratitis* species have wing banding that deviates from the normal pattern (i.e. *C. divaricata* (Munro, 1933), *C. flexuosa* (Walker, 1853), *C. munroanum* (Bezzi, 1926), *C. taitaensis* De Meyer and Copeland, 2016, *C. whartoni* De Meyer and Copeland, 2009) but none of them is of economic significance. The typical wing banding is also shared by some *Trirhithrum* and *Neoceratitis* species. The latter two groups can be separated from *Ceratitis* by the banding being dark black to black-brown combined with the presence of a posterior apical band (Figure 12) or at least a triangular extension “tooth” attached to the anterior apical band (Figure 13). *Ceratitis* species usually have a yellow to brown wing banding (Figure 2, Figure 11). | *Category : TECHNICAL* **(200) EPPO (20 Sep 2022 5:03 PM)** We may be wrong, but the reference for terminology used in this document is not mentioned while it would be very useful to avoid confusion. For example, for someone being more familiar with White & Elson-Harris (1992) this band is known as “preapical crossband”. | **Modified**  A sentence for terminology has been added. Source of White et al. 1999 stated. This clarifies terminology here. |
| 121 | The majority of *Ceratitis* species have a typical wing banding pattern consisting of an anterior apical band, a discal band, and a subapical band (Figure 3). In some cases, an additional posterior apical band is present (Figure 11). A few *Ceratitis* species have wing banding that deviates from the normal pattern (i.e. *C. divaricata* (Munro, 1933), *C. flexuosa* (Walker, 1853), *C. munroanum* (Bezzi, 1926), *C. taitaensis* De Meyer and Copeland, 2016, *C. whartoni* De Meyer and Copeland, 2009) but none of them is of economic significance. The typical wing banding is also shared by some *Trirhithrum* and *Neoceratitis* species. The latter two groups can be separated from *Ceratitis* by the banding being dark black to black-brown combined with the presence of a posterior apical band (Figure 12) or at least a “tooth-shaped” triangular extension ~~“tooth” attached to~~ on the anterior apical band (Figure 13). *Ceratitis* species usually have ~~a~~ yellow to brown wing ~~banding~~ bands (Figure 2, Figure 11). | *Category : EDITORIAL* **(27) United States of America (17 Aug 2022 9:11 PM)** clarity | **Incorporated** |
| 122 | Phylogenetic studies have indicated that at least some *Trirhithrum* species cluster within the *Ceratitis* group (see Virgilio *et al.*, 2015). Thus, the generic concept of both *Ceratitis* and *Trirhithrum*, and the species to be included in each of these higher taxa, needs revision. | *Category : TECHNICAL* **(203) EPPO (20 Sep 2022 5:03 PM)** Does it refer to group or genera? | **Modified**  Text states genus |
| 124 | For the purposes of this protocol, a number of characters useful for the identification of adult flies have been retrieved from the different published revisions of subgenera (De Meyer 1996, 1998, 2000; De Meyer and Freidberg, 2005) and from the subsequent inclusion in the identification tool developed by Virgilio, White and De Meyer (2014). The diagnostic character states for the six ~~species of~~ economically important *Ceratitis* species included in this protocol are listed in Table 2, with reference to relevant images illustrating the states. | *Category : EDITORIAL* **(286) New Zealand (30 Sep 2022 7:41 AM)** | **Incorporated** |
| 124 | For the purposes of this protocol, a number of characters useful for the identification of adult flies have been retrieved from the different published revisions of subgenera (De Meyer 1996, 1998, 2000; De Meyer and Freidberg, 2005) and from the subsequent inclusion in the identification tool developed by Virgilio, White and De Meyer (2014). The diagnostic character states for the six species of economically important *Ceratitis* species included in this protocol are listed in Table 2, with reference to relevant images illustrating the states. | *Category : EDITORIAL* **(204) EPPO (20 Sep 2022 5:03 PM)** Please check correct citation in text for 3 authors: Virgilio, White and De Meyer (2014) OR Virgilio et al. 2014? | **Incorporated** |
| 124 | For the purposes of this protocol, a number of characters useful for the identification of adult flies have been retrieved from the ~~different published~~ different revisions of subgenera (De Meyer 1996, 1998, 2000; De Meyer and Freidberg, 2005) and from the subsequent inclusion in the identification tool developed by Virgilio, White and De Meyer (2014). The diagnostic character states for the six species of economically important *Ceratitis* ~~species included in this protocol are listed in Table 2, with reference to relevant images illustrating the states.~~ fruit files included in this protocol are listed in Table 2, with reference to relevant images illustrating the states. | *Category : EDITORIAL* **(141) United States of America (29 Aug 2022 7:45 PM)** delete unnecessary word; better language | **Modified**  Incorporated deletion of “published.”  Did not change “species” to “fruit flies” to keep consistency in protocol. |
| 124 | For the purposes of this protocol, a number of characters useful for the identification of adult flies have been retrieved from the different published revisions of subgenera (De Meyer 1996, 1998, 2000; De Meyer and Freidberg, 2005) and from the subsequent inclusion in the identification tool developed by ~~Virgilio~~Virgilio et al., ~~White and De Meyer (2014)~~2014. The diagnostic character states for the six species of economically important *Ceratitis* species included in this protocol are listed in Table 2, with reference to relevant images illustrating the states. | *Category : EDITORIAL* **(28) United States of America (17 Aug 2022 9:13 PM)** citation | **Incorporated** |
| 127 | **Species** | *Category : EDITORIAL* **(205) EPPO (20 Sep 2022 5:03 PM)** Would it be possible to apply a systematic grouping here instead of an alphabetical ordering, so e.g. having C anonae next to C. fasciventris | **Considered but not incorporated**  Original table had systematic grouping but was adjusted to alphabetical from previous suggestions. Alphabetical matches other protocols. |
| 129 | ***C. anonae*** | *Category : TECHNICAL* **(143) United States of America (29 Aug 2022 7:49 PM)** while the species seem to be arranged left to right alphabetically, perhaps it is worthwhile arranging them: C. capitata, C. cosyra and then the otherfour FAR complex | **Considered but not incorporated**  Original table had systematic grouping but was adjusted to alphabetical from previous suggestions. Alphabetical matches other protocols. |
| 135 | Both sexes, scutum, postpronotal lobe | *Category : TECHNICAL* **(266) Canada (28 Sep 2022 9:42 PM)** Only referring to the postpronotal lobe and not the scutum, so remove scutum. And the pprn lobe is not part of the scutum. | **Incorporated** |
| 135 | Both sexes, ~~scutum,~~ postpronotal lobe | *Category : TECHNICAL* **(51) United States of America (18 Aug 2022 8:09 PM)** Postpronotal lobe is not part of scutum | **Incorporated** |
| 135 | Both sexes, scutum, postpronotal lobe | *Category : TECHNICAL* **(50) United States of America (18 Aug 2022 8:08 PM)** wouldn't this be assumed? | **Considered but incoporated**.  Because of many sexual dimporhisms in genus, this text is retained to be explicit. |
| 136 | Unicolorous (as in Figure 14 & Figure 15) | *Category : EDITORIAL* **(142) United States of America (29 Aug 2022 7:46 PM)** could omit the word ‘as in” throughout the table | **Incorporated** |
| 136 | Unicolorous (as in ~~Figure~~Figures 14 & ~~Figure~~15) | *Category : EDITORIAL* **(52) United States of America (18 Aug 2022 8:10 PM)** the table would be shorter and easier to use if this corrected through. | **Incorporated** |
| 137 | Pale with black median spot (as in ~~Figure~~Figures 16 & ~~Figure~~17) | *Category : EDITORIAL* **(53) United States of America (18 Aug 2022 8:11 PM)** see previous comment | **Incorporated** |
| 152 | Yellow-orange to orange ground colour with distinct black markings (as in Figure 22) (black ~~markings can~~ markings sometimes ~~be~~ strongly reduced) | *Category : EDITORIAL* **(54) United States of America (18 Aug 2022 8:13 PM)** clarity | **Incorporated** |
| 159 | Connected with discal band (as in Figure 25)~~,~~ ; or at most partially separated (as in Figure 26) | *Category : TECHNICAL* **(55) United States of America (18 Aug 2022 8:16 PM)** technical clarity | **Incorproated** |
| 164 | With black-brown ~~transverse~~ band (as in Figure 27) | *Category : TECHNICAL* **(56) United States of America (18 Aug 2022 8:17 PM)** Delete? Bands are transverse by definition (McAlpine 1981). | **Incorproated** |
| 170 | Male, head, ~~lower~~ anterior orbital seta | *Category : TECHNICAL* **(57) United States of America (18 Aug 2022 8:19 PM)** correct word | **Incorporated** |
| 177 | Male, ~~leg~~ leg, fore femur | *Category : EDITORIAL* **(58) United States of America (18 Aug 2022 8:20 PM)** clarity | **Incorporated** |
| 184 | Male, ~~leg~~ leg, mid femur | *Category : EDITORIAL* **(59) United States of America (18 Aug 2022 8:20 PM)** clarity | **Incorporated** |
| 191 | Male, ~~leg~~ leg, mid tibia | *Category : EDITORIAL* **(60) United States of America (18 Aug 2022 8:21 PM)** clarity | **Incorporated** |
| 192 | Lateral margins with row of long black stout setae (feathering) for more than three-quarters of entire length (as in Figure 38) | *Category : TECHNICAL* **(61) United States of America (18 Aug 2022 8:21 PM)** What are lateral margins? Legs have anterior, dorsal, posterior and ventral sides. | **Incorporated** |
| 199 | Pale to brownish ~~coloured~~ over entire length (as in Figure 38) | *Category : EDITORIAL* **(62) United States of America (18 Aug 2022 8:23 PM)** unnecessary wording | **Incorporated** |
| 200 | Pale ~~coloured~~ over entire length (as in Figure 39) | *Category : EDITORIAL* **(63) United States of America (18 Aug 2022 8:24 PM)** unnecessary | **Incorporated** |
| 201 | ~~Pale coloured~~ Pale over entire length (as in Figure 39) | *Category : EDITORIAL* **(64) United States of America (18 Aug 2022 8:24 PM)** unnecessary | **Incorporated** |
| 202 | Usually pale ~~coloured~~, at most area between feathering partially darker yellow to ~~brownish coloured~~ brownish (as in Figure 40) | *Category : EDITORIAL* **(65) United States of America (18 Aug 2022 8:24 PM)** unnecessary. | **Incorporated** |
| 203 | Pale except area between feathering where darker coloured; dark colour not reaching ~~lateral margins~~ in ~~upper~~ basal part (red arrow in Figure 41) | *Category : TECHNICAL* **(66) United States of America (18 Aug 2022 8:26 PM)** see earlier comments | **Incorporated** |
| 204 | Pale except area between feathering where darker coloured; dark colour reaching ~~lateral margins in upper~~ on basal part (red arrow in Figure 42) | *Category : TECHNICAL* **(67) United States of America (18 Aug 2022 8:28 PM)** see above | **Incorporated** |
| 206 | ~~With~~ Setulae partly dark ~~pilosity in lower~~ on ventral half (as in Figure 44) | *Category : TECHNICAL* **(68) United States of America (18 Aug 2022 8:40 PM)** Setulae would be more clear than pilosity? | **Modified**  Revised for clairty but retaining common word usage for fly taxonomy |
| 207 | ~~Whole pale pilosity~~ Setulae entirely pale (as in Figure 43) | *Category : TECHNICAL* **(69) United States of America (18 Aug 2022 8:41 PM)** see comment before | **Modified**  Revised for clairty but retaining common word usage for fly taxonomy |
| 212 | Female, ~~leg~~ leg, fore femur | *Category : EDITORIAL* **(70) United States of America (18 Aug 2022 8:42 PM)** consistency with above | **Incorporated** |
| 213 | Posteriorly with few dark hairs between posterior and posterodorsal row of setae (as in Figure 33) | *Category : TECHNICAL* **(206) EPPO (20 Sep 2022 5:03 PM)** Captation of Figure 33 say C. rosa (not C. anonae) For C. rosa the figure is for C. quinaria  Check correspondence between figures and table | **Incorporated**  Caption for Fig 33 is C. anonae |
| 213 | Posteriorly with few dark hairs between posterior and posterodorsal ~~row~~ rows of setae (as in Figure 33) | *Category : EDITORIAL* **(71) United States of America (18 Aug 2022 8:42 PM)** plural | **Incorporated** |
| 219 | **4.2** **Morphological identification of third instars larvae** | *Category : EDITORIAL* **(97) Thailand (26 Aug 2022 4:20 AM)** | **Modified**  Included “larvae” but that change required hyphen for thrid-instar and making instar singular (to describe the larvae) |
| 220 | As explained at the beginning of section 4, identification of flies based on examination of the third-instar life stage is not sufficient to complete accurate species identification under all circumstances. Larval descriptions are not available for all species that could be confused for a pest, and descriptions are based on laboratory-reared colonies that might not represent the true variation of the species (Steck and Ekesi, 2015). However, a diagnosis to the genus or species that is based solely on larval morphology could be appropriate when screening for a pest where its presence is expected based on prior information and closely related species that could be mistaken for the pest are absent. Molecular analysis (section 4.3) should be performed to complete the identification of a larva when the diagnosis is intended to confirm a new record of pest presence. | *Category : TECHNICAL* **(208) EPPO (20 Sep 2022 5:03 PM)** Last sentence: Molecular tests wiill not be sufficient for cryptic species where rearing to adults is necessary especially to confirm a first record. This should be mentioned. | **Incorporated** |
| 220 | As explained ~~earlier in~~ at the beginning of section 4, identification of flies based on examination of the third-instar life stage is not sufficient to complete accurate species identification under all circumstances. Larval descriptions are not available for all species that could be confused for a pest, and descriptions are based on laboratory-reared colonies that might not represent the true variation of the species (Steck and Ekesi, 2015). However, a diagnosis to the genus or species that is based solely on larval morphology could be appropriate when screening for a pest where its presence is expected based on prior information and closely related species that could be mistaken for the pest are absent. Molecular analysis (section 4.3) should be performed to complete the identification of a larva when the diagnosis is intended to confirm a new record of pest presence. | *Category : EDITORIAL* **(207) EPPO (20 Sep 2022 5:03 PM)** Better wording? | **Incorporated** |
