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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/142 (1)

Diagnostics
Diagnostic

PM 7/142 (1) *Bactrocera latifrons*

Specific scope

This Standard describes a diagnostic protocol for *Bactrocera latifrons*.[1]

This Standard should be used in conjunction with PM 7/76 Use of EPPO diagnostic protocols.

Specific approval and amendment

Approved in 2019–09

1. Introduction

Bactrocera latifrons is a pest of Solanaceae and to a lesser extent of Cucurbitaceae, and also, although more rarely, of other families of economic interest. The major host plants in Solanaceae are *Capsicum* spp. (*C. annuum*, *C. chinensis* and *C. frutescens*) and *Solanum* spp. (*S. lycopersicum*, *S. melongena* and *S. aethiopicum*). Host plants of other families are considered as minor. More information on host plants can be found in McQuate & Liquido (2013).

Bactrocera latifrons, also named 'solanum fruit fly', is native to south and southeast Asia (Pakistan, India, Sri Lanka, Bangladesh, Thailand, Cambodia, Lao, Vietnam, China, Taiwan, Brunei, Philippines, Malaysia and Singapore) and Indonesia (Kalimantan and Sulawesi) (Drew & Romig, 2013). The species has also been detected in 1984 in Yonaguni Island (an island in the southwest of Japan facing Taiwan) (Ishida *et al*., 2005) and has spread to the entire island (Shimizu *et al*., 2007).

In 1983, *B. latifrons* was recorded from Hawaii (Vargas & Nishida, 1985). For the continental United States of America, a few isolated outbreaks have been reported in California and eradicated (EPPO, 2018).

In Africa, *B. latifrons* was detected in three countries: Tanzania in 2006 (Mwatawala *et al*., 2007), Kenya in 2007 (De Meyer *et al*., 2012) and Burundi in 2016 (on *Solanum aethiopicum*; L. Ndayizeye, 2018, pers. comm.). In Europe, the solanum fruit fly is regularly detected during import inspections (EPPO 2016a, 2017a).

B. latifrons was recommended for regulation by EPPO in 2017 and the most likely areas at risk of establishment outdoors in the EPPO region are the Mediterranean Basin, Portugal and the south of the Black Sea coast (EPPO, 2017b).

Additional information on the distribution and biology of the pest can be found in EPPO/CABI (1997) and EPPO (2018).

2. Identity

Name: *Bactrocera (Bactrocera) latifrons* (Hendel, 1915).
Common Name: Solanum fruit fly.
Synonyms: *Chaetodacus latifrons* Hendel, *Chaetodacus antennalis* Shiraki (Thompson, 1998) and *Dacus* (*Strumeta*) *latifrons* (Hendel) (Hardy & Adachi, 1954). *Dacus parvulus* Hendel is often cited as a synonym but Drew & Romig (2013) restated it as separate species with morphological criteria.
Taxonomic position: Diptera Brachycera Tephritidae Dacinae Dacini [nomenclature and taxonomy suggested by Fauna Europaea (https://fauna-eu.org/) are used as the reference].
EPPO Code: DACULA.
Phytosanitary categorization: EPPO A1 List no. 404.

3. Detection

Fruit flies are mostly detected as larvae in fruits.[2] Holes are visible on the fruits. Eggs might be found inside the fruit at the point where oviposition puncture marks are visible on

[1]Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

[2]In the biological sense.

the surface. Larvae will leave the fruits to pupate, and so consequently puparium may also be detected in packaging.

The only male attractants known are Latilure (α-ionol/α-ionone) but without a good efficacy (NAPPO, 2006; Ishida *et al*., 2008) McQuate, Keum, Sylva, Li, & Jang (2004) suggests using latilure in combination with cade oil as the latter would enhance the attractiveness of the former. However, Mziray *et al*. (2010) compared this combined lure with protein bait in Tanzania and found protein bait to be more attractive still.

