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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/109 (2)

Diagnostics
Diagnostic

PM 7/109 (2) *Epitrix cucumeris, Epitrix papa, Epitrix subcrinita, Epitrix tuberis*

Specific scope

This Standard describes a diagnostic protocol for adults of *Epitrix cucumeris*, *Epitrix papa, Epitrix subcrinita* and *Epitrix tuberis*.

Specific approval and amendment

First approved in 2011-09.
Revision approved in 2016-01.

1. Introduction

Epitrix subcrinita, *Epitrix tuberis* and *Epitrix cucumeris* are flea beetles belonging to the North American species of *Epitrix* developing on *Solanum tuberosum* (Solanaceae). *Epitrix cucumeris* is established in the Azores Islands (Portugal) where it was collected for the first time in Faial Island around 1979 (Boavida & Germain, 2009). In mainland Portugal, *E. cucumeris* was detected with another species new to science, *Epitrix papa*, in approximately 2004 when unusual damage to potato crops was observed (Oliveira *et al.*, 2008). *Epitrix papa* was initially misidentified as *Epitrix similaris*, another North American species (Orlova-Bienkowskaja, 2015). Both species have also been detected in Spain. To date, *E. tuberis* and *E. subcrinita* have not been detected in the EPPO region.

Nine species of *Epitrix* were recorded as present in the EPPO region before these detections (Warchalowski, 2003), these are *Epitrix abeillei, Epitrix allardi, Epitrix atropae, Epitrix caucasica, Epitrix dieckmanni, Epitrix hirtipennis, Epitrix intermedia, Epitrix priesneri* and *Epitrix pubescens*. Until recently, among the seven *Epitrix* species present in Europe (Warchalowski, 2003) none was causing damage on *S. tuberosum*, although *E. hirtipennis,* known as a pest of tobacco in Italy (Sannino *et al.*, 1984), is occasionally recorded on potato. This species also has a North American origin. Two other species which are present in the non-European part of the EPPO region, *Epitrix allardi* and *Epitrix priesneri*, are not covered in this diagnostic protocol.

Further information on the host range, geographical distribution and biology of the four species recommended for regulation can be found in the EPPO Global Database (https://gd.eppo.int/). This protocol focuses on detection of *Epitrix* species in potato.

2. Identity

Name: *Epitrix cucumeris* (Harris)
Taxonomic position: Coleoptera: Chrysomelidae: Alticinae
EPPO code: EPIXCU
Phytosanitary categorization: EPPO A2, EU Commission decision 2016/1359/EU

Name: *Epitrix papa* Orlova-Bienkowskaja
Taxonomic position: Coleoptera: Chrysomelidae: Alticinae
EPPO code: EPIXPP
Phytosanitary categorization: EPPO A2, EU Commission decision 2016/1359/EU

Name: *Epitrix subcrinita* (LeConte)
Taxonomic position: Coleoptera: Chrysomelidae: Alticinae
EPPO code: EPIXSU
Phytosanitary categorization: EPPO A1, EU Commission decision 2016/1359/EU

Name: *Epitrix tuberis* Gentner
Taxonomic position: Coleoptera: Chrysomelidae: Alticinae
EPPO code: EPIXTU
Phytosanitary categorization: EPPO A1, EU Commission decision 2016/1359/EU

3. Detection

Symptoms on potato

Adults of *Epitrix* species may be found on all aboveground parts of the plant as well as on the soil surface.

They mainly feed on the upper surface of leaves, and less often on the lower surface. Adult beetles cut characteristic shot-like holes (1.0–1.5 mm in diameter) (Fig. 1).

Epitrix cucumeris: larvae inhabit the soil around potato roots; occasionally they may enter the tubers, leaving roughened trails or tiny tunnels filled with corky tissue.

Epitrix papa and *Epitrix tuberis*: larvae are more injurious, affecting potato tubers which then show long sinuous corky lesions and small holes. These lesions are caused by larvae which feed under the epidermis, digging galleries that usually remain superficial and do not affect the flesh of the tuber (Fig. 2).

Epitrix subcrinita: adults can cause damage on foliage similar to that caused by other *Epitrix* species, However, there is a great uncertainty concerning tuber damage caused by larvae.

4. Identification

Identification is commonly based on the examination of adult specimens. A protocol for DNA barcoding based on the mitochondrial cytochrome *c* oxidase I (COI) gene is described in PM 7/129 *DNA barcoding as an identification tool for a number of regulated pests* (EPPO, 2016), and allows the identification of *E cucumeris, E. papa, E. subcrinita* and *E. tuberis*

Fig. 1 Shot-like holes on potato leaves (courtesy: Germicopa, FR).

Fig. 2 Potato tuber with corky lesions, small superficial warts and a larva (courtesy: Germicopa, FR).

4.1 Morphological identification

Morphological identification requires the examination of both male and female adults. A stereomicroscope and a compound microscope are needed for this purpose. For reliable identification the habitus of the female spermatheca and the male aedeagus should be examined. (See Appendix 1 for the preparation of male genitalia and female spermatheca.)

For a key to the families of Coleoptera see Lawrence *et al.* (2002).

For a key to the subfamilies of Chrysomelidae and the *Epitrix* genus see Warchalowski (2003).

Epitrix species (based on Doeberl, 2000):

Elytra have puncture rows, intervals are entirely covered with erect setae, the pronotum basally is slightly narrower than the elytral base and narrowed anteriorly. A pronotal ante-base transverse impression is developed and the two basal short longitudinal furrows are distinct. Procoxal cavities are closed posteriorly with the first abdominal sternite as long as following three combined.