| 220 | As explained earlier in section 4, identification of flies based on examination of the third-instar life stage is not sufficient to complete accurate species identification under all circumstances. Larval descriptions are not available for all species that could be confused for a pest, and descriptions are based on laboratory-reared colonies that might not represent the true variation of the species (Steck and Ekesi, 2015). However, a diagnosis to the genus or species that is based solely on larval morphology could be appropriate when screening for a pest where its presence is expected based on prior information and closely related species that could be mistaken for the pest are absent. Molecular analysis (section 4.3) should be performed to complete the identification of a larva when the diagnosis is intended to confirm a new record of pest presence. | *Category : TECHNICAL* **(73) United States of America (18 Aug 2022 8:44 PM)** Rare? | **Considered but not incorporated**  Rarity is not easily defined in a protocol and could result in misinterpretation of methods |
| 220 | As explained earlier in section 4, identification of ~~flies~~ Ceratitis species based on examination of the third-instar life stage is not sufficient to complete accurate species identification under all circumstances. Larval descriptions are not available for all species that could be confused for a pest, and descriptions are based on laboratory-reared colonies that might not represent the true variation of the species (Steck and Ekesi, 2015). However, a diagnosis to the genus or species that is based solely on larval morphology could be appropriate when screening for a pest where its presence is expected based on prior information and closely related species that could be mistaken for the pest are absent. Molecular analysis (section 4.3) should be performed to complete the identification of a larva when the diagnosis is intended to confirm a new record of pest presence. | *Category : TECHNICAL* **(72) United States of America (18 Aug 2022 8:43 PM)** this section is about larvae, not flies. | **Considered but not incorporated**  Larvae are a life stage of a fly. |
| 221 | Morphological characters of third instars are published for several *Ceratitis* species. These descriptions can be used to discriminate among species that have been studied. ~~These descriptions~~ They can also be used to provide additional support to ~~an~~ the identification of one of those studied species if the identification is based on ~~other~~ molecular methods. In this protocol, a description of third instars for the genus *Ceratitis* is provided that has been extrapolated from published species descriptions: this may be of value in supporting identifications. | *Category : EDITORIAL* **(209) EPPO (20 Sep 2022 5:03 PM)** 1) For simplification. 2) Better wording? 3) Should "other methods" be replaced with "molecular methods", or are there other methods than the morphological or molecular ones? | **Incorporated** |
| 221 | Morphological characters of third instars are published for several *Ceratitis* species. These descriptions can be used to discriminate among species that have been studied. These descriptions can also be used to provide additional support to an identification of one of those studied species if the identification is based on other methods. In this protocol, a description of third instars for the genus *Ceratitis* is provided that has been extrapolated from published species descriptions: this may be of value in supporting identifications. | *Category : TECHNICAL* **(144) United States of America (29 Aug 2022 7:50 PM)** Include references. | **Considered but not incorporated**  Inclusion of references would increase size of protocol without improving the protocol to diagnose species. The literature continues to grow for this work and citaitons would only be examples. Relevant papers used in the diagnostic sections of the protocol are cited herein. |
| 221 | Morphological characters of third instars are published for several *Ceratitis* species. These descriptions can be used to discriminate among species that have been studied. These descriptions can also be used to provide additional support to an identification ~~of one of those studied species if the identification is~~ based on other methods. In this protocol, a description of third instars for the genus *Ceratitis* is provided that has been extrapolated from published species descriptions: this may be of value in supporting identifications. | *Category : EDITORIAL* **(74) United States of America (18 Aug 2022 8:45 PM)** improved clarity | **Incorporated** |
| 222 | When a larva is detected in fruit, identification of the instar stage is not always certain. The fully developed second instar and newly moulted third instar of a fly species can be the same length: the third-instar *Ceratitis*, for example, can be as small as 3.2 mm in length for some species (Steck and Ekesi, 2015). Typical relative sizes of the egg and three instars are shown in Figure 45. The best characters to separate instars in all species are the 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 third and second instars is the relative size of the mouthhook subapical tooth: in the third instar the subapical tooth is very small compared to the apical tooth (Figure 46), but in the second instar it is subequal (Figure 47). | *Category : TECHNICAL* **(288) New Zealand (30 Sep 2022 7:43 AM)** why is this relevant to whether the instar sizes overlap | **Modified**  It is relevant because third instars and 2nd instars of species can share identical sizes. The 3rd instar can overlap with second instar of *Ceratitis* species”  The text is updated to state: “*Ceratitis*, for example, can be as small as 3.2 mm in length for some species which can overlap with second instar of *Ceratitis* species (Steck and Ekesi, 2015)” |
| 222 | When a larva is detected in fruit, identification of the instar stage is not always certain. The fully developed second instar and newly moulted third instar of a fly species can be the same length: the third-instar *Ceratitis*, for example, can be as small as 3.2 mm in length for some species (Steck and Ekesi, 2015). Typical relative sizes of the egg and three instars are shown in Figure 45. The best characters to separate instars in all species are the 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 third and second instars is the relative size of the mouthhook subapical tooth: in the third instar the subapical tooth is very small compared to the apical tooth (Figure 46), but in the second instar it is subequal (Figure 47). | *Category : EDITORIAL* **(287) New Zealand (30 Sep 2022 7:42 AM)** this term seems to be inconsistently hyphenated throughout. suggest a global check for consistency | **Modifed**  The dcoument was checked to ensure third instar was used when instar was a noun and hyphenated with third-instar was adjectvie of a noun (i.e.,larvae). |
| 222 | When a larva is detected in fruit, identification of the instar stage is not always certain. The fully developed second instar and newly moulted third instar of a fly species can be the same length: the third-instar *Ceratitis*, for example, can be as small as 3.2 mm in length for some species (Steck and Ekesi, 2015). Typical relative sizes of the egg and three instars are shown in Figure 45. The best characters to separate instars in all species are the absolute sizes of the cephaloskeleton and spiracles: they never overlap between ~~instars~~instars (see Figure 46 on how size is measured). However, these data are not published for second or first instars of most species. Another differentiating feature between third and second instars is the relative size of the mouthhook subapical tooth: in the third instar the subapical tooth is very small compared to the apical tooth (Figure 46), but in the second instar it is subequal (Figure 47). | *Category : EDITORIAL* **(210) EPPO (20 Sep 2022 5:03 PM)** Suggested addition | **Incorporated** |
| 222 | When a larva is detected in fruit, identification of the instar stage is not always certain. The fully developed second instar and newly moulted third instar of a fly species can be the same length: the third-instar *Ceratitis*, for example, can be as small as 3.2 mm in length for some species (Steck and Ekesi, 2015). Typical relative sizes of the egg and three instars are shown in Figure 45. The best characters to separate instars in all species are the 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 third and second instars of Ceratitis is the relative size of the mouthhook subapical tooth: in the third instar the subapical tooth is very small compared to the apical tooth (Figure 46), but in the second instar it is subequal (Figure 47). | *Category : TECHNICAL* **(75) United States of America (18 Aug 2022 8:49 PM)** is this the species described? | **Incorporated** |
| 223 | Larvae can be examined using a dissecting stereomicroscope, compound optical microscope and scanning electron microscope (SEM). General examination for initial screening can be accomplished using the stereomicroscope, but slide-mounted specimens under a compound microscope or SEM are needed to complete genus and species diagnoses. The most detailed images and illustrations reported in the literature are from SEM examination of specimens. Therefore, diagnoses based on optical microscopy require photographed images that provide evidence of structures observed in SEM images. | *Category : TECHNICAL* **(211) EPPO (20 Sep 2022 5:03 PM)** Should 'diagnoses' be replaced by 'identification second and fourth sentence?  Comment on the last sentence  Should this always be the case? This is not practical and a strong requirement. Are there also circumstances in which it is not needed to prepare slides and collect photographed images? The sentence is not understood. Can it be clarified? We would suggest deletion. | **Incorporated**  Replaced diagnoses with identification. Deleted final sentence |
| 223 | Larvae can be examined using a dissecting stereomicroscope, compound optical microscope and scanning electron microscope (SEM). General examination for initial screening can be accomplished using the stereomicroscope, but slide-mounted specimens under a compound microscope or SEM are needed to complete genus and species diagnoses. The most detailed images and illustrations reported in the literature are from SEM examination of specimens. Therefore, diagnoses based on optical microscopy require photographed images that provide evidence of structures observed in SEM images. | *Category : TECHNICAL* **(78) United States of America (18 Aug 2022 8:53 PM)** Why would you have to have an image? Couldn’t you just observe the characters through the microscope? But the resolution of the microscope or images would need to be sufficient to see these characters | **Incorporated** |
| 224 | **4.2.1** **Preparation of ~~third-instar~~ third-instars larvae for identification** | *Category : EDITORIAL* **(104) Thailand (26 Aug 2022 4:28 AM)** | **Considered but not incorporated**  Did not modify in order to match headings for para 219, and if larvae were included then instar would be singular as adejective of noun |
| 225 | Larvae can be prepared for morphological examination by first killing them in very hot or boiling water and then storing them in 70% ethanol. Rinsing larvae in cool, distilled water with a drop of mild dishwashing detergent before killing in hot water helps clean specimens for subsequent examination. The live larvae are then placed in water at >65 °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, 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 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 an SEM. | *Category : TECHNICAL* **(212) EPPO (20 Sep 2022 5:03 PM)** Larvae may also turn black depending on the conditions prior to their detection. E.g. when specimens were collected from fruits that were in cold storage, larvae will turn black after hot water treatment. In those cases, it is better to incubate the larvae until they are active again. Incubation for several days may also allow for better development of the oral ridge area in late third instars and thus allow for a more reliable ID. Can this information be added? | **Considered but not incorporated**  It is our understanding that blackening is a consequence of ezymatic reaction that occurs after death. The drafting team is not aware of effects of immediate boiling after cold storage on blackening of larvae.  Not including this information does not preclude its use as the protcol describes the steps to kill (not additional pre-killing procedures).  Additional research and publications on this topic are recommended. |
| 228 | Morphological examination of larvae can be performed on unmounted specimens using a ~~stereomicroscope~~stereo-microscope. After intact larvae are removed from alcohol and blotted dry, their external features such as oral ridges, anterior and posterior spiracles, and anal lobes can be examined. Counts of oral ridges and lobes of the anterior spiracle can be made, as well as observations of characters such as shapes of spiracles and anal lobes, orientation and length and width measurements of posterior spiracular slits, and presence or absence of dorsal spinules and caudal ridges. Specimens should be re-wetted with alcohol as needed to prevent ~~shrivelling~~shriveling. | *Category : TECHNICAL* **(213) EPPO (20 Sep 2022 5:03 PM)** A method of studying the oral ridges is as follows. Under a stereo microscope, in alcohol, use a fine brush to remove fruit pulp from the ridges if necessary. (Cleaning is often not needed with larvae that are still highly active). Dry the larva shortly. Place a slide under a transmitted light compound (not stereo) microscope (light comes from below) and place a piece of Kleenex tissue on the slide. Then place the larva on the tissue and you can check for accessory plates and the shape of the teeth on the oral ridges at 100x magnification. (A related extra comment from experts was that often the oral ridge is not visible anymore on slide mounted specimen embedded in Canada balsam). Can it be considered? | **Incorporated**  New text has been added for observing unmounted larva under compund microscope.    The note on Canada balsam (CB) is apprieciated. CB is used in a subsequent method as an option but not required. It appears that different labs have different experiences.  Section is now optical microscope. But stereomicroscope spelling is used throughout text and retained for consistency. Shrivelling is preferred British spelling, we believe. IPPC Secretariat will apply the correct style. |
| 232 | Cleared specimens can be placed in ~~glycerin~~ glycerine on a glass depression slide with a cover slip for examination or imaging and recording of measurement data under a compound microscope (Figure 48). 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. 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~~ need to be labelled. | *Category : EDITORIAL* **(289) New Zealand (30 Sep 2022 7:44 AM)** | **Modified**  Changed glycerine to British spelling.  The text “should be labelled” is reatined to be less prescriptive. Storage times of permanent mounts determined by institution based on determination. |