Larvae can be reared to the adult stage for species identification. Rearing of larvae is described in White & Elson-Harris (1992). A presumptive diagnosis may be feasible on the 3rd instar (Balmès & Mouttet, 2017) and molecular tests can also be performed on larvae (see section 4.2).

If collected larvae are to be preserved for morphological identification, they should be placed in boiling water for a few seconds (until it becomes immobile). It should then be transferred to either 70% ethanol for use for morphological identification or to 95% ethanol for use for molecular tests. Other procedures can be used.

Adults collected on traps can be used for identification.

4. Identification

Identification is commonly based on the examination of adult specimens. A protocol for DNA barcoding based on the COI gene is described in PM 7/129 DNA *barcoding as an identification tool for a number of regulated pests* (EPPO, 2016b) and can be used in support of identification for all life stages.

4.1. Morphological identification

Morphological examination requires a stereo microscope with 10× magnification for external examination of the adult to 200× for examination of the larvae (for the preparation of the larvae see Appendix 1 Part A) and of the adult female's aculeus (for the preparation of the aculeus see Appendix 1 Part B). A reliable morphological identification to species level can only be made by examination of an adult specimen (either male or female) using the key presented in Table 1. A description of the larvae is also provided and may allow a presumptive diagnosis (see section 4.1.1). Definitions and illustration of terms used and not specifically defined and illustrated in this protocol can be found in White & Elson-Harris (1992).

4.1.1. Larva

A key for the 3rd-instar larvae is available in White & Elson-Harris (1992). This key can be used for identification to the genus level, but it should be noted that several *Ceratitis* larvae may also be assigned to the *Bactrocera* genus using this key.

Examination of the 3rd-instar larvae in combination with knowledge about the origin and the host, as well as the evidence provided by previously identified specimens from earlier and similar consignments, may allow a presumptive diagnosis (Balmès & Mouttet, 2017).

Table 1. Identification of the adult of *B. latifrons* [(after White & Elson-Harris (1992), Kapoor (1993) and Drew & Romig (2016)] (This simplified key will allow the distinction of *B. latifrons* from most species of agronomic and/or economic importance)

1	Subcostal vein abruptly bent and dorsal side of vein R1 with setulae (Fig. 9)	Tephritidae 2
	Subcostal vein not abruptly bent or dorsal side of vein R1 lacks setulae	Other families
2	Abdominal segments not fused (Fig. 10)	3
	Abdominal segments fused	*Dacus*
3	Scutellum not bilobed and with 2 apical setae (Fig. 8)	4
	Scutellum bilobed	Other species
4	Scutum with prescutellar acrostichal and anterior supra-alar setae (Fig. 6), and without medial orange and/or yellow vitta. Male with pecten on tergite 3 (Fig. 10)	*Bactrocera (Bactrocera) subgenera*
		5
	Scutum different	Other subgenera
5	Mesonotum with lateral postsutural yellow vittae (Fig. 6). Head with black markings (Fig. 5)	6
	Mesonotum without lateral postsutural yellow vittae	Other species
6	Face with black spot in each antennal furrow (Fig. 5)	7
	Face with transverse dark markings	*B. correcta*
7	Wing without any cross band (Fig. 9)	8
	Wing different	Other species
8	Wing with a distinct costal band from the end of vein Sc to just beyond the end of vein R4+5 (Fig. 9). Scutellum entirely pale coloured, except sometimes for a narrow black line across the base (Fig. 8)	9
	Wing and scutellum different	Other species
9	Apex of costal band distinctly expended into a spot (Fig. 9). Thorax predominantly black (Fig. 6) but not laterally (Fig. 7). Abdomen predominantly red-brown, but very variable (Fig. 10). Aculeus with apex trilobed and around 1.7 mm length (Fig. 11)	*Bactrocera latifrons*
	Apex of costal band different. Thorax, abdomen different coloured	Other species

