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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM7/134 (1)

Diagnostics
Diagnostic

PM 7/134 (1) *Dacus ciliatus*

Specific scope

This Standard describes a diagnostic protocol for *Dacus ciliatus*.[1]

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

Specific approval and amendment

Approved in 2018–09.

1. Introduction

Dacus ciliatus is a serious pest of Cucurbitaceae including *Cucumis melo*, *Cucumis metuliferus*, *Cucumis sativus*, *Cucurbita maxima*, *Citrullus lanatus*, *Citrullus colocynthis*, *Coccinia grandis*, *Momordica balsamina*, *Momordica charantia*, *Trichosanthes cucumerina*, *Lagenaria siceraria*, *Lagenaria aegyptiaca*, *Luffa acutangula*, *Sechium edule* (White & Elson-Harris, 1992) and *Benincasa* sp. (Anses, LSV pers. comm., 2016). For a complete list of Cucurbitaceae hosts in Africa see White (2006). Some species of records reported in Munro (1984) on Leguminosae (*Phaseolus*) and Malvaceae (*Gossypium*) need to be reconfirmed and are probably based on erroneous data.

Dacus ciliatus is widespread in Africa and Asia. Details on its distribution are available in the EPPO Global Database (EPPO, 2018). This species has the potential to establish outdoors in the Mediterranean area, as well as in protected cultivation in other areas. It should be noted that in 2015 *Dacus frontalis*, another African pest of Cucurbitaceae closely related to *D. ciliatus*, was detected in Tunisia (Hafsi *et al.*, 2015), and it is consequently important to be able to identify these two species and discriminate between them.

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

2. Identity

Name: *Dacus* (*Didacus*) *ciliatus* Loew, 1862

Common name: Ethiopian fruit fly, lesser pumpkin fly or cucurbit fly

Synonyms: *Dacus sigmoides* Coquillett, *Dacus brevistylus* Bezzi, *Dacus apoxanthus* var. *decolor* Bezzi, *Dacus insistens* Curran, *Dacus cocciniae* Premlata & Singh and *Tridacus mallyi* Munro (Thompson, 1998), *Didacus brevistylus* (Bezzi), *Didacus ciliatus* (Loew), *Leptoxyda ciliata* (Loew), *Dacus sexmaculatus* Walker (White & Elson-Harris, 1992) (White, 2006).

Taxonomic position: Diptera, Brachycera, Tephritidae, Dacinae, Dacini

Nomenclature and taxonomy suggested by Fauna Europaea are used as the reference

EPPO Code: DACUCI

Phytosanitary categorization: EPPO A2 List No 238

3. Detection

Fruit flies are mostly detected as larvae in fruits. Holes are visible on the fruits. Eggs might be found inside the fruit at the point where an oviposition puncture is visible on the surface. Larvae will leave the fruits to pupate, and so consequently pupae may also be detected in packaging.

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 for the third instar (see 4.1.1) and molecular tests can also be performed on larvae (see 4.2).

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

Adults collected on traps can be used for identification.

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

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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, 2016) and can be used for all life stages.

4.1 Morphological identification

Morphological examination requires a stereo microscope with a magnification of ×10 for external examination of the adult to ×200 for examination of the larvae (for the preparation of the larvae see part A of Appendix 1) and of the aculeus of the adult female (for the preparation of the aculeus see part B of Appendix 1). 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 4.1.1). Definitions and illustration of terms used but not specifically defined and illustrated in this protocol can be found in White & Elson-Harris (1992).

4.1.1. Larvae

A key for third instar larvae is available in White & Elson-Harris (1992). This key allows identification to the genus level, but not discrimination between different species.

Examination of third 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 (Balmes & Mouttet, 2017).

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. 2);

Anterior spiracle in a lateral position on each side of the first thoracic segment (Fig. 3);

Posterior spiracle on the surface of the last segment of the abdomen, unpigmented and without spine or lobe;

Two posterior spiracles with 3 spiracular openings or slits, arranged more or less parallel to each other (Fig. 4).

4.1.1.2. Partial description of third larval instar of Dacus ciliatus (after White & Elson-Harris, 1992 and Carroll et al., 2004). Medium to large, length 9.0–10.5 mm, width 1.5–2.0 mm.

Head: antenna 2 segmented. Oral ridges present with 12–13 rows, some branched.