4.1.1 Eggs

Minute, whitish, approximately elliptical (0.5 mm long) (see Neilson & Finlayson, 1953).

4.1.2 Larvae

Whitish, slender, cylindrical, mature larvae are about 5 mm long with a brown head (Fig. 2).

Fig. 3 Appearance of pupae of *Epitrix* sp. (courtesy: Boavida, INIAV, I.P.).

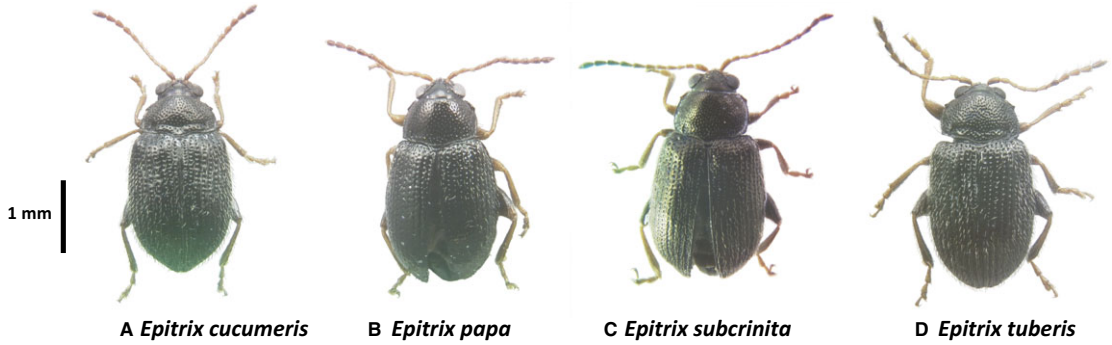

Fig. 4 Adult habitus.

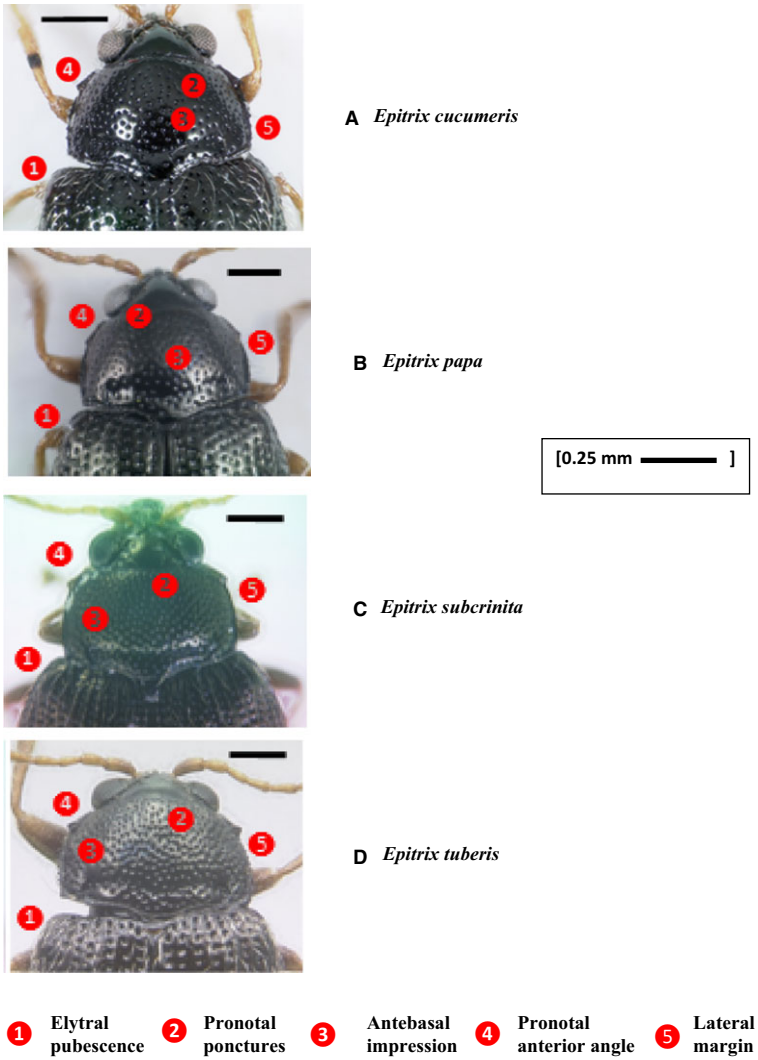

Fig. 5 Prothorax.

4.1.3 Pupa

The pupal stage is in the soil. Pupae are uniformly white, about 2.5 mm long and 1.5 mm wide across the mesothorax (Fig. 3).

4.1.4 Adults

Epitrix cucumeris (based on Gentner, 1944; Orlova-Bienkowskaja, 2015) (Fig. 4):

- Body length from 1.56 to 1.92 mm
- The upper side is always shiny black
- The antennae, tibia and tarsi are pale testaceous; femora are dark testaceous and often lighter towards the apex
- The pronotum shows a tight punctuation with well-marked points, separated by a distance greater than their diameter; anterior sides are angular, dentate, with rather denticulate edges. The ante-basal impression is well marked and deep (Fig. 5a). Elytra are often more densely pubescent than in the other three species
- The spermatheca is narrowed in its central part. It is slightly curved and the ductus is very long and forms a large curve very clearly detached (Fig 6a)
- The median lobe of the aedeagus is distinctly narrowed at the apex; its tip is spatulate (Fig. 7a).

Epitrix papa (based on Orlova-Bienkowskaja