| 234 | For observation using an SEM, the specimens (stored in alcohol) should first be completely dehydrated by running through a series of ethanol rinses – 70%, 80%, 95%, and two or three changes of absolute ethanol – followed by one or two rinses in ethyl acetate and air-dried (or critical-point dried after the alcohol dehydration series), then coated with gold–palladium and mounted on a stub (Carroll and Wharton, 1989). If the larval specimen has not been cut or punctured before the ethanol rinses, then two to three lateral punctures should be made with a minuten pin to allow alcohol to permeate the tissues. The duration of each ethanol rinse for a larva with punctures should be at least two hours. If the midsection of the larval specimen has been excised and removed (section 4.3.1), then alcohol permeates the tissue more quickly and each rinse step should have a duration of 15 minutes. Similar techniques can be found elsewhere (e.g.  Frías *et al.*, 2006; Frías, Selivon and Hernández-Ortiz, 2008; Frías Lassere, Hernández Ortiz and López Muñoz, 2009). | *Category : EDITORIAL* **(214) EPPO (20 Sep 2022 5:03 PM)** Harmonize the references in the last sentence. | **Incorporated**. |
| 235 | **4.2.2** **Characters to identify ~~third-instar~~ third-instars larvae of genus *Ceratitis*** | *Category : EDITORIAL* **(98) Thailand (26 Aug 2022 4:21 AM)** | **Considered but not incoirporated**  Did not modify in order to match headings for para 219,and if larvae included then instar is singular as adejective of noun |
| 236 | Diagnosis: dorsolateral pair of sensilla parallel to maxillary ~~palp~~palpus; preoral lobes elongate and petal-like, preoral organ ringed with petal-like lobes; preoral teeth absent; mouthhook apical tooth ventrally grooved, secondary conical, subapical tooth present, mouthhook basally elongate, dental sclerite present; oral ridges with scalloped edges, accessory plates present in single series; anterior spiracle tubules in a single sinuous row, flat to convex centrally; rimae of posterior spiracles approximately 2.5–3.5 times longer than wide; caudal ridge present; thin, dark, sclerotized line on caudal segment absent; live, mature third instars display skipping (jumping) behaviour. Important exceptions are noted below under the individual species notes. | *Category : EDITORIAL* **(267) Canada (28 Sep 2022 9:45 PM)** as spelled below | **Incorporated** |
| 236 | Diagnosis: dorsolateral pair of sensilla parallel to maxillary palp; preoral lobes elongate and petal-like, preoral organ ringed with petal-like lobes; preoral teeth absent; mouthhook apical tooth ventrally grooved, secondary conical, subapical tooth present, mouthhook basally elongate, dental sclerite present; oral ridges with scalloped edges, accessory plates present in single series; anterior spiracle tubules in a single sinuous row, flat to convex centrally; rimae of posterior spiracles approximately 2.5–3.5 times longer than wide; caudal ridge present; thin, dark, sclerotized line on caudal segment absent; live, mature third instars display skipping (jumping) behaviour. Important exceptions are noted below under the individual species notes. | *Category : TECHNICAL* **(84) United States of America (18 Aug 2022 9:02 PM)** I would include these in the diagnosis (as suggested for mouthhook); otherwise it isn’t very useful if you have to check all the species diagnoses as well as the generic diagnosis for each character | **Modified**  Text is updated to clarify subapical tooth and accessory plate can be absent. |
| 236 | Diagnosis: dorsolateral pair of sensilla parallel to maxillary palp; preoral lobes elongate and petal-like, preoral organ ringed with petal-like lobes; preoral teeth absent; mouthhook apical tooth ventrally grooved, secondary conical, subapical tooth present, mouthhook basally elongate, dental sclerite present; oral ridges with scalloped edges, accessory plates present in single series; anterior spiracle tubules in a single sinuous row, flat to convex centrally; rimae of posterior spiracles approximately 2.5–3.5 times longer than wide; caudal ridge present; thin, dark, sclerotized line on caudal segment ventral to spiracles absent; live, mature third instars display skipping (jumping) behaviour. Important exceptions are noted below under the individual species notes. | *Category : TECHNICAL* **(83) United States of America (18 Aug 2022 9:01 PM)** was this the meaning? | **Incorporated**. |
| 236 | Diagnosis: dorsolateral pair of sensilla parallel to maxillary palp; preoral lobes elongate and petal-like, preoral organ ringed with petal-like lobes; preoral teeth absent; mouthhook apical tooth ventrally grooved, secondary conical, subapical tooth present, mouthhook basally elongate, dental sclerite present; oral ridges with scalloped edges, accessory plates present in single series; anterior spiracle tubules in ~~a~~ single sinuous row, in profile flat to convex centrally; rimae of posterior spiracles approximately 2.5–3.5 times longer than wide; caudal ridge present; thin, dark, sclerotized line on caudal segment absent; live, mature third instars display skipping (jumping) behaviour. Important exceptions are noted below under the individual species notes. | *Category : TECHNICAL* **(82) United States of America (18 Aug 2022 8:59 PM)** clarification | **Incorporated**. |
| 236 | Diagnosis: dorsolateral pair of sensilla parallel to maxillary palp; preoral lobes elongate and petal-like, preoral organ ringed with petal-like lobes; preoral teeth absent; mouthhook apical tooth ventrally grooved, secondary conical, subapical tooth present, mouthhook basally elongate, dental sclerite present; oral ridges with scalloped edges, accessory plates present in single series; anterior spiracle tubules in a single sinuous row, flat to convex centrally; rimae of posterior spiracles approximately 2.5–3.5 times longer than wide; caudal ridge present; thin, dark, sclerotized line on caudal segment absent; live, mature third instars display skipping (jumping) behaviour. Important exceptions are noted below under the individual species notes. | *Category : TECHNICAL* **(81) United States of America (18 Aug 2022 8:58 PM)** Absent in Med fly? | **Modified**.  Adjusted text to indicate “when present” to indicate not present in all species. |
| 236 | Diagnosis: dorsolateral pair of sensilla parallel to maxillary palp; preoral lobes elongate and petal-like, preoral organ ringed with petal-like lobes; preoral teeth absent; mouthhook apical tooth ventrally grooved, secondary conical, subapical tooth ~~present~~usually present but often minute or absent in C. capitata and C. rosa), mouthhook basally elongate, dental sclerite present; oral ridges with scalloped edges, accessory plates present in single series; anterior spiracle tubules in a single sinuous row, flat to convex centrally; rimae of posterior spiracles approximately 2.5–3.5 times longer than wide; caudal ridge present; thin, dark, sclerotized line on caudal segment absent; live, mature third instars display skipping (jumping) behaviour. Important exceptions are noted below under the individual species notes. | *Category : TECHNICAL* **(80) United States of America (18 Aug 2022 8:55 PM)** to specify, since it varies in Med fly | **Modified**.  Text now provides clarity that species do not always have subapical tooth. |
| 236 | Diagnosis: dorsolateral pair of sensilla parallel to maxillary palp; preoral lobes elongate and petal-like, preoral organ ringed with petal-like lobes; preoral teeth absent; mouthhook apical tooth ventrally grooved, secondary conical, subapical tooth present, mouthhook basally elongate, dental sclerite present; oral ridges with scalloped edges, accessory plates present in single series; anterior spiracle tubules in a single sinuous row, flat to convex centrally; rimae of posterior spiracles approximately 2.5–3.5 times longer than wide; caudal ridge present; thin, dark, sclerotized line on caudal segment absent; live, mature third instars display skipping (jumping) behaviour. Important exceptions are noted below under the individual species notes. | *Category : TECHNICAL* **(79) United States of America (18 Aug 2022 8:54 PM)** unclear | **Modified**.  Text adjusted to specify conical in shape as opposed to ventrally grooved. |
| 237 | Fruit fly larval descriptive terminology has evolved over the years. Useful references include Teskey (1981), Steck and Wharton (1988), White and Elson-Harris (1992), White *et al.* (1999), Carroll *et al.* (2004), Rodriguez *et al.* (2021) and Steck *et al.* (forthcoming). The figures in this protocol illustrate the usage employed here and the diagnostic and key features listed above and below. | *Category : EDITORIAL* **(145) United States of America (29 Aug 2022 7:51 PM)** In preparation, in review? or in press? | **Incorporated**  New citation:  2022.  Reference 641 should be updated to: Steck, G.J., Ndlela, S., Somma, L.A., Diaz, J., Moore, M.J. & Awad, J. 2022. Description of immature stages of *Dacus bivittatus* (Diptera: Tephritidae). *Proceedings of the Entomological Society of Washington*, 124: 1-22. |
| 240 | The preoral lobes are present just anterior to the mouth opening, and laterally adjacent to them are the preoral organ and associated lobes. In *Ceratitis* larvae, the preoral organ is a small cylindrical lobe bearing sensilla that is ringed by several petal-like lobes, referred to as the preoral lobes, that extend medially (Figure 53). They differ from *Dacus* and *Zeugodacus* (Figure 54), in which the preoral lobes are elongated with toothed margins identical to the oral ridges, and from those of *Anastrepha* (Figure 55), in which the sensilla of the preoral organ are on the lateral ends of an elongate, undifferentiated preoral lobe. These features can be observed in detail under an SEM and sometimes crudely under a dissecting or compound microscope. | *Category : TECHNICAL* **(128) Kenya (29 Aug 2022 8:39 AM)** They differ from Dacus and Zeugodacus (Figure 54), in which the preoral lobes are elongated with toothed margins identical to the oral ridges, and from those of Anastrepha (Figure 55), in which the sensilla of the preoral organ are on the lateral ends of an elongate, undifferentiated preoral lobe (cite source). | **Incorporated**  New citation:  2022.  Reference 641 should be updated to: Steck, G.J., Ndlela, S., Somma, L.A., Diaz, J., Moore, M.J. & Awad, J. 2022. Description of immature stages of *Dacus bivittatus* (Diptera: Tephritidae). *Proceedings of the Entomological Society of Washington*, 124: 1-22. |
| 240 | The preoral lobes are present just anterior to the mouth opening, and laterally adjacent to them are the preoral organ and associated lobes. In *Ceratitis* larvae, the preoral organ is a small cylindrical lobe bearing sensilla that is ringed by several petal-like lobes, referred to as the preoral lobes, that extend medially (Figure 53). They differ from larvae of *Dacus* and *Zeugodacus* (Figure 54), in which the preoral lobes are elongated with toothed margins identical to the oral ridges, and from those of *Anastrepha* (Figure 55), in which the sensilla of the preoral organ are on the lateral ends of an elongate, undifferentiated preoral lobe. These features can be observed in detail under an SEM and sometimes crudely under a dissecting or compound microscope. | *Category : TECHNICAL* **(47) United States of America (17 Aug 2022 9:46 PM)** "They" - Meaning Ceratitis larvae? | **Incorporated**. |
| 242 | Most of the cephaloskeleton is internal and not visible until the specimen is cleared. Only part of the mouthhook is visible externally. In *Ceratitis* species, the mouthhook has a large apical tooth and a small secondary tooth. However, the secondary tooth may be imperceptibly small (visible only under an SEM) or entirely absent in some specimens of *C. capitata* and *C. rosa*. The secondary tooth is always absent in *Anastrepha* and pest species of *Bactrocera* (except *B.*(*Notodacus*)*xanthodes*) (Figure 58, Figure 59). The ventral shape of the apical tooth can easily be seen under an SEM, but it is not apparent under a light microscope. It is ventrally grooved in *Ceratitis* but tusk-like in *Dacus* and some *Zeugodacus* spp. (Figure 58, Figure 59, Figure 60). The posterior part of the mouthhook is extended into an elongate neck beyond the ventral protuberance in *Ceratitis* and other Dacinae but is truncate posteriorly in Trypetinae (*Anastrepha*, *Rhagoletis*). A dental sclerite is present in *Ceratitis* and other Dacinae but there is no dental sclerite in Trypetinae (*Anastrepha*, *Rhagoletis*). The neck and dental sclerite can be observed under a compound microscope (Figure 61, Figure 62). | *Category : TECHNICAL* **(217) EPPO (20 Sep 2022 5:03 PM)** Secondary tooth = subapical tooth? If yes, explain e.g. (= subapical tooth) or use only one of the terms throughout the document. Please consider also for next sentence and in Table 3 | **Incoporated**  Secondary tooth = subapical and subapical is preferred techncial term. It has been updated to subapical throughout text. |
| 242 | Most of the cephaloskeleton is internal and not visible until the specimen is cleared. Only part of the mouthhook is visible externally. In *Ceratitis* species, the mouthhook has a large apical tooth and a small secondary tooth. However, the secondary tooth may be imperceptibly small (visible only under an SEM) or entirely absent in some specimens of *C. capitata* and *C. rosa*. The secondary tooth is always absent in *Anastrepha* and pest species of *Bactrocera* (except *B.*(*Notodacus*)*xanthodes*) (Figure 58, Figure 59). The ventral shape of the apical tooth can easily be seen under an SEM, but it is not apparent under a light microscope. It is ventrally grooved in *Ceratitis* but tusk-like in *Dacus* and some *Zeugodacus* spp. (Figure 58, Figure 59, Figure 60). The posterior part of the mouthhook is extended into an elongate neck beyond the ventral protuberance in *Ceratitis* and other Dacinae but is truncate posteriorly in Trypetinae (*Anastrepha*, *Rhagoletis*). A dental sclerite is present in *Ceratitis* and other Dacinae but there is no dental sclerite in Trypetinae (*Anastrepha*, *Rhagoletis*). The neck and dental sclerite can be observed under a compound microscope (Figure 61, Figure 62). | *Category : TECHNICAL* **(216) EPPO (20 Sep 2022 5:03 PM)** Legend of fig 58 says small subapical teeth, but they appear to be in a basal position? | **Considered but not incorporated**  Subapical is correct term. Subapical tooth may appear “basal” in image, but only the the apical part of mouthhook is shown. The basal portion of mouthhook is not visible in intact specimens – see full mouthhook in cleared specimen-Fig 46. |