Cephalopharyngeal skeleton (Fig. 5): mouthhook with a stout pre-apical tooth; dental sclerite present; parastomal bars elongate, free from hypopharyngeal sclerite.

Anterior spiracles: elevated, with 14–16 tubules in a single uniform row (Fig. 6).

Thoracic and abdominal segments: rows of spinules encircling anterior portion of segment T1, T3 and segments A1 and A8 with 2 pairs of small tubercles and sensilla.

Anal area: lobes small, surrounded by small spinules.

Posterior spiracles: spiracular slits 3.5–4.0 times as long as broad; spiracular hairs short, less than half the length of a spiracular slit, with 4–19 hairs per bundle.

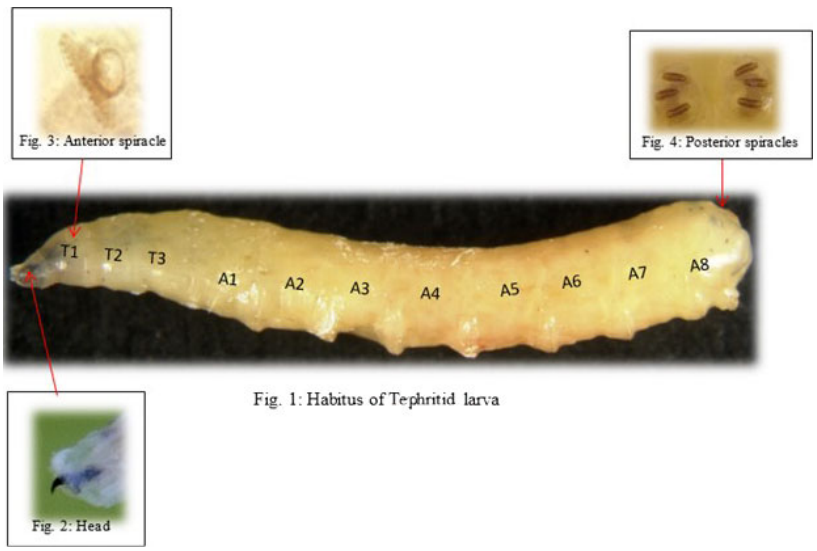

Fig. 1-4 Habitus of tephritid larva. Head. Anterior spiracle. Posterior spiracles.

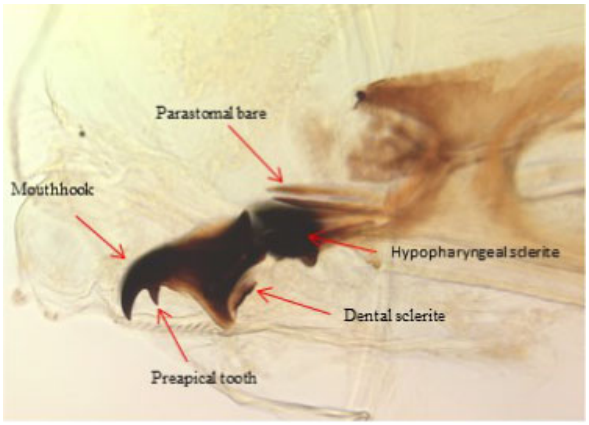

Fig. 5 Larva – cephalopharyngeal skeleton.

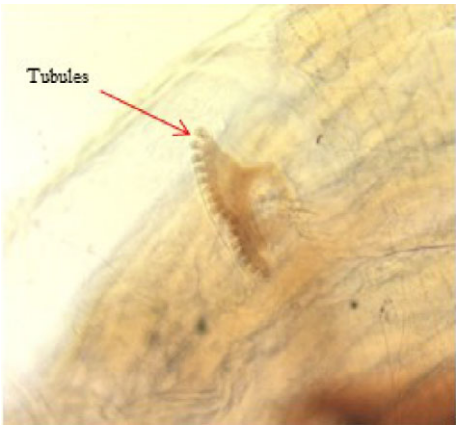

Fig. 6 Larva – anterior spiracle.

4.1.2. Adults

4.1.2.1 Description of the adult (after Munro, 1984; Drew et al., 1998; Drew & Romig, 2013 and Carroll et al., 2002 and White, 2006). Predominantly pale orange-brown to red-brown in colour (Fig. 7).