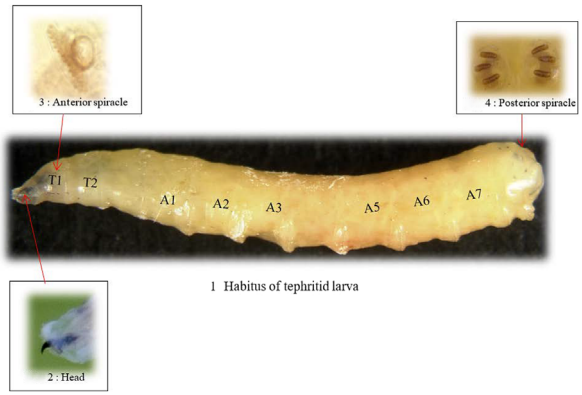

Fig. 1 Habitus of tephritid larva. (detail 2 - Head, detail 3 - Anterior spiracles, detail 4 - Posterior spiracles)

4.1.1.1. Description of a tephritid larva after Smith (1989) and Stehr (1991).

- Body cylindrical and rounded with a small tapering head, 3 thoracic and 8 abdominal segments (Fig. 1).
- Head without sclerotization, but with the cephalopharyngeal skeleton partially visible by transparency (Fig. 1, detail 2).
- Anterior spiracle in dorsolateral position on each side of the first thoracic segment (Fig. 1, detail 3).
- Posterior spiracle on the surface of the last segment of the abdomen, not elevated from the body in fresh specimens (alcohol-preserved specimens dehydrate and may appear to have elevated posterior spiracle). Peritreme unpigmented and without spine or lobe.
- 2 posterior spiracles with 3 spiracular openings or slits, arranged more or less parallel to each other (Fig. 1, detail 4).

4.1.1.2. Partial description of 3rd-instar larva of Bactrocera latifrons. Medium-sized, length 7.0–8.5 mm, width 1.2–1.5 mm (Fig. 1).

Head: Antenna 2 segmented. Oral ridges present with 9–14 rows. 6–10 small accessory plates present.

Cephalopharyngeal skeleton (Fig. 2): mouthhooks stout, with apex relatively rounded but without preapical tooth; presence of a hole in mouthhook (localization indicated by a red circle in Fig. 2); dental sclerite present; parastomal bars elongate, free from hypopharyngeal sclerite.

Anterior spiracles: elevated, sometimes concave medially, with 13–18 tubules (usually at least 14 tubules) in a single row (Fig. 3).

Thoracic and abdominal segments: A broad, encircling, anterior band of discontinuous rows of small spinules surrounding each thoracic segment. T1 with 6–10 rows of small, sharply pointed spinules; T2 and T3 with 3–7 rows of small spinules decreasing laterally. A8 with intermediate areas large and sensilla well developed. (Visible with SEM or phase contrast.)

Anal area: Large lobes, protuberant, surrounded by 3–6 rows of small, sharply pointed spinules, becoming more concentrated and stouter below anal opening. (Visible with SEM or phase contrast.)

Posterior spiracles: Each spiracular slit about 3 times as long as broad. Spiracular hairs broad, flat; dorsal and ventral bundles of 16–22 hairs branched in apical third to a quarter; lateral bundles of 6–11 hairs. (SEM or phase contrast is required to observe these characters.)

4.1.2. Adults

Characters to identify the subgenus *Bactrocera (Bactrocera)* are presented in section 4.2.1 of the IPPC Diagnostic Protocol (IPPC, 2019).

4.1.2.1. Description of the adult [after Carroll et al. (2002), De Meyer & White (2004) and Drew & Romig (2013)]. The specimen may be observed in alcohol or dry. Observation of colours is best achieved on specimens in ethanol as colours are more contrasted.

Adult appears bicolored: thorax predominantly dark and abdomen predominantly red-brown (Fig. 4). For detailed descriptions see below.