| 242 | Most of the cephaloskeleton is internal and not visible until the specimen is cleared. Only part of the mouthhook is visible externally. In *Ceratitis* species, the mouthhook has a large apical tooth and a small secondary tooth. However, the secondary tooth may be imperceptibly small (visible only under an SEM) or entirely absent in some specimens of *C. capitata* and *C. rosa*. The secondary tooth is always absent in *Anastrepha* and pest species of *Bactrocera* (except *B.*(*Notodacus*)*xanthodes*) (Figure 58, Figure 59). The ventral shape of the apical tooth can easily be seen under an SEM, but it is not apparent under a light microscope. It is ventrally grooved in *Ceratitis* but tusk-like in *Dacus* and some *Zeugodacus* spp. (Figure 58, Figure 59, Figure 60). The posterior part of the mouthhook is extended into an elongate neck beyond the ventral protuberance in *Ceratitis* and other Dacinae but is truncate posteriorly in Trypetinae (*Anastrepha*, *Rhagoletis*). A dental sclerite is present in *Ceratitis* and other Dacinae but there is no dental sclerite in Trypetinae (*Anastrepha*, *Rhagoletis*). The neck and dental sclerite can be observed under a compound microscope (Figure 61, Figure 62). | *Category : TECHNICAL* **(215) EPPO (20 Sep 2022 5:03 PM)** Also present in Bactrocera trilineata (not a big pest species, but listed in White and Helson-Harris and sometimes intercepted in Europe) | **Incorporated.**  Sentence modified to: “The subapical tooth is always absent in *Anastrepha* and most pest species of *Bactrocera* (exceptions with subapical tooth include *B.*(*Notodacus*) *xanthodes* and *B.*(*Javadacus*) *trilineata*) (Figure 58, Figure 59).” |
| 243 | A lateral lip of the oral opening, apparently a single structure but usually deeply invaginated to give the appearance of being two adjacent lips [inner (medial lateral lip) and outer (lateral lateral lip)], is present in SEM images of nearly all tephritid larvae described to date but varies in extent (Figure 63, Figure 64). Lateral to the outer lateral lip is a series of elongate ridges called the oral ridges, which may funnel liquids into the mouth during feeding. Oral ridges occur in larvae of all fruit-infesting tephritids, but they vary in number and their edges may be smooth, serrate, scalloped or fringed (Figure 65, Figure 66, Figure 67). Details of these features are best observed with an SEM as they may be damaged during preparation for slide mounting or difficult to get into a good viewing position on a slide. | *Category : TECHNICAL* **(46) United States of America (17 Aug 2022 9:44 PM)** "inner and outer" -Medial and lateral?  "smooth" - entire? (term used in Anastrepha papers) | **Modified**  Replaced smooth with entire as recommended.  Provided additional text on technical terms of orientation but retained original text. Inner and outer were used in first description of this feature (Steck et al. 2022). The suggestion is technically correct, but not commonly used of easily understood by tephritid workers. If used, one would have to refer to the inner part as the “medial lateral lip” and the outer part as “lateral lateral lip” and that is not in literature or common for experts of this group. |
| 243 | A lateral lip of the oral opening, apparently a single structure but usually deeply invaginated to give the appearance of being two adjacent lips (inner and outer), is present in SEM images of nearly all tephritid larvae described to date but varies in extent (Figure 63, Figure 64). Lateral to ~~the outer~~ the lateral lip is a series of elongate ridges called the oral ridges, which may funnel liquids into the mouth during feeding. Oral ridges occur in larvae of all fruit-infesting tephritids, but they vary in number and their edges may be smooth, serrate, scalloped or fringed (Figure 65, Figure 66, Figure 67). Details of these features are best observed with an SEM as they may be damaged during preparation for slide mounting or difficult to get into a good viewing position on a slide. | *Category : TECHNICAL* **(45) United States of America (17 Aug 2022 9:43 PM)** Medial and lateral. | **Considered but not incorporated**.  Inner and outer were used in first description of this feature (Steck et al. 2022). The text change to para 243 helps inform users of protocol on locations. |
| 245 | Anterior spiracles are located dorsolaterally on the first thoracic segment. They have an internal trunk that flares apically to ~~expose~~ one or more external rows of tubules that are short with a rounded top bearing a thin slit to allow passage of air. Individual tubules are very similar among all fruit fly larvae. However, the number of tubules, their arrangement and the overall dimensions of the spiracles may be useful in diagnosing some fruit fly species. The apical row of tubules in *Ceratitis* and other Dacinae in profile are typically fan-shaped with a flat or convex top, compared with *Anastrepha* in which the row or rows of tubules are distinctly bilobed. The anterior spiracles should be observed on cleared specimens on slides under a light microscope (Figure 68, Figure 69). | *Category : TECHNICAL* **(44) United States of America (17 Aug 2022 9:38 PM)** technical correction | **Incorporated**. |
| 246 | The last larval abdominal segment has a pair of posterior spiracles located posterodorsally (Figure 70) and anal lobes located ventrally. In the Dacinae (including *Ceratitis*), a caudal ridge is present on the tubercle in the area between the posterior spiracles and anal lobe. Presence of a caudal ridge can be used to separate the subfamily Dacinae from Trypetinae, in which it is absent (Figure 71, Figure 72). The caudal ridge is usually apparent in dorsal, caudal and lateral views, although it may be easier to see from some angles than others. The caudal ridge can be observed using either a dissecting microscope with high resolution or an SEM. | *Category : TECHNICAL* **(43) United States of America (17 Aug 2022 9:36 PM)** The ridge is on a protuberance (I forget the name, ventral tubercle?) that is also present in other (all?) pest genera. I think this should be explained further as it is a subtle character and often difficult to see, at least under a stereoscope. Users shouldn’t confuse the tubercle with the ridge. | **Incorporated.** |
| 247 | Some Dacinae have a thin, dark, sclerotized line below the caudal ~~ridge~~ ridges that is visible under a dissecting microscope, but not under an SEM. It is known to occur in numerous *Zeugodacus* species (Figure 73). It has not been observed in any *Ceratitis* larvae described to date. | *Category : TECHNICAL* **(42) United States of America (17 Aug 2022 9:32 PM)** Really ventral to the tubercles or sometimes just the space between them? | **Modified.**  Incorporated suggested edit.  To answer question: Dark line is below the caudal ridges; Do not know if it is on or off the tubercle itself. |
| 250 | Useful diagnostic features given in Steck and Ekesi (2015) and Steck *et al.* (forthcoming) are included in Table 3. If all of the character states in Table 3 are observed, the insect is consistent with a diagnosis as *Ceratitis capitata*, but molecular analysis should be performed to confirm that identification (section 4.3.5). Steck and Ekesi (2015) stated that “*C*. *capitata* larvae can be separated from most individuals of the FAR complex by the absence of oral ridge accessory plates and the presence of dorsal spinules on T3” (see Figure 65 for oral ridge). Also, the subapical tooth of the mouthhook is absent or minute when present and usually not apparent with a light microscope, and the single, wide lateral lip seen in *C. capitata* (Figure 64) has not been observed in larvae of any other *Ceratitis* species described to date. | *Category : SUBSTANTIVE* **(221) EPPO (20 Sep 2022 5:03 PM)** We wonder if the requirement for molecular tests is compatible with urgent identifications performed during import controls where perishable fruits are not released until the diagnosis is made ? Barcoding takes at least 3-4 days unless you have in house sequencing facilities.  Also not in line with paragraph [220] : “a diagnosis to the genus or species that is based solely on larval morphology could be appropriate when screening for a pest where its presence is expected based on prior information and closely related species that could be mistaken for the pest are absent. Molecular analysis (section 4.3) should be performed to complete the identification of a larva when the diagnosis is intended to confirm a new record of pest presence”  We think that this statement (above) makes sense for import controls.  Also not in line with paragraph [102]: "Morphological examination of a third instar can provide diagnostic information but may not allow an identification to be completed without additional molecular diagnostic information". This statement with 'may' indicates that identification can be possible.   Can this be discussed with the drafting team and cases where molecular tests are needed be specified.  The figure 65 is pointing to the absence of oral ridges accessory plates, it not typical for C. capitata as the margin shown on this picture is smooth | **Modified.**  [1] consistency in document sections [para 102, 220, and 250] on ability to diagnose species using larval **Modified**. Inconsistency is in sentence “If all of the character states in Table 3 are observed, the insect is *consistent with a diagnosis* as *Ceratitis capitata*, but molecular analysis *should be* performed to confirm that identification.” Changed to: “If all of the character states in Table 3 are observed, the insect is *identified as Ceratitis capitata for routine screening and surveillance*, but molecular analysis *must be* performed to complete an identification for first records for a first record.”  Para 102 has new language to ensure consistency: “Morphological examination of a third instar can provide diagnostic information but for first records of a species additional molecular diagnostic information will be needed.”  [2] General concern that methods for reliable identification of species at immature life stage does not fit operational needs because of time required to diagnose specimen.  **Considered but not incorporated.** Theprotocol provides guidance for completing identification and expectations for new or first records will require DNA technology. Once less expensive or timely methods are available they can be included.  [3] Figure 65 replaced to show scalloped edges of oral ridges.  **Incoporated** as new Fig 65 with both smooth and non smooth forms. |
| 250 | Useful diagnostic features given in Steck and Ekesi (2015) and Steck *et al.* (forthcoming) are included in Table 3. If all of the character states in Table 3 are observed, the insect is consistent with a diagnosis as *Ceratitis capitata*, but molecular analysis should be performed to confirm that identification (section 4.3.5). Steck and Ekesi (2015) stated that “*C*. *capitata* larvae can be separated from most individuals of the FAR complex by the absence of oral ridge accessory plates and the presence of dorsal spinules on T3” (see Figure 65 for oral ridge). Also, the subapical tooth of the mouthhook is absent or minute when present and usually not apparent with a light microscope, and the single, wide lateral lip seen in *C. capitata* (Figure 64) has not been observed in larvae of any other *Ceratitis* species described to date. | *Category : TECHNICAL* **(220) EPPO (20 Sep 2022 5:03 PM)** We are afraid this may lead to confusion if you write it this way; fig 65 suggests the oral ridge is smooth but that is atypical in medfly, as also stated in table 3. Moreover, oral ridges in medfly are often narrowing to the lateral sides ending in a very thin line which is strongly curved forward. As seen under compound. | **Modified**.  Figure 65 replaced to include both appearances to avoid misinterpretation.  Narrowing, curved oral ridges laterally are not apparent in all available SEM images. |
| 250 | Useful diagnostic features given in Steck and Ekesi (2015) and Steck *et al.* (forthcoming) are included in Table 3. If all of the character states in Table 3 are observed, the insect is consistent with a diagnosis as *Ceratitis capitata*, but molecular analysis should be performed to confirm that identification (section 4.3.5). Steck and Ekesi (2015) stated that “*C*. *capitata* larvae can be separated from most individuals of the FAR complex by the absence of oral ridge accessory plates and the presence of dorsal spinules on T3” (see Figure 65 for oral ridge). Also, the subapical tooth of the mouthhook is absent or minute when present and usually not apparent with a light microscope, and the single, wide lateral lip seen in *C. capitata* (Figure 64) has not been observed in larvae of any other *Ceratitis* species described to date. | *Category : EDITORIAL* **(219) EPPO (20 Sep 2022 5:03 PM)** insert (see Figure 65) here? | **Incorporated**.  Note that this insertion is within quotation. |
| 250 | Useful diagnostic features given in Steck and Ekesi (2015) and Steck *et al.* (forthcoming) are included in Table 3. If all of the character states in Table 3 are observed, the insect is consistent with a diagnosis as *Ceratitis capitata*, but molecular analysis should be performed to confirm that identification (section 4.3.5). Steck and Ekesi (2015) stated that “*C*. *capitata* larvae can be separated from most individuals of the FAR complex by the absence of oral ridge accessory plates and the presence of dorsal spinules on T3” (see Figure 65 for oral ridge). Also, the subapical tooth of the mouthhook is absent or minute when present and usually not apparent with a light microscope, and the single, wide lateral lip seen in *C. capitata* (Figure 64) has not been observed in larvae of any other *Ceratitis* species described to date. | *Category : SUBSTANTIVE* **(218) EPPO (20 Sep 2022 5:03 PM)** We are not sure that all characters can clearly be observed without SEM… (ex. preoral organ and lobes?) If not, it would not be possible to perform an identification of larvae following strictly the IPPC protocol, unless you have a SEM? We believe that most phytosanitary diagnostic labs do not have SEM… | **Considered but not incorporated.**  SEM is required to make final identificaiton using morphology.  If the use of SEM is not possible, the identification can be done by rearing larvae to adults or by molecular methods. |
| 250 | Useful diagnostic features given in Steck and Ekesi (2015) and Steck *et al.* (forthcoming) are included in Table 3. If all of the character states in Table 3 are observed, the insect is consistent with a diagnosis as *Ceratitis capitata*, but molecular analysis should be performed to confirm that identification (section 4.3.5). Steck and Ekesi (2015) stated that “*C*. *capitata* larvae can be separated from most individuals of the FAR complex by the absence of oral ridge accessory plates and the presence of dorsal spinules on T3” (see Figure 65 for oral ridge). Also, the subapical tooth of the mouthhook is absent or minute when present and usually not apparent with a light microscope, and the single, wide lateral lip seen in *C. capitata* (Figure 64) has not been observed in larvae of any other *Ceratitis* species described to date. | *Category : TECHNICAL* **(129) Kenya (29 Aug 2022 8:42 AM)** Useful diagnostic features given in Steck and Ekesi (2015) and Steck et al. (forthcomingun published data) are included in Table 3. | **Modified.**  Adjusted to “personal communcation” to be consistent with style protocol. |