Fig. 7 Male habitus.

Head—Structure: height 1.3 mm, higher than long; antenna long; first flagellomere elongate, rounded apically (Fig. 8); arista longer than first flagellomere, bare or with short rays.

Chaetotaxy: ocellar seta absent or minute setula-like; post-ocellar seta absent; frontal setae 1–2 pairs or 3 pairs (anterior tends to be minute and posterior tends to disappear); orbital setae 0–1 pair, orbital seta reclinate, acuminate.

Coloration: face yellow with moderate dark round spots in each antennal furrow (Fig. 8).

Thorax—Chaetotaxy: anterior supra-alar setae absent; intra-alar seta well developed, similar to post-alar seta; prescutellar acrostichal seta absent; anterior notopleural seta present or absent (Fig. 9); posterior notopleural seta present; one pair of scutellar setae present apically (Fig. 10).

Coloration: scutum orange-brown, or red-brown; without central or lateral vittae.

Pleura red-brown without dark markings; post-pronotal lobe whitish or yellowish; posterior half of notopleuron whitish or yellowish; distinct pale vertical anepisternal stripe extending halfway between posterior half of notopleuron and anterior notopleural seta (Fig. 9). Katatergite with distinct whitish or yellowish spot.

Scutellum densely setulose; yellow brownish (at most with a narrow dark basal line). Setulae on scutellum short, decumbent; unicolorous (clearer than the scutellum), acuminate (Fig. 10).

Fig. 8 Head.

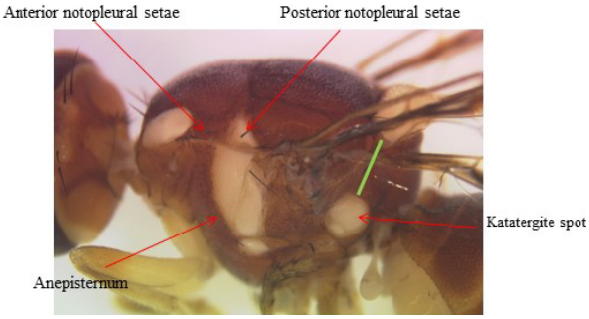

Fig. 9 Lateral view of thorax.

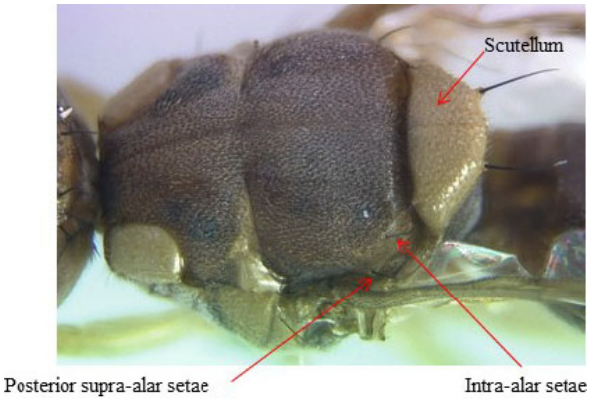

Fig. 10 Scutum and scutellum.

Abdomen: abdomen ovate or parallel sided. Abdominal tergites fused (Figs 11, 12 and 16). Tergites 1 and 2 red-brown. Posterior margin of tergite 2 greyish beige. Tergites 3–5 red-brown with usually a moderate round black spot on the anterior margin of tergite 3 (Fig. 16 yellow circle). Male with pecten of dark setae on tergite 3 (Fig. 12). Medial T-shaped mark absent (see Fig. 16).

Fig. 11 Abdomen (female).

Fig. 12 Abdomen (male).

Aculeus: pointed, apex tapered evenly, length 1.5–1.6 mm (Fig. 13).

Legs: femora slender. Fore femur without setae and without ventral spines. Mid femur and hind femur without spine-like setae. Mid tibia with apical spur. Male and female femora pale; or female mid- and hind femora tending to bicoloured (pale basally, reddish-brown apically)

Wings: (Fig. 14) length 4.4–6.0 mm. Wing pattern mostly brownish. Costal band dark extending from cell sc beyond vein R_{4+5} but not to vein M. Apex of the costal band distinctly expanded into a spot. Anal band present, extending nearly to the wing margin along cell cu*p* extension. Vein R_1 dorsal setation without a bare section on the opposite end of vein Sc. Cell bc without microtrichia and in cell c microtrichia present only apically. Cell bm broad, parallel sided; ratio of length to width 2; ratio of cell bm width to cell *cup* width 2. Vein M anterodistally curved. Posterodistal corner of cell dm distinctly acute, or approximately at a right angle. Cell *cup* extension very long, equal or longer than length of vein A_1+CuA_2.