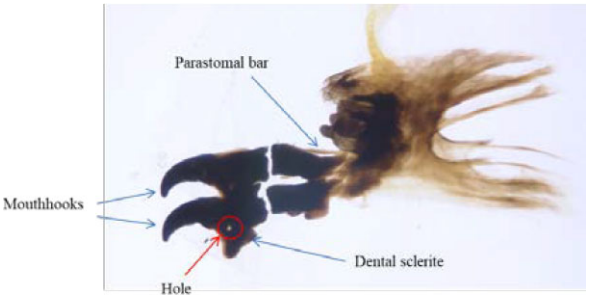

Fig. 2 Larva: cephalopharyngeal skeleton.

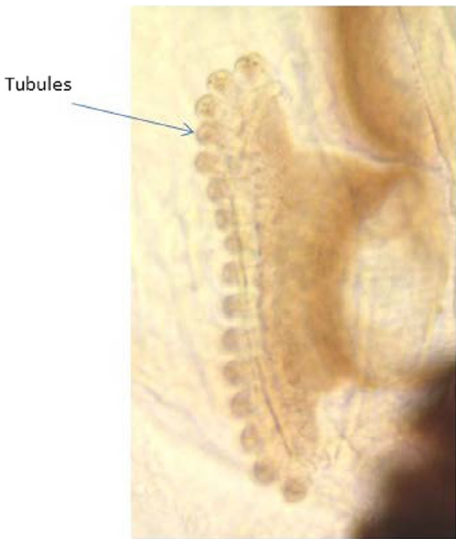

Fig. 3 Larva: left anterior spiracle.

Head: (Fig. 5) Height 1.5 mm. Higher than long. Chaetotaxy reduced: frontal setae 2 pairs. Orbital setae 1 pair reclinate, acuminate. Ocellar seta absent or minute like setulae. Postocellar seta hardly detectable. Large oval dark spots in each antennal furrow. Antenna longer than face. First flagellomere rounded apically. Arista longer than first flagellomere, bare or with short hairs, distinctly shorter than greatest aristal width.

Fig. 4 Adult: male habitus.

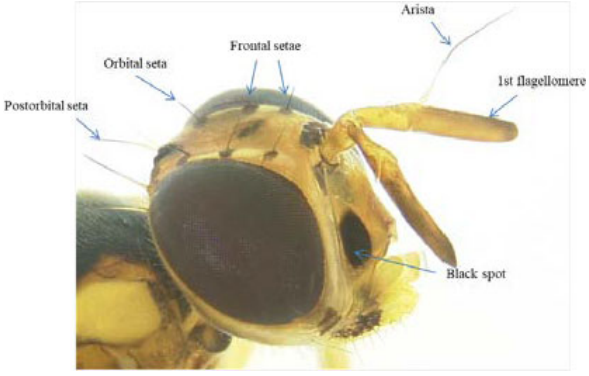

Fig. 5 Adult: head.

Thorax: Anterior notopleural seta present. Presutural supra-alar seta absent. Anterior supra-alar seta present. Posterior supra-alar seta present. Prescutellar acrostichal seta present. Scutum predominantly black. Postpronotal lobe yellow. Notopleural callus yellow. Lateral post sutural vittae present. Medial post-sutural vitta absent (Fig. 6). Anepisternum with a stripe from notopleural callus to (or almost to) katepisternum, extended onto katepisternum; stripe very broad (anteriorly extending to, or almost to, postpronotal lobe). Both anatergite and katatergite with a simple xanthine across (Fig. 7).

Scutellum yellow except a basal dark line. Apical scutellar setae present (Fig. 8).

Legs: Femora all entirely of one colour, or at least one femur markedly darker in apical part than basal part. Dark mark on fore and middle femur 0–30% of length of femur. Dark mark on hind femur 0–20% of length of femur (Carroll *et al.*, 2002).