| 250 | Useful diagnostic features given in Steck and Ekesi (2015) and Steck *et al.* (forthcoming) are included in Table 3. If all of the character states in Table 3 are observed, the insect is consistent with a diagnosis as *Ceratitis capitata*, but molecular analysis should be performed to confirm that identification (section 4.3.5). Steck and Ekesi (2015) stated that “*C*. *capitata* larvae can be separated from most individuals of the FAR complex by the absence of oral ridge accessory plates and the presence of dorsal spinules on T3” (see Figure 65 for oral ridge). Also, the subapical tooth of the mouthhook in C. capitata is absent or minute when present and usually not apparent with a light microscope, and the single, wide lateral lip seen in *C. capitata* (Figure 64) has not been observed in larvae of any other *Ceratitis* species described to date. | *Category : TECHNICAL* **(41) United States of America (17 Aug 2022 9:30 PM)** ? it seems from above that the tooth can be similar in C. rosa. This sentence should be reworded; it could be interpreted as unique to capitata, but that’s not the case? | **Modified**  Incorporated edit and also included statement that this state can be true for C. rosa. |
| 256 | **Table 3.** Diagnostic morphological characters of third instars of *Ceratitis* species | *Category : EDITORIAL* **(223) EPPO (20 Sep 2022 5:03 PM)** Here as well, a systematic arrangement of the colums might be better, thus having the FAR complex species together. | **Considered but not incorporated**  Alphabetical order is consistent with other protocols and rearrangement will not improve interpretation of the table. |
| 256 | **Table 3.**Diagnostic morphological characters of third instars of *Ceratitis* species | *Category : EDITORIAL* **(222) EPPO (20 Sep 2022 5:03 PM)** Congratulations on the wonderful larvae illustrations! Please provide Figure-numbers (where possible) like in Table 2 E.g.: at [295] Fig. 63; [296] Fig.64 | **Modified.**  Figures are provided under the Character field in the table to direct to Ceratitis image. |
| 256 | **Table 3.** Diagnostic morphological characters of third instars of *Ceratitis* species | *Category : TECHNICAL* **(16) United States of America (17 Aug 2022 8:44 PM)** Of the characters in Table 3, only “lateral lips”, “accessory plates”, and “dorsal spinules” provide unambiguous differentiation of the listed taxa. As described, C. anonae, C. rosa, C. fasciventris and C. quilicii cannot be differentiated by any of these characters or by the combination of all of them. | **Considered but not incorporated**  These values provide two purposes: 1) It demonstrates that overlap among species in most characters prevent use in diagnosis; 2) The ranges provided for characters can be used to diagnose C. fasciventris given that specimens can fall outside ranges of the other species |
| 260 | ***C. anonae*** | *Category : TECHNICAL* **(146) United States of America (29 Aug 2022 7:53 PM)** See comment for Table 1 re: arranging the species | **For Consideration by TPDP** |
| 266 | Dorsolateral ~~sensilla~~ sensilla, orientation to maxillary palp | *Category : EDITORIAL* **(34) United States of America (17 Aug 2022 9:20 PM)** Add comma after main character if you want to be consistent with format of adult table. | **Incorporated.** |
| 280 | ~~Mouthhook Secondary~~ outhhook secondary tooth | *Category : EDITORIAL* **(35) United States of America (17 Aug 2022 9:21 PM)** non cap | **Incorporated.** |
| 294 | Lateral lips, number and width | *Category : TECHNICAL* **(36) United States of America (17 Aug 2022 9:22 PM)** consistency with [295]-[300] | **Incorporated.** |
| 301 | Oral ~~ridges~~ ridges, number | *Category : TECHNICAL* **(37) United States of America (17 Aug 2022 9:24 PM)** consistency with [302]-[307] | **Incorporated.** |
| 308 | Oral ~~ridge~~ ridge, margins | *Category : EDITORIAL* **(38) United States of America (17 Aug 2022 9:25 PM)** general consistency | **Incorporated.** |
| 315 | Accessory plates, number, size and dentition | *Category : TECHNICAL* **(39) United States of America (17 Aug 2022 9:26 PM)** consistency with [316]-[321] | **Incorporated.** |
| 329 | ~~Number~~ Anterior spiracle, number of ~~anterior spiracle~~ tubules | *Category : EDITORIAL* **(40) United States of America (17 Aug 2022 9:27 PM)** general consistency | **Incorporated.** |
| 331 | 9–12 | *Category : TECHNICAL* **(225) EPPO (20 Sep 2022 5:03 PM)** 8-12 from observation from the NIVIP laboratory (NL)) According to the expert the reference of White & Elson and Harris is obsolete on this characteristic. You could refer to pers. communication of that is allowed in DP? For the comments on the Tubules numbers could the different sources be given? | **Modified.**  The soruce for these values is Steck & Ekesi 2015 (stated in preceeding section). The issue of exceptions to ranges is a problem. Outliers having unusual morphologies and incorrectly identified larvae could contribute. The values in 2015 study are reported as estimated ranges. A note is included [329] and footnote [350] to explain variability. |
| 331 | 9–12 | *Category : TECHNICAL* **(224) EPPO (20 Sep 2022 5:03 PM)** - White & Elson-Harris (1992): 8-10 tubules - Carroll (2004): 7-11 tubules | **Considered but not incorporated**  The soruce for these values is Steck & Ekesi 2015 (stated in preceeding section). The issue of exceptions to ranges is a problem. Outliers having unusual morphologies and incorrectly identified larvae could contribute. The values in 2015 study are reported as estimated ranges. A note is included [329] and footnote [350] to explain variability. |
| 351 | **4.3** **Molecular identification of *Ceratitis*specimens** | *Category : TECHNICAL* **(147) United States of America (29 Aug 2022 7:55 PM)** Perhaps "species" is a better word? | **Incorporated** |
| 351 | **4.3** **Molecular identification of *Ceratitis*specimens** | *Category : SUBSTANTIVE* **(121) China (28 Aug 2022 5:06 PM)** Add related information as “FARQ complex and similar species can be identified based on re-sequencing. Add ”Zhang et al., 2021”as reference. | **Incorporated** |
| 351 | 4.3 Molecular identification of *Ceratitis* specimens | *Category : SUBSTANTIVE* **(20) United States of America (17 Aug 2022 8:51 PM)** In general, given that they are largely inconclusive, unvalidated, or both, the molecular methods are described in far too much detail (~10 pages). Who is the audience of this protocol and what are they trying to accomplish? Are all of the taxa described here generally viewed as quarantine in imported fruit? If so, then telling which is which is largely an academic exercise. Do we actually NEED to tell them apart as a matter of plant protection? | **Modified**  The text has been reduced. However, the methods do provide means to correctly identify some species and provide methods to diagnose immature life stages. Despite all being pests, correct ID is important when documenting risks and where some Ceratitis species are present but others are not. |
| 353 | Specimen identification based on comparison of DNA sequences of a fragment of the *COI* gene of animals is commonly referred to as DNA barcoding (Hebert *et al.*, 2003; Floyd *et al.*, 2010). This diagnostic technique has been applied to tephritid fruit flies in several studies to demonstrate the ~~general~~ performance of the technology (Armstrong and Ball, 2005; Virgilio *et al.*, 2010; Jiang *et al.*, 2014). Development of DNA barcode data into an identification method for specific pests has been examined ~~formally~~ for *C. capitata* (Barr *et al.*, 2012) and *C. cosyra* (Virgilio *et al.*, 2017). The DNA barcoding method is not sufficient to complete species-level identifications for within the members of the FAR species complex: *C*. *anonae*, *C. fasciventris*, *C*. *rosa* and *C*. *quilicii* (Virgilio *et al.*, 2019). | *Category : EDITORIAL* **(149) United States of America (29 Aug 2022 8:56 PM)** "general", "formally" could be deleted. FAR complex is added for consistency | **Incorporated** |
| 353 | Specimen identification based on comparison of DNA sequences of a fragment of the *COI* gene of animals is commonly referred to as DNA barcoding (Hebert *et al.*, 2003; Floyd *et al.*, 2010). This diagnostic technique has been applied to tephritid fruit flies in several studies to demonstrate the general performance of the technology (Armstrong and Ball, 2005; Virgilio *et al.*, 2010; Jiang *et al.*, 2014). Development of DNA barcode data into an identification method for specific pests has been examined formally for *C. capitata* (Barr *et al.*, 2012) and *C. cosyra* (Virgilio *et al.*, 2017). The DNA barcoding method is not sufficient to complete species-level identifications for *C*. *anonae*, *C. fasciventris*, *C*. *rosa*and *C*. *quilicii* (Virgilio *et al.*, 2019). | *Category : TECHNICAL* **(148) United States of America (29 Aug 2022 8:49 PM)** "Species" might be preferable? | **Incorporated** |
| 354 | In the case of *C. capitata*, the method does not separate *C. capitata* from its sister species, *C*. *caetrata*. As a result, identification of a specimen as *C. capitata* using DNA barcoding is dependent on considering a reduced taxonomic scope in the diagnosis process. This reduced scope is achieved by excluding *C. caetrata* as a possible outcome in the diagnosis, where possible, on the basis of its restricted host range, which includes indigenous wild fruits but not commercially grown fruits, and its limited geographical distribution: *C*. *caetrata* has not been detected outside of Kenya (De Meyer, 2001; De Meyer *et al.*, 2002, 2004). The inability to separate *C. capitata* and *C. caetrata* is also true of the real-time PCR method developed for diagnosis of *C. capitata* based on *COI* gene sequence differences (described in section 4.3.5.2). | *Category : TECHNICAL* **(151) United States of America (29 Aug 2022 9:00 PM)** This sentence does not add any new info, as it is already said that the DNA barcode (COI gene) sequence could not separate these two species. Certainly, real-time PCR based on the DNA barcoding region should not bring any additional information. | **Considered but not incorporated**  It is important to provide clarity on this specifcity as the lack of statement could lead to misinterpretation of confidence. |
| 354 | In the case of *C. capitata*, the method does not separate *C. capitata* from its sister species, *C*. *caetrata*. As a result, identification of a specimen as *C. capitata* using DNA barcoding is dependent on considering a reduced taxonomic scope in the diagnosis process. This reduced scope is achieved by excluding *C. caetrata* as a possible outcome in the diagnosis, where possible, on the basis of its restricted host range, which includes indigenous wild fruits but not commercially grown fruits, and its limited geographical distribution: *C*. *caetrata* has not been detected outside of Kenya (De Meyer, 2001; De Meyer *et al.*, 2002, 2004). The inability to separate *C. capitata* and *C. caetrata* is also true of the real-time PCR method developed for diagnosis of *C. capitata* based on *COI* gene sequence differences (described in section 4.3.5.2). | *Category : TECHNICAL* **(150) United States of America (29 Aug 2022 8:59 PM)** Are there any newer references? | **Considered but not incorporated.**  As it is not a pest species, only studies on native species include the pest. No new information to counter those detailed studies. |
| 354 | In the case of *C. capitata*, the method does not separate *C. capitata* from its sister species, *C*. *caetrata*. As a result, identification of a specimen as *C. capitata* using DNA barcoding is dependent on considering a reduced taxonomic scope in the diagnosis process. This reduced scope is achieved by excluding *C. caetrata* as a possible outcome in the diagnosis, where possible, on the basis of its restricted host range, which includes indigenous wild fruits but not commercially grown fruits, and its limited geographical distribution: *C*. *caetrata* has not been detected outside of Kenya (De Meyer, 2001; De Meyer *et al.*, 2002, 2004). The inability to separate *C. capitata* and *C. caetrata* is also true of the real-time PCR method developed for diagnosis of *C. capitata* based on *COI* gene sequence differences (described in section 4.3.5.2). | *Category : TECHNICAL* **(17) United States of America (17 Aug 2022 8:45 PM)** Given that this protocol is for six particular species of Ceratitis s. l., and not for lots of other ones that are not big pests, it seems like a bit of a digression to talk about how C. capitata can’t be distinguished from C. caetrata. If caetrata is not a pest, then it won’t be intercepted anyway. | **Considered but not incorporated.**  This is important for labs working in areas where they co-occur. |
| 356 | Analyses of *C. cosyra* specimens using microsatellite DNA (Virgilio *et al.*, 2015) and mitochondrial DNA (Barr *et al.*, 2012; Frey *et al.*, 2013; Virgilio *et al.*, 2017) support the hypothesis of cryptic species under the name *C. cosyra*. Virgilio *et al.* (2017) distinguished at least two lineages named *C*. *cosyra* group 1 and *C*. *cosyra* group 2. These two groups do not form one monophyletic lineage based on analysis of the *COI* gene. Of the *C*. *cosyra* specimens included in molecular studies, group 1 is the dominant lineage because it is reported from a greater number of specimens and from collections over a wider geographical distribution range. The DNA barcoding method can identify a fly to species *C*. *cosyra* group 1 or to *C*. *cosyra* group 2 based on high DNA sequence similarity (Virgilio *et al.*, 2017). | *Category : TECHNICAL* **(152) United States of America (30 Aug 2022 8:54 PM)** this is a redundant and unnecessary statement; You have already talked about this at the beginning of this paragraph. (lines 1-5) | **Considered but not incorporated**  The previous sentences state that molecualr methods can discern cryptic species but do not state if the COI DNA barcode gene region can achieve that itself. This provides a clear statement on ability. |
| 356 | Analyses of *C. cosyra* specimens using microsatellite DNA (Virgilio *et al.*, 2015) and mitochondrial DNA (Barr *et al.*, 2012; Frey *et al.*, 2013; Virgilio *et al.*, 2017) support the hypothesis of cryptic species under the name *C. cosyra*. Virgilio *et al.* (2017) distinguished at least two lineages named *C*. *cosyra* group 1 and *C*. *cosyra* group 2. These two groups do not form one monophyletic lineage based on analysis of the *COI* gene. Of the *C*. *cosyra* specimens included in molecular studies, group 1 is the dominant lineage because it is reported from a greater number of specimens and from collections over a wider geographical distribution range. The DNA barcoding method can identify a fly to species *C*. *cosyra* group 1 or to *C*. *cosyra* group 2 based on high DNA sequence similarity (Virgilio *et al.*, 2017). | *Category : TECHNICAL* **(18) United States of America (17 Aug 2022 8:46 PM)** “these two groups do not form one monophyletic lineage … “ with respect to what? This is quite confusing. It is not clear whether this is taxonomic or population genetic-level variation. Is it significant from a quarantine perspective? | **Incorporated.**  Provided explanation that with respect to toehr Ceratitis species. It is not popualtional. |