4.1.2.2. Key to adults. For identification of the family Tephritidae see Oosterbroek, 2006

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, 2016) and can support the identification of *Dacus ciliatus.* Sequences are available in different databases; those in Q-bank are curated (http://www.q-bank.eu/arthropods/). For African species sequences are available in http://projects.bebif.be/fruitfly/index.html

5. Reference material

Links to specimens are available in Q-bank (http://www.q-bank.eu/arthropods/) and http://projects.bebif.be/fruitfly/index.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 is also

Table 1. Simplified key for the identification of adult *Dacus ciliatus* (after White & Elson-Harris, 1992; Drew *et al.*, 1998 and Drew & Romig, 2013) and separation from the most commonly related species detected at import (for a complete key see White, 2006)

1	Subcostal vein bent at right angles toward the costa and dorsal side of vein R1 with setulae (Fig. 14)	Tephritidae 2
	Subcostal vein not abruptly bent or dorsal side of vein R1 lacks setulae	Other families
2	Abdominal segments fused* (Fig 16). Male usually with a pecten (Fig. 12)	Genus *Dacus* 3
	Abdominal segments not fused (Fig 16)	Other Tephritidae
3	Abdominal tergites 1+2 broader than long. Apex of the antenna reaching or going beyond the lower margin of the face (Fig. 8)	4
	Abdominal tergites 1+2 longer than broad, giving a strongly wasp-waisted appearance. Apex of the antenna reaching at maximum the lower margin of the face	Other Tephritidae
4	Scutum without anterior supra-alar seta (Fig. 9)	Subgenus *Didacus* 5
	Scutum with anterior supra-alar seta	Other Subgenus
5	Scutum without any yellow or orange vittae (Fig. 9)	6
	Scutum with medial and/or lateral yellow vittae (Fig. 15)	Other species
6	Posterolateral area of thorax with a yellow spot in front of the halter base, which is virtually confined to the katatergite. Spot separated from scutellum by at least its own diameter (Fig. 10, green line)	*Dacus ciliatus*
	Posterolateral area of thorax with a diagonal yellow stripe ventral to the scutellum which extends across both the katatergite and anatergite. Stripe only separated from the scutellum by about 1/3 its length (Fig. 17)	7
7	Mid femora yellow in basal half, orange in apical half; fore and hind tibia entirely yellow	*Dacus frontalis*
	All femora yellow in basal half, orange in apical half.	*Dacus vertebratus*

*This character is difficult to observe and requires experience. Figure 16 is provided to illustrate this character.

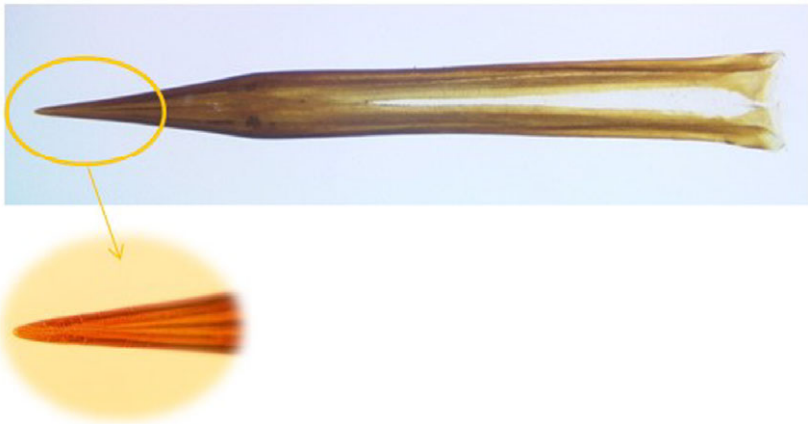

Fig. 13 Aculeus and detail.