Wings (Fig. 9): Length 4.4–6.1 mm. Cells bc and c hyaline and without a complete covering of microtrichia. Cell bm without microtrichia. Costal band dark, extending from cell sc to beyond R4+5. Apex of costal band distinctly expended into a spot. Vein R2+3 generally straight. Crossvein r-m hyaline. Crossvein dm-cu hyaline. Wing without cross band. Cross vein r-m meeting anterior border of cell dm beyond middle of cell. Distance between crossvein r-m and costa shorter than r-m. Anal band present, reaching wing margin along cell cup extension.

Abdomen: Abdomen ovate or parallel sided. Abdominal tergites separate. Abdomen in lateral view arched, dome-like, rather rigid. Abdominal tergite 1 broader at apex than at base, without a prominent hump laterally. Male with pecten (comb of setae) on tergite 3 (Fig. 10). Abdominal tergites T3–T5 predominantly orange-brown or red-brown, with or without black pattern. Large variability has been observed in some populations with very distinct and broad dark markings on the abdomen (Doorenweerd & Leblanc, in prep.). T2 lighter than T3–T5, mostly whitish to yellowish.

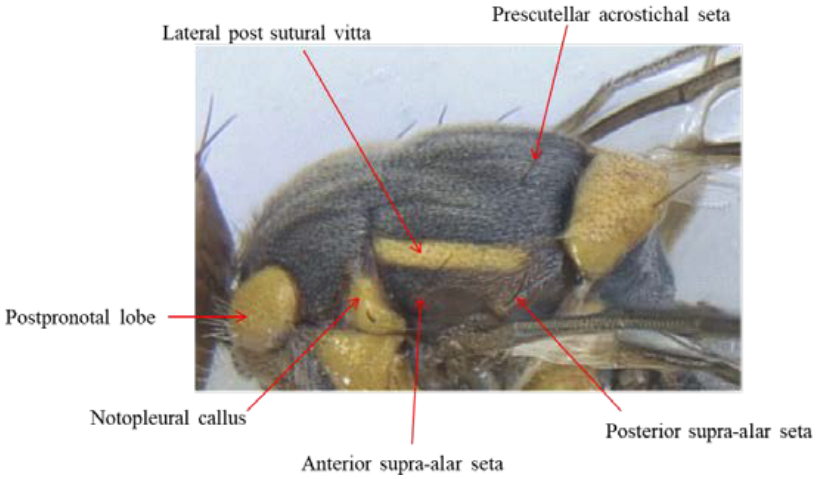

Fig. 6 Adult: thorax (dry).

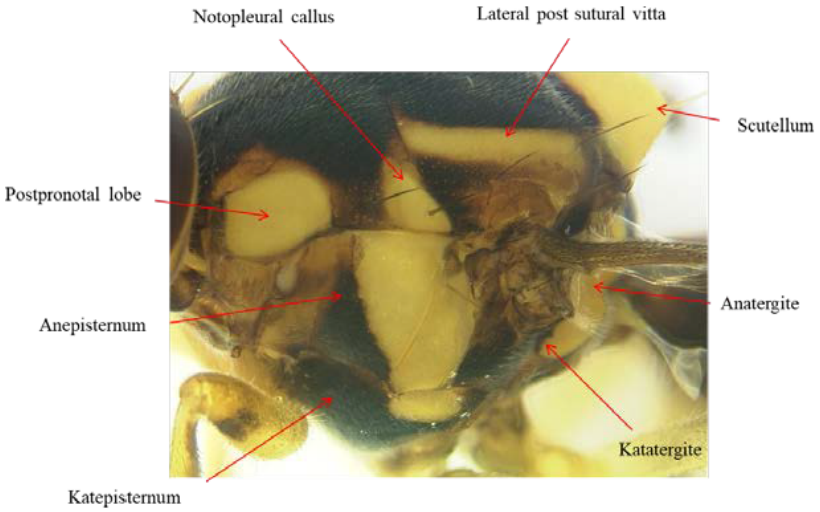

Fig. 7 Adult: thorax, lateral view (in alcohol).