| 357 | Phylogenetic analysis of *C. cosyra* using mitochondrial *~~COI~~*DNA sequences (COI gene) has identified additional specimens that do not cluster into group 1 or group 2. These sequences were from specimens that either could not be confirmed to be *C. cosyra* using morphology or had multiple pseudogene copies of the *COI* gene that preclude diagnostic analysis of the data (Barr *et al.*, 2012; Virgilio *et al.*, 2017). As summarized by Virgilio *et al.* (2017), the species limits of *C*. *cosyra* and potential cryptic species that look like *C. cosyra* are not yet known. Insufficient information is available to conclude that a fly is not *C*. *cosyra* based on dissimilarity to records reported from either group 1 or group 2. Presence of two or more dissimilar copies of *COI* gene in a *C*. *cosyra* specimen has been reported (Barr *et al.*, 2012) and when sequenced could generate results that do not match the DNA barcoding sequence records for groups 1 and 2. | *Category : TECHNICAL* **(153) United States of America (30 Aug 2022 8:58 PM)** redundant with the statements described at the beginning of the paragraphs (lines 2-4) | **Modified.**  Deleted final sentence based on redundancy.  Mitochndrial DNA description for COI is provided in previous para to ensure clarity. |
| 358 | The pests *C*. *rosa*,*C*. *anonae*, *C. fasciventris* and *C*. *quilicii* are closely related species that together comprise the FAR species complex (Barr and McPheron, 2006; Virgilio *et al.*, 2008, 2013, 2019). These four species cannot be separated from each other using the DNA barcoding method (Virgilio *et al.*, 2010; Barr *et al.*, 2012; Virgilio *et al.*, 2012). The *COI* records for these four species form a monophyletic clade in phylogenetic trees indicating that identification of the FAR complex is possible using the DNA barcode data (Barr and McPheron, 2006; Virgilio *et al.*, 2008, 2019), but there are limitations to using tree-based identification methods for the data (Meier *et al.*, 2006; DeSalle and Goldstein, 2019). Reliable identification of flies to the level of the FAR complex based on percentage divergence between *COI* sequences has not been demonstrated. This is because the observed genetic distances separating FAR complex DNA barcode records can be high and similar to the minimum distances separating FAR specimens from other species (Barr *et al.*, 2012). The application of conservative genetic distance estimates can be used to support a tree-based analysis for the identification of specimens in the FAR complex. | *Category : TECHNICAL* **(155) United States of America (30 Aug 2022 9:02 PM)** What is conservative genetic distance? Any example? | **Modified.**  Text has been removed for this and previous sentence because process are described in section 3.4.7.1 |
| 358 | The pests *C*. *rosa*, *C*. *anonae*, *C. fasciventris* and *C*. *quilicii* are closely related species that together comprise the FAR species complex (Barr and McPheron, 2006; Virgilio *et al.*, 2008, 2013, 2019). ~~These four~~ The members within FAR species complex: C. rosa, C. anonae, C. fasciventris and C. quilicii cannot be separated from each other using the DNA barcoding method (Virgilio *et al.*, 2010; Barr *et al.*, 2012; Virgilio *et al.*, 2012). The *COI* records for these four species form a monophyletic clade in phylogenetic trees indicating that identification of the FAR complex is possible using the DNA barcode data (Barr and McPheron, 2006; Virgilio *et al.*, 2008, 2019), but there are limitations to using tree-based identification methods for the data (Meier *et al.*, 2006; DeSalle and Goldstein, 2019). Reliable identification of flies to the level of the FAR complex based on percentage divergence between *COI* sequences has not been demonstrated. This is because the observed genetic distances separating FAR complex DNA barcode records can be high and similar to the minimum distances separating FAR specimens from other species (Barr *et al.*, 2012). The application of conservative genetic distance estimates can be used to support a tree-based analysis for the identification of specimens in the FAR complex. | *Category : TECHNICAL* **(154) United States of America (30 Aug 2022 9:01 PM)** this has been already explained earlier. Instead, restructure the next sentence, as suggested. | **Modified.**  In addition to the suggested change, modified text now reduces first two senetences because of redundant informaiton from para 353 |
| 358 | The pests *C*. *rosa*, *C*. *anonae*, *C. fasciventris* and *C*. *quilicii* are closely related species that together comprise the FAR species complex (Barr and McPheron, 2006; Virgilio *et al.*, 2008, 2013, 2019). These four species cannot be separated from each other using the DNA barcoding method (Virgilio *et al.*, 2010; Barr *et al.*, 2012; Virgilio *et al.*, 2012). The *COI* records for these four species form a monophyletic clade in phylogenetic trees indicating that identification of the FAR complex is possible using the DNA barcode data (Barr and McPheron, 2006; Virgilio *et al.*, 2008, 2019), but there are limitations to using tree-based identification methods for the data (Meier *et al.*, 2006; DeSalle and Goldstein, 2019). Reliable identification of flies to the level of the FAR complex based on percentage divergence between *COI* sequences has not been demonstrated. This is because the observed genetic distances separating FAR complex DNA barcode records can be high and similar to the minimum distances separating FAR specimens from other species (Barr *et al.*, 2012). The application of conservative genetic distance estimates can be used to support a tree-based analysis for the identification of specimens in the FAR complex. | *Category : TECHNICAL* **(19) United States of America (17 Aug 2022 8:47 PM)** Again, if the methods to tell them apart don’t work, then why bother talking about it. Just say “molecular methods to distinguish among species of the FAR complex have not been validated.” | **Modified.**  The text is intedend to provide information on how identification to the level of species complex might be achieved. Text was revised to be clearer and achieve that purpose. |
| 359 | Once a fly is identified as a member of the FAR complex based on morphology or mitochondrial DNA ~~barcode data~~(barcode data), additional analysis ~~using~~ of nuclear DNA (using 16 microsatellite ~~DNA markers~~ markers) can distinguish the four species (Delatte *et al.*, 2014; Virgilio *et al.*, 2013, 2019). The microsatellite DNA technique requires comparison of PCR-amplified alleles to alleles of reference material to correctly score the size of the allele fragments and complete computational analysis of admixture coefficients to determine the fly’s identity. Reference material of these species is not readily available, and the method has not been replicated in multiple laboratories yet. Consequently, this method is not provided in detail in the current protocol. | *Category : EDITORIAL* **(156) United States of America (30 Aug 2022 9:05 PM)** clarity | **Incorporated**. |
| 360 | **4.3.1** **DNA extraction for molecular tests** | *Category : TECHNICAL* **(157) United States of America (30 Aug 2022 9:06 PM)** More appropriate heading of this paragraph would be ‘Sample preservation, preparation, and DNA extraction’ for the molecular test, And the contents should be organized accordingly, i.e., first sample preservation and sample preparation and then DNA extraction. | **Incorporated.** |
| 362 | In cases where molecular and morphological methods are to be used, it is therefore recommended that a portion of the larva (such as abdominal segment 4 or 5) be excised ~~for the extraction~~(see section 4.2.1), or a hind leg be removed (see beginning of section 4), and stored in ethanol for DNA extraction. The remaining specimen can be prepared for morphological work. It is important to ensure that the legs of adults are available for examination as the characters present on the legs are used to identify *Ceratitis* species. Further examples of methods are provided by Plant Health Australia (2016). | *Category : EDITORIAL* **(226) EPPO (20 Sep 2022 5:03 PM)** 1) Deletion of "for the extraction" : Simplification suggested because of "for DNA extraction" at the end of the sentence. 2) and 3) : addition of "(see section 4.2.1)" and "(see beginning of section 4)" : Because more precisions are given in paragraphs 226 and 106. 4) Addition of the comma before "and stored in ethanol for DNA extraction": Because the tissue excised from the larva should also be kept in ethanol (see paragraph 226). | **Incorporated.** |
| 362 | In cases where molecular and morphological methods are to be used, it is therefore recommended that a portion of the larva (such as abdominal segment 4 or 5) be excised for the extraction, or a hind leg be removed and stored in ethanol for DNA extraction. The remaining specimen can be prepared for morphological work. It is important to ensure that the legs of adults are available for morphological examination as the characters present on the other legs are used to identify *Ceratitis* species. Further examples of methods are provided by Plant Health Australia (2016). | *Category : TECHNICAL* **(158) United States of America (30 Aug 2022 9:07 PM)** technical clarification | **Incorporated.** |
| 364 | For the test result to be considered reliable, appropriate controls should be considered for each series of nucleic acid extractions and PCR amplifications of the target pest. As a minimum, a positive nucleic acid control, a negative amplification control (no template control), and a negative extraction control should be used for a *~~COI~~* PCR test used to conduct DNA barcoding or for a real-time PCR test. | *Category : TECHNICAL* **(159) United States of America (30 Aug 2022 9:08 PM)** correction | **Incorporated.** |
| 376 | **Table 4.** Master mix composition, thermal cycling parameters and amplicons for PCR to amplify *COI* barcode from *Ceratitis capitata* | *Category : TECHNICAL* **(160) United States of America (30 Aug 2022 9:09 PM)** correction | **Incorporated.** |
| 409 | **Expected amplicons** | *Category : TECHNICAL* **(162) United States of America (30 Aug 2022 9:11 PM)** it would be better to provide a gel electrophoresis photo (if available) to demonstrate the PCR amplification result clearly. | **Considered but not incorporated**.  This is not included in a DP unless there is a reason why gel size would be complicated to interpret for the trainned end user. IPPC protocols assume users have knolwedge of general methods. |
| 418 | The DNA sequencing of PCR products should be carried out using each PCR primer to generate two DNA sequence reads in alternate directions. In addition to the output of base sequence data reported as text, the chromatogram and Phred scores used to determine base calls should also be examined during the editing process and stored with records. The two sequences should be aligned to create a consensus sequence and then visually examined to identify conflicting information. Chromatograms should be edited to resolve conflicting signals using the visual examination. Sites that are not corroborated by data in both sequences because of differences in lengths should be removed or 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, then the site should be assigned as an ambiguous base (i.e., N) in the consensus sequence. If conflict is the result of ambiguity at a site because of two sequences and each has a high Phred score (>30), then the site should be assigned as an ambiguous base (i.e., N). Diagnosis should only be performed on edited sequences having less than 0.5% ambiguous bases. The final sequence length of the query sequence should be at least 500 bp in length for data interpretation. Additional information on data-editing processes is available in EPPO (2016). | *Category : EDITORIAL* **(163) United States of America (30 Aug 2022 9:12 PM)** definite article | **Modified.**  Removed reference to visual examination in case alternative methods are developed. |
| 420 | DNA barcode analysis should be performed using copies of the *COI* gene that are orthologous. Paralogous copies of *COI* and other mitochondrial genes have been reported for *Ceratitis* species, including *C. capitata* and *C. cosyra* (Barr *et al.*, 2006, 2012). Evidence of pseudogenes in a specimen or the presence of multiple, paralogous *COI* copies in a specimen can make it more difficult to interpret results (Blacket, Semeraro and Malipatil, 2012). It is possible for paralogous copies of a mitochondrial gene to be preferentially amplified instead of the orthologous copy during PCR (Barr *et al.*, 2006, Barr and McPheron 2006). Virgilio *et al.* (2012) included a record for *C*. *capitata* (DQ011888) that is inconsistent with estimated intraspecific variation for the species and is possibly a misidentified specimen or a paralogous copy of the *COI* gene. | *Category : TECHNICAL* **(164) United States of America (30 Aug 2022 9:13 PM)** In paragraph [419], you have talked about if the barcode/quarry sequences do not match the expected species, then they would be considered copies of that species' pseudogenes or separate species.  Moreover, I think the content of this paragraph has already been discussed in section 4.3 [ paragraph 357] and looks redundant. Also, in this section, we are providing guidelines for DNA editing and the quality control process. Therefore, I think this paragraph could be shortened or even omitted. | **Modified.**  Use of the protocol might not be peformed by experts on Ceratitis and this paragraph provides additional warning about the need for checking for orthologous copies. It is relevant for this group of pests.  However, it is logical to reduce and move that text to end of the next paragraph (421). |
| 421 | Before completing a diagnosis, the query nucleotide sequence should be translated into an amino acid sequence and compared to the amino acid translation of *Ceratitis* records (sections 4.3.5, 4.3.6 or 4.3.7) to detect evidence of premature stop codons and reading-frame shifts (frameshifts) that suggest a pseudogene has been amplified and sequenced. Paralogous copies of *COI* such as pseudogenes should not be interpreted using the DNA barcoding methods included in this protocol. ~~It~~ However, it can be difficult to detect pseudogenes and other paralogs of the *COI* gene because DNA barcode records can lack evidence of insertions or deletion in the nucleotide alignment and disruptions to amino acid translation codes (Buhay, 2009). In addition to detecting frameshift mutations, the protocol includes steps to assist in paralogous copy recognition based on high rates of ambiguous calls (i.e., conflicting calls of multiple peaks) and high mutation rates for a specimen observed as a long branch in the clade of a phylogenetic tree. | *Category : TECHNICAL* **(165) United States of America (30 Aug 2022 9:14 PM)** Should this be done to check the quality of the DNA to see if it qualifies for barcoding analysis as mentioned in paragraph [429]. Or it should be done to identify pseudogene even after the DNA is qualified for barcode analysis. | **Incorporated.**  Ideally this would be done before analysis but many researchers discover the information while in prcoess of analysis. The language is to be non-prescriptive in order. If it is done sometime during process, the ID is appropriate. |