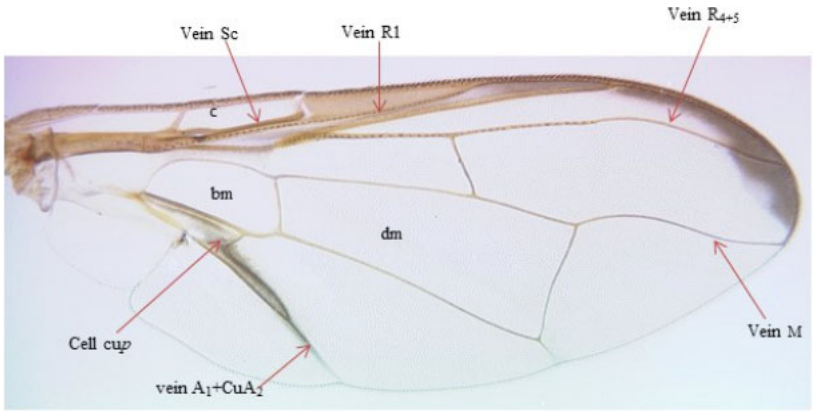

Fig. 14 Wing.

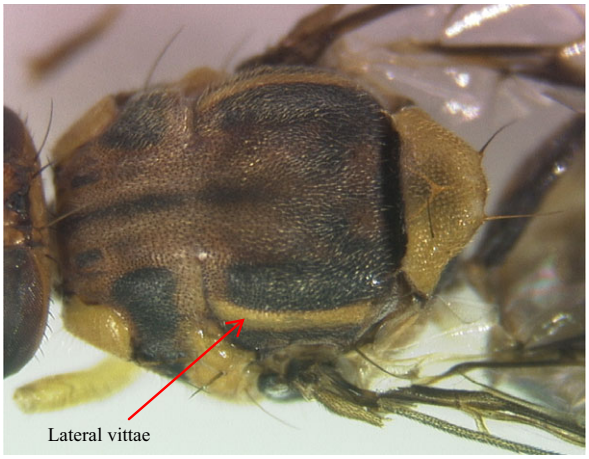

Fig. 15 Scutum and scutellum of *Bactrocera dorsalis*.

Fig. 16 Abdominal tergites not fused or fused black spot on T3 (yellow circle).

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, France. 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.

Acknowledgements

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

It was reviewed by the Panel on Diagnostics in Entomology.

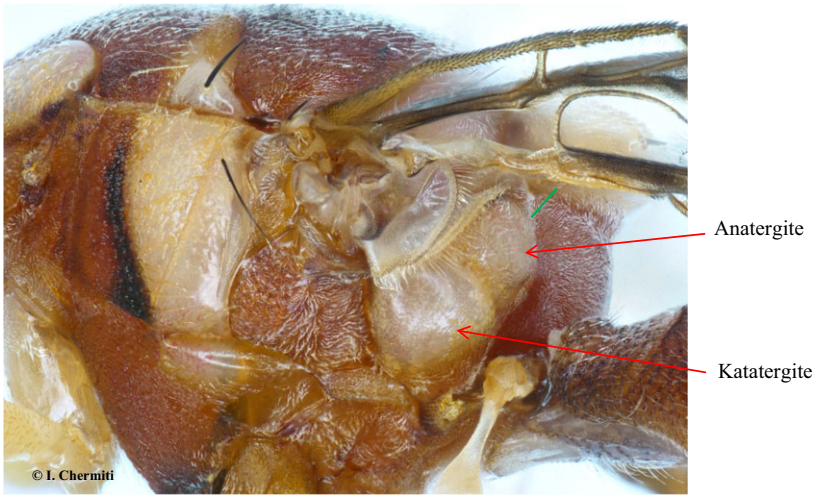

Fig. 17 *Dacus frontalis* – lateral view of thorax.

Fig. 17 was provided by Ibrahim Chermiti, High Agronomic Institute of Chott-Mariem, University of Sousse, 4042 Chott-Mariem, Sousse, Tunisia.

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Appendix 1

A: preparation of larvae for observation using a stereo microscope and compound microscope with ×100 magnification (Balmes & 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°C 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.

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

(1) Break off the abdomen of the female and place it in a 10% potassium solution for 1 h at room temperature or 20–30 min at between 60°C and 80°C;
(2) 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;
(3) Transfer the aculeus to distilled water for several minutes and mount on a glass slide in a drop of glycerol with a cover slip.