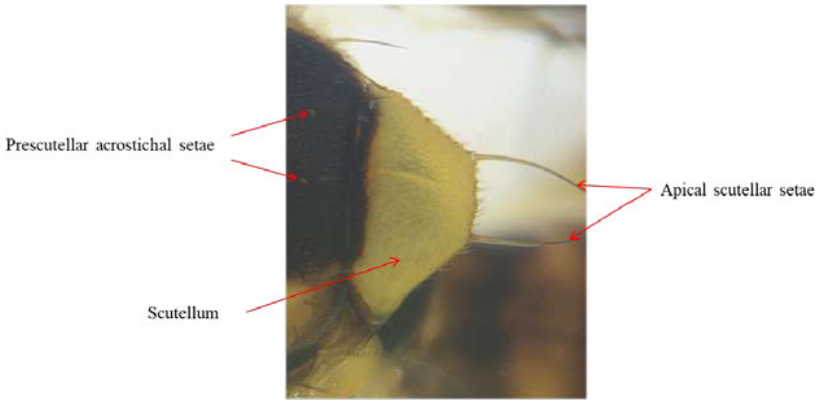

Fig. 8 Adult: scutellum.

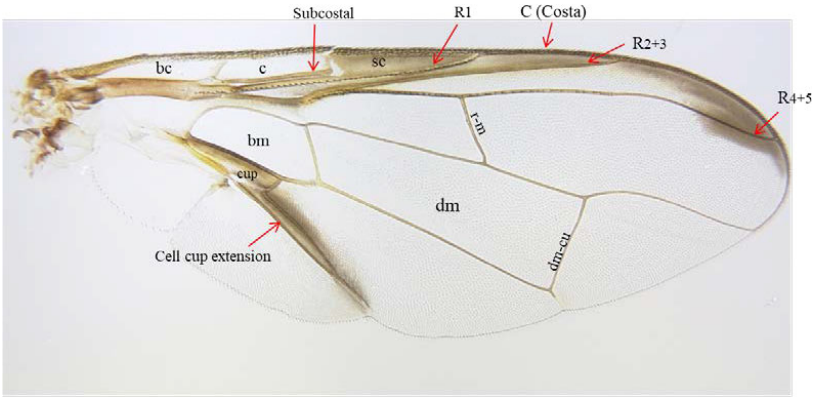

Fig. 9 Wing.

Female: Aculeus length 1.4–1.7 mm with apex trilobed (Fig. 11).

4.1.2.2. Key to adults. For identification of the Family Tephritidae see Oosterbroek (2006).

Characters to identify the subgenus *Bactrocera (Bactrocera)* are presented in section 4.2.1 of the IPPC Diagnostic Protocol (IPPC, 2019). The key for adult identification in Table 1 will allow the distinction of *B. latifrons* from most species of agronomic or/and economic importance.

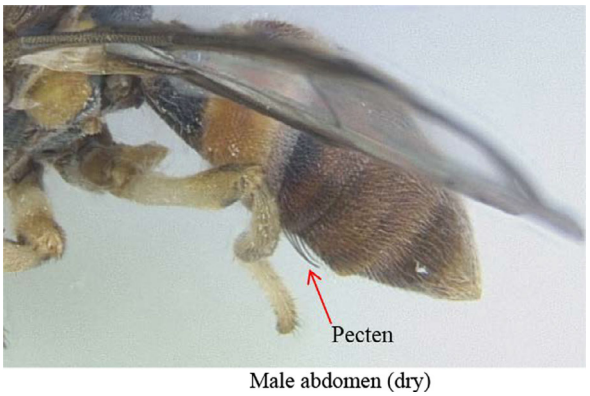

Fig. 10 Male abdomen (dry).

4.2. Molecular methods: sequencing

A protocol for DNA barcoding based on COI is described in Appendix 1 of PM 7/129 DNA *barcoding as an identification tool for a number of regulated pests:* DNA barcoding Arthropods (EPPO, 2016b) and can support the identification of *Bactrocera latifrons*.