| 425 | ~~The~~ Barr *et al.* (2012) ~~study~~ demonstrated that an uncorrected p-distance measure of 2% was appropriate to capture intraspecific variation and that a barcode gap existed between *C*. *capitata* and the close relative *C*. *pinax*. After exclusion of an atypical sequence (DQ011888), ~~analysis by~~ Virgilio et al. (2012) also reported an expected divergence of 2% using p-distance or Kimura 2-parameter distance. In both studies, the next most similar species (*C. catoirii*, *C. malgassa* and *C. pinax*) were greater than 5% distant from the *C*. *capitata* and *C*. *caetrata* DNA records. ~~The~~ Barr *et al.* (2012) ~~study~~ also examined the dataset using DNA characters states and determined that a clade including *C*. *capitata* and *C*. *caetrata* can be diagnosed from other species*.* The DNA barcoding method described in this protocol to identify *C*. *capitata* is based on these studies and describes one reliable approach to diagnose *C*. *capitata* without reliance on databases that can change over time. The PCR method for amplification of the *COI* target is provided in section 4.3.3. | *Category : EDITORIAL* **(166) United States of America (30 Aug 2022 9:17 PM)** better language | **Incorporated.** |
| 427 | If quality conditions are met, the consensus sequence of the query should be aligned to the *COI* records reported in Barr *et al.* (2012) and available from GenBank as PopSet407912263. This can be accomplished using an algorithm such as CLUSTAL and visual examination of alignment. The alignment should be visually examined for insertion and deletion events caused by the query sequence. The alignment should be translated into amino acids using genetic code for insect mitochondria and examined for evidence of frameshifts or premature stops. If either is observed, the query sequence is treated as a pseudogene. If there is no evidence that the consensus sequence is a pseudogene, then the query sequence can be diagnosed based on agreement of two analyses: a tree-based visualization and a separate genetic-distance measure. | *Category : TECHNICAL* **(167) United States of America (30 Aug 2022 9:18 PM)** Should this be done, even the alignments (quarry and reference sequences) are matched with each other. In that case, is there still any chance of being detected as a pseudogene?   Also, shouldn’t this process be part of the quality control processes? Or it should be done again even after the query sequence is qualified for DNA barcode analysis after quality control. Also, this paragraph is redundant with section 4.3.4 9 [paragraph 421] | **Modified.**  Removed sentences as suggested and added refence to seciton 4.3.4 where quality measurement is explained |
| 428 | The alignment can be used to generate a maximum parsimony (MP) tree or, if multiple MP trees are determined to be equally parsimonious in a search, a strict consensus tree of all MP trees. This provides an assessment of character-based similarities between the query and the records in the alignment. The query sequence is interpreted to be a *C. capitata* sequence if the query sequence is in a clade that consists exclusively of *C. capitata* and *C. caetrata*sequences. If the query sequence does not form a clade including any *C. capitata* and *C. caetrata*sequences in the MP tree, this is evidence in support of the sequence being a species other than *C. capitata* or *C. caetrata*. It is possible for paralogous copies of *COI* to form a clade with reference sequences and complicate interpretation. A comparison of the query records to reported genetic-distance values between orthologous copies of the pest can assist in detecting possible pseudogenes or confirming the MP-based interpretation. | *Category : TECHNICAL* **(255) South Africa (28 Sep 2022 7:36 AM)** Maximum parsimony is gradually falling out of favour with taxonomists and in phylogeny. In its place have emerged maximum likelihood and Bayesian analyses. Proposal: These paragraphs should therefore be expanded to include both sets of these analyses. | **Incorporated**.  Text has been changed to allow for any character-based phylgenetic approach. |
| 428 | The alignment can be used to generate a maximum parsimony (MP) tree or, if multiple MP trees are determined to be equally parsimonious in a search, a strict consensus tree of all MP trees. This provides an assessment of character-based similarities between the query and the records in the alignment. The query sequence is interpreted to be a *C. capitata* sequence if the query sequence is in a clade that consists exclusively of *C. capitata* and *C. caetrata*sequences. If the query sequence does not form a clade including any *C. capitata* and *C. caetrata*sequences in the MP tree, this is evidence in support of the sequence being a species other than *C. capitata* or *C. caetrata*. It is possible for paralogous copies of *COI* to form a clade with reference sequences and complicate interpretation. A comparison of the query records to reported genetic-distance values between orthologous copies of the pest can assist in detecting possible pseudogenes or confirming the MP-based interpretation. | *Category : TECHNICAL* **(168) United States of America (30 Aug 2022 9:21 PM)** Even after quality control step? Aren’t’ we excluding the paralogues copies or pseudogenes during the quality control process? | **Considered but not incorporated.**  Correct. A sequence could lack frameshift mutation and pass to next step in diagnosis. The only way to note elevated mutation rates of such genes is through comparison with other sequences. A paralogous copy could group with the species but have an elevated rate of substitutions. Quality control is not limited to one screening step. |
| 429 | Next, to confirm a positive identification in the MP tree result, the edited sequence should be aligned to three reference sequences: GeneBank accessions GQ154188 (*C*. *capitata*), GQ154186 (*C*. *caetrata*) and GQ154194 (*C*. *catoirii*) from reference specimens at the Royal Museum for Central Africa. The pairwise, uncorrected percent differences among the four sequences should be computed and the results used to determine if the follow conditions are true: | *Category : TECHNICAL* **(169) United States of America (30 Aug 2022 9:22 PM)** clarification | **Incoporated.** |
| 435 | If the results do not match either of these two outcomes, then the query fly cannot be identified. In this situation, the genetic results are inconsistent with genetic-distance estimates from prior datasets. It is possible that the sequence is an alternate, paralogous copy of the *COI*gene. | *Category : TECHNICAL* **(95) United States of America (24 Aug 2022 9:55 PM)** Non-target (different species)? Aren’t we excluding paralogous copies/pseudogene copies by translating amino acids during the quality control process? | **Considered but not incorporated.**  Translation of AA is not suffcient to detect paralogous copies. If the results do not match the outcomes, then it is not consistent with variation estimated for other non-target species in the genus. It is possible that a yet to be studied species is the determination. But this result should be treated as inconclusive. |
| 444 | The master mix and PCR amplification conditions are described in Table 5. | *Category : TECHNICAL* **(170) United States of America (30 Aug 2022 9:25 PM)** clarification | **Incorporated.** |
| 445 | **Table 5.** Master mix composition, thermal cycling parameters and amplicons for real-time PCR to identify *C. capitata* | *Category : TECHNICAL* **(171) United States of America (30 Aug 2022 9:25 PM)** clarification, and for consistency with above tables. | **Incorporated.** |
| 482 | Failure to generate a real-time PCR product consistent with the *C*. *capitata* target is not sufficient to determine that the specimen is not *C*. *capitata*, as it is possible that the nucleic acid sample of the specimen was not appropriate for real-time PCR. In these circumstances, an additional PCR-based test of the extracted DNA, such as the conventional PCR to amplify *COI* described in section 4.3.3, must therefore also be performed to confirm that nucleic acid quality and quantity did not impact the result. Dhami *et al.* (2016) demonstrated that commercially available eukaryotic 18S real-time PCR control kits can also be used to confirm suitability of the extraction for diagnosis of *C. capitata*. The *COI* conventional PCR and 18S real-time PCR options must also include positive and negative controls. The positive controls and experimental samples for the 18S real-time PCR are only positive if they generate a product within 35 cycles and have a sigmoidal shaped growth curve. The relative sensitivity of the *COI*conventional PCR and the *COI*and 18S real-time PCR have not been reported. | *Category : TECHNICAL* **(94) United States of America (24 Aug 2022 9:52 PM)** Is there any reason why DNA samples may not be appropriated? Low concentration or high concentration of DNA? I could be better to add an example photo if available | **Modified**.  Text modified to add example of why specimen would not be appropriate.  A photo is not included to be consistent with other protocols and expectation of familiarity of trained expert using protocols. |
| 488 | The master mix and PCR amplification conditions are described in Table 6. | *Category : TECHNICAL* **(172) United States of America (30 Aug 2022 9:28 PM)** for consistency | **Incorporated.** |
| 489 | **Table 6.** Master mix composition, thermal cycling parameters and amplicons for PCR to amplify ITS-1 from *Ceratitis capitata* | *Category : TECHNICAL* **(173) United States of America (30 Aug 2022 9:28 PM)** for consistency | **Incorporated.** |
| 544 | If the negative controls generate amplicons, then the results are not valid. If the positive control fails to generate the expected product, then the results are not valid. Amplicon size differences can be scored on 1.4% agarose gels. The results for the query fly should be compared to those of a known *C. capitata* and *C. caetrata* to compare amplicon size or to one of the species and a molecular ladder that can discriminate the band sizes in the range. | *Category : TECHNICAL* **(93) United States of America (24 Aug 2022 9:50 PM)** Adding a gel electrophoresis photo would be better (if available) | **Considered but not incorporated.**  A photo is not included to be consistent with other protocols and expectation of familiarity of trained expert using protocols. |
| 546 | The name *C*. *cosyra* currently includes multiple cryptic species (Virgilio *et al.*, 2017). Molecular identification can be completed for two lineages within the species referred to as *C*. *cosyra* group 1 and *C*. *cosyra* group 2 using DNA ~~barcoding (section 4.3.6.1)~~barcoding. | *Category : EDITORIAL* **(227) EPPO (20 Sep 2022 5:03 PM)** Unnecessary as it is the following section? | **Incorporated.** |
| 551 | If quality conditions are met, the consensus sequence of the query should be aligned to the *COI* records reported by Virgilio *et al.* (2017) and available at this link: . The dataset and the query sequence can be aligned using an algorithm such as CLUSTAL and visual examination of alignment. The alignment should be visually examined for insertion and deletion events caused by the query sequence. The alignment should be translated into amino acids using genetic code for insect mitochondria and examined for evidence of frameshifts or premature stops. If either is observed, the query sequence is treated as a pseudogene. If there is no evidence that the consensus sequence is a pseudogene, then the query sequence can be diagnosed based on agreement of two analyses: a tree-based visualization and a separate genetic-distance measure. | *Category : TECHNICAL* **(92) United States of America (24 Aug 2022 9:49 PM)** This paragraph is redundant with the statement provided in sections 4.3.4 [paragraph 421]; 4.3.5.1 [427]; 4.3.6.1 [551] and 4.3.7.1 [562]. As this statement has been provided in section 4.3.4, we could give a reference to that section when necessary to avoid redundancy. | **Modified**.  Text was reduced to remove redundancy with quality check. The redundant informaiton between barcode anlyses for species were not removed because user would expect instructions under section. |
| 552 | The alignment can be used to generate an MP tree or, if multiple MP trees are determined to be equally parsimonious in a search, a strict consensus tree of all MP trees. This provides an assessment of character-based similarities between the query and the records in the alignment. The query sequence is interpreted to be a *C. cosyra*sequence if the query sequence is in a clade that consists exclusively of *C. cosyra*sequences. If the query sequence does not form a clade including any *C. cosyra* sequences in the MP tree, this should not be interpreted as evidence that the sequence is *not C. cosyra*, because the species appears to form polyphyletic lineages in trees and might be a cryptic species (Virgilio *et al.*, 2017). It is also possible for paralogous copies of *COI* to form a clade with reference sequences and complicate interpretation. A comparison of the query records to reported genetic-distance values between orthologous copies of the pest can assist in detecting possible pseudogenes or confirming the MP-based interpretation. | *Category : TECHNICAL* **(91) United States of America (24 Aug 2022 9:48 PM)** Even after quality control step? Aren’t’ we excluding the paralogues copies or pseudogenes during the quality control process? | **Considered but not incorporated.**  Translation of AA is not suffcient to detect paralogous copies. If the results do not match the outcomes, then it is not consistent with variation estimated for other non-target species in the genus. It is possible that a yet to be studied species is the determination. But this result should be treated as inconclusive. |
| 553 | Next, to confirm a positive identification in the MP tree result, the edited sequence should be aligned to a *C*. *cosyra* group 1 record ~~(GQ154202)~~ (GeneBank accessions: GQ154202) and *C*. *cosyra* group 2 record ~~(GQ154204)~~ (GeneBank accessions: GQ154204) from reference specimens at the Royal Museum for Central Africa. The pairwise, uncorrected percent differences among the three sequences should be computed and the results used to determine the identification. | *Category : TECHNICAL* **(90) United States of America (24 Aug 2022 9:48 PM)** more detailed info | **Incorporated.** |