Sequences are available in different databases such as http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxon=bactrocera+latifrons&searchTax=Search+Taxonomy

For African species sequences are available in http://projects.bebif.be/fruitfly/index.html/.

A real-time PCR for the identification of *Bactrocera latifrons* by real-time PCR using SYBR Green chemistry was published in 2014 (Yu *et al*., 2004), but there is so far limited experience with this test in the EPPO region.

5. Reference material

Links to African specimens are available at http://projects.bebif.be/fruitfly/taxoninfo.html?xml:id=371 or http://fruitflykeys.africamuseum.be/en/pdf_keys.html.

6. Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

7. Performance criteria

When performance criteria are available, these are provided with the description of the test. Validation data are also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int) and it is recommended that this database is consulted as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

8. Further information

Further information on this organism can be obtained from:

V. Balmès. ANSES - LSV– Unité d'Entomologie et Plantes Invasives, 755 avenue du campus d'Agropolis CS30016, 34988 Montferrier sur Lez (FR). E-mail: valerie.balmes@anses.fr**.**

9. Feedback on this diagnostic protocol

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this protocol that you wish to share, please contact diagnostics@eppo.int.

10. Protocol revision

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

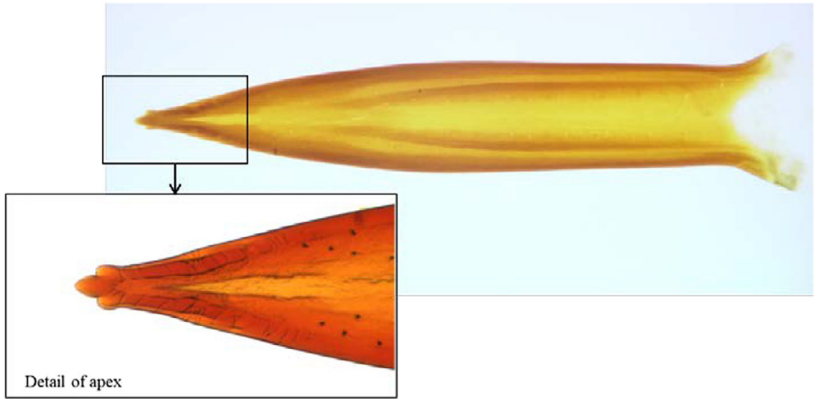

Fig. 11 Female aculeus.

Acknowledgements

This protocol was originally drafted by Ms V. Balmès, ANSES - LSV– Unité d'Entomologie et Plantes Invasives, 755 avenue du campus d'Agropolis CS30016, 34988 Montferrier sur Lez (FR). E-mail: valerie.balmes@anses.fr.

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Appendix 1

Part A Preparation of larvae for observation using a stereo microscope and compound microscope with 100× magnification (Balmès & Mouttet, 2017)

(1) Cut the anterior part of the larva with fine scissors or pins and place it in a 10% potassium solution for 1 h at room temperature or 15–20 min at between 60 and 80°C.

(2) Put the larva in distilled water and flatten the body contents by gentle pressure with a spatula (use a mandrel with flattened fishing thread).

(3) Transfer the larva into clean distilled water for several minutes.

(4) The larva can be mounted on a slide in a drop of glycerol with a cover slip or prepared for permanent mounting.

Part B Preparation of aculeus for examination using a stereo microscope and compound microscope with 200× or 400× magnification

Break off the abdomen of the female and place it in a 10% potassium solution, 1 hour at room temperature or 20–30 minutes at between 60 and 80°C.

When the abdominal sclerites are smooth enough, remove them, leaving only the aculeus. Use a pin to separate the aculeus and take care to not damage the tip of the aculeus.

Transfer the aculeus to distilled water for several minutes and mount it on a glass in a drop of glycerol with a cover slip.