| 556 | If the results do not match either of these two outcomes, then the query fly cannot be identified. In this situation, the genetic results are inconsistent with genetic-distance estimates from prior datasets. It is possible that the sequence is an alternate, paralogous copy of the *COI* gene. Identification of the query fly as *C*. *capitata* (section 4.3.5) or a FAR complex species (section 4.3.7) can be examined using this protocol. | *Category : TECHNICAL* **(228) EPPO (20 Sep 2022 5:03 PM)** Can you clarify what 'query fly' means. is it the specimen being identified?  if yes may be clearer to simply state this. but is hits sentence needed? it is not included in the last paragraph of 4.3.7.1 DNA barcoding the FAR complex  We do not understand 'identification.... can be examined..." | **Incorporated.**  Changed query fly to specimen |
| 558 | Molecular methods can diagnose a query fly to the level of the FAR complex using DNA barcoding (section 4.3.7.1). As explained ~~in~~ at the beginning of section 4.3, molecular identification of FAR complex specimens to the level of species (i.e., *C*. *anonae*, *C. fasciventris*, *C*. *rosa* and *C*. *quilicii*) require microsatellite DNA examination but details for that procedure are not provided in this protocol. Identification of a fly to the FAR complex is a prerequisite for subsequent microsatellite DNA diagnosis. | *Category : EDITORIAL* **(229) EPPO (20 Sep 2022 5:03 PM)** More precise wording. | **Incorporated.** |
| 558 | Molecular methods can diagnose a query fly to the level of the FAR complex using DNA barcoding (section 4.3.7.1). As explained in section 4.3, molecular identification of FAR complex specimens to the level of species (i.e., *C*. *anonae*, *C. fasciventris*, *C*. *rosa* and *C*. *quilicii*) require microsatellite DNA examination but details for that procedure are not provided in this protocol. Identification of a fly to the FAR complex is a prerequisite for subsequent microsatellite DNA diagnosis. | *Category : TECHNICAL* **(130) Kenya (29 Aug 2022 8:47 AM)** As explained in section 4.3, molecular identification of FAR complex specimens to the level of species (i.e., C. anonae, C. fasciventris, C. rosa and C. quilicii) require microsatellite DNA examination but details for that procedure are is not provided in this protocol. | **Considered but not incorporated**.  The text in comment is near identical to original text except for substitution of “is” for “are.” |
| 562 | If quality conditions are met, the consensus sequence of the query should be aligned to the *COI* records reported in both Barr *et al.* (2012) and Virgilio *et al.* (2010). These are stored in GenBank as PopSet407912263 and PopSet339262093, respectively. The two datasets and the query sequence can be aligned using an algorithm such as CLUSTAL and visual examination of alignment. The alignment should be visually examined for insertion and deletion events caused by the query sequence. The alignment should be translated into amino acids using genetic code for insect mitochondria and examined for evidence of frameshifts or premature stops. If either is observed, the query sequence is treated as a pseudogene. If there is no evidence that the consensus sequence is a pseudogene, then the query sequence can be diagnosed based on agreement of two analyses: a tree-based visualization and a separate genetic distance measure. | *Category : TECHNICAL* **(87) United States of America (24 Aug 2022 9:41 PM)** This paragraph is redundant with the statement provided in sections 4.3.4; 4.3.5.1; 4.3.6.1 and 4.3.7.1. As this statement has been provided in section 4.3.4, we could give a reference to that section when necessary to avoid redundancy. | **Modified**.  Text was reduced to remove redundancy. |
| 563 | The alignment can be used to generate an MP tree or, if multiple MP trees are determined to be equally parsimonious in a search, a strict consensus tree of all MP trees. This provides an assessment of character-based similarities between the query and the records in the alignment. The query sequence is interpreted to be a FAR complexsequence if the query sequence is in a clade that consists exclusively of sequences of FAR complex species. If the query sequence does not form a clade including any FAR complex sequences in the MP tree, this is evidence in support of the sequence being a species other than those in the FAR complex. It is also possible for paralogous copies of *COI* to form a clade with reference sequences and complicate interpretation. A comparison of the query records to reported genetic-distance values between orthologous copies of the pest can assist in detecting possible pseudogenes or confirming the MP-based interpretation. | *Category : TECHNICAL* **(88) United States of America (24 Aug 2022 9:43 PM)** This paragraph is redundant with the statement provided in sections 4.3.4; 4.3.5.1; 4.3.6.1 and 4.3.7.1. As this statement has been provided in section 4.3.4, we could give a reference to that section when necessary to avoid redundancy. | **Modified**.  Text was reduced to remove redundancy with quality check. |
| 564 | To confirm that genetic distances between the query and the FAR complex sequences are consistent with prior estimates of genetic variation, the query sequence should be aligned to the following two FAR complex records from reference specimens at the Royal Museum for Central Africa: *C. anonae* ~~(GQ154176)~~ (GeneBank accessions: GQ154176) and *C~~.~~. rosa* ~~(GQ154252)~~(GeneBank accessions: GQ154252). The pairwise, uncorrected percent difference between the query and the two FAR complex records should be computed and the results used to determine the identification. | *Category : TECHNICAL* **(89) United States of America (24 Aug 2022 9:44 PM)** specific info added | **Incorporated.** |
| 569 | In cases where other contracting parties may be adversely affected by the diagnosis, records and evidence (in particular, preserved or slide-mounted specimens, photographs of distinctive taxonomic structures, DNA extracts and photographs of gels, DNA sequence files with chromatograms, aligned DNA sequences, as appropriate) should be kept for at least one year in a manner that ensures traceability. | *Category : TECHNICAL* **(290) New Zealand (30 Sep 2022 7:47 AM)** is it standard practice? we keep interceptions for 3 years | **Considered but not included.**  This is standard language for DPs. Increasing time could be inconsistent with acceptable practices at other labs. |
| 569 | In cases where other contracting parties may be adversely affected by the diagnosis, the records and evidence of the results of the diagnosis (in particular, preserved or slide-mounted specimens, photographs of distinctive taxonomic structures, DNA extracts and photographs of gels, DNA sequence files with chromatograms, aligned DNA sequences, as appropriate) should be kept for at least one ~~year in a manner that ensures traceability~~year. | *Category : EDITORIAL* **(230) EPPO (20 Sep 2022 5:03 PM)** 1) and 2) More precise wording, corresponding to the one used in paragraph 221 of the draft diagnostic protocol for Mononychellus tanajoa. 3) Suggested deletion of "in a manner that ensures traceability", which seems to be obvious and therefore not necessary. However, if it is kept because it is deemed necessary, for consistency please add it also in in paragraph 221 of the draft diagnostic protocol for Mononychellus tanajoa. | **Modified**.  Incorporated most changes but did not delete standard language of “in a manner that ensures traceability.” |
| 577 | The first draft of this protocol was written by Marc De Meyer (Royal Museum for Central Africa, Belgium (see preceding section)), Massimiliano Virgilio (Royal Museum for Central Africa, Belgium (see preceding section)), Norman Barr ~~(USDA-APHIS~~ (USDA-APHIS, United States of America (see preceding section)) and Gary Steck (Florida Department of Agriculture and Consumer Services, United States of America (see preceding section)). | *Category : EDITORIAL* **(231) EPPO (20 Sep 2022 5:03 PM)** For consistency within the paragraph. | **Incorporated.** |
| 582 | **Barr, N.B.** 2009. Pathway analysis of *Ceratitis capitata* (Diptera: Tephritidae) using mitochondrial DNA. *Journal of Economic Entomology*, 102: ~~401-411~~401–411. | *Category : EDITORIAL* **(232) EPPO (20 Sep 2022 5:03 PM)** | **Incorporated.** |
| 583 | **Barr, N.B., Copeland, R.S., De Meyer, M., Masiga, D., Kibogo, H.G., Billah, M.K., Osir, E., Wharton, R.A. & McPheron, B.A.** 2006. Molecular diagnostics of economically important *Ceratitis* fruit fly species (Diptera: Tephritidae) in Africa using PCR and RFLP analyses. *Bulletin of Entomological Research*, 96: 505–521. | *Category : EDITORIAL* **(33) United States of America (17 Aug 2022 9:19 PM)** citation | **Incorporated.** |
| 629 | **Kandybina, M. N.** 1977. Lichinki plodovykh mukh-pestrokrylok (Diptera, Tephritidae). [Larvae of fruit-infesting fruit flies (Diptera, Tephritidae)]. Opred. Faune SSSR No. 114: ~~1-210~~1–210. [In Russian; unpublished English translation, 1987, produced by National Agricultural Library, Beltsville, Maryland, U.S.A.] | *Category : EDITORIAL* **(233) EPPO (20 Sep 2022 5:03 PM)** Typo ("–" instead of "-" for the range of pages). | **Incorporated.** |
| 644 | **Teskey, H.J.** 1981. Morphology and terminology: larvae. In: J.~~R~~F. McAlpine, B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth & D.M. Wood, ~~eds~~coords. *Manual of Nearctic Diptera, Volume 1*. Research Branch Agriculture Canada, Monograph 27: 65–88. | *Category : EDITORIAL* **(268) Canada (28 Sep 2022 9:53 PM)** | **Incorporated.** |
| 655 | 9. Figures | *Category : TECHNICAL* **(86) United States of America (24 Aug 2022 9:40 PM)** Are these lists of figures necessary as you have provided captions for each figure from [732] and afterward? | **Incorporated.** |
| 729 | ~~Figures 1–44~~**Figures 1–44**, *Source:* Jonathan Brecko and Annelies. Kayenbergh, © Royal Museum for Central Africa, Belgium. | *Category : EDITORIAL* **(234) EPPO (20 Sep 2022 5:03 PM)** If the sources are not indicated under each figure in the final version, please put "Figures 1–44" in bold for better visibility. | **Incorporated.** |
| 730 | ~~Figures 45–73~~**Figures 45–73**, *Source*: Gary Steck, Louis A. Somma and Jessica Diaz, Florida Department of Agriculture and Consumer Services, United States of America. | *Category : EDITORIAL* **(235) EPPO (20 Sep 2022 5:03 PM)** If the sources are not indicated under each figure in the final version, please put "Figures 45–73" in bold for better visibility. | **Incorporated** |
| 740 | picturebox.gif | *Category : EDITORIAL* **(107) Thailand (26 Aug 2022 4:37 AM)** Is figure 5 a duplicate of figure 19? and this figure should be rotated vertically to make it identical to other images. | **Modified.**  Image 19 rotated as recommended. Redundancy in protocol with Fig 5 and 19 intended to facilitate comparison of images when used for species identification. |
| 742 | **Figure 5.** Scutellum *Ceratitis capitata*; apical markings fused. | *Category : TECHNICAL* **(32) United States of America (17 Aug 2022 9:17 PM)** Very nice images generally! But why orient this one horizontally, different from 6-10? | **Incorporated**. |
| 765 | picturebox.gif | *Category : EDITORIAL* **(105) Thailand (26 Aug 2022 4:32 AM)** Is figure 18 a duplicate of figure 2? | **Modified.**  Image 19 rotated as recommended. Redundancy in protocol with Fig 5 and 19 intended to facilitate comparison of images when used for species identification. |
| 769 | picturebox.gif | *Category : EDITORIAL* **(106) Thailand (26 Aug 2022 4:35 AM)** Figure19 - 21 should be rotated vertically to make it identical to other images. | **Incorporated.** |
| 771 | **Figure 19.** Scutellum *Ceratitis capitata;* apical spots merged into one marking. | *Category : TECHNICAL* **(31) United States of America (17 Aug 2022 9:16 PM)** Why orient 19-21 differently than most others? | **Incorporated.** |
| 777 | picturebox.gif | *Category : EDITORIAL* **(110) Thailand (26 Aug 2022 5:04 AM)** Figure 23-26 should use a pointing arrow on "anterior apical band" and "discal band". | **Incorporated.** |
| 819 | **Figure 43.** Female anepisternum *Ceratitis rosa,* along ventral margin without dark hairs, pilosity completely yellow (indicated by circle). | *Category : TECHNICAL* **(269) Canada (28 Sep 2022 9:56 PM)** This is encircling mostly the katepisternum. Needs to be moved upwards | **Incorporated.** |
| 822 | **Figure 45.** Egg, first, second, and third instars of ~~[~~*~~Dacus bivittatus~~Dacus bivittatus*~~]~~  showing differences in sizes. | *Category : EDITORIAL* **(236) EPPO (20 Sep 2022 5:03 PM)** Dacus bivittatus not in square brackets and not underlined. | **Incorporated.** |
| 825 | **Figure 46.** Cephaloskeleton of *Ceratitis fasciventris*, third instar. Subapical tooth on mouthhook is much smaller than apical tooth. Dental sclerite present (arrow). Bar = length of cephaloskeleton. | *Category : EDITORIAL* **(270) Canada (28 Sep 2022 9:57 PM)** The subapical tooth should be shown with a red arrow. | **Incorporated.** |
| 827 | picturebox.gif | *Category : EDITORIAL* **(109) Thailand (26 Aug 2022 4:57 AM)** This figure should use a pointing arrow on "cephaloskeleton", "Anterior spiracle" and "Anal lube". | **Incorporated.** |
| 833 | **Figure 50.** Maxillary palpus, dorsolateral pair of sensilla (circle), and antenna of *Ceratitis capitata*, SEM. | *Category : TECHNICAL* **(241) Colombia (21 Sep 2022 5:16 AM)** It would be very useful to provide a photograph of this character for C. capitata in a compound microscope, due to the absence of SEM in several countries. | **Incorporated.**  This is already in Figure 51 |
| 850 | **Figure 58.** Mouthhooks of *Ceratitis rosa*, with grooved ventral surface and small subapical teeth (circles). | *Category : TECHNICAL* **(30) United States of America (17 Aug 2022 9:15 PM)** It looks far from the apex in this image? | **Considered but not incorporated.**  Another view is provided in Figure 46. Figure 58 provides useful view of character. |
| 857 | picturebox.gif | *Category : EDITORIAL* **(108) Thailand (26 Aug 2022 4:51 AM)** This figure should use a pointing arrow on "truncate posterior end" to know which part it is. | **Incorporated.**  New Figure 62 is provided with arrows for end. |
| 866 | **Figure 65.** Oral ridges of *Ceratitis capitata* with entire margins, no accessory plates. | *Category : TECHNICAL* **(242) Colombia (21 Sep 2022 5:17 AM)** It would be very useful to provide a photograph of this character for C. capitata in a compound microscope, due to the absence of SEM in several countries. | **Considered but not incorporated.**  An image does not exist and attempts to generate figure using digital camera was not successful by author team. |
| 867 | **Figure 66.** Oral ridges of *Ceratitis cosyra* with scalloped margins, one series of accessory plates (arrows). | *Category : TECHNICAL* **(29) United States of America (17 Aug 2022 9:14 PM)** This is the other side of the body (right vs left) than in figs 65 and 67? That’s fine, but maybe indicate this so there’s no confusion. | **Incorporated.** |
| 881 | ridges present (arrows). | *Category : TECHNICAL* **(243) Colombia (21 Sep 2022 5:17 AM)** It would be very useful to provide a photograph of this character for C. capitata in a compound microscope, due to the absence of SEM in several countries. | **Incorporated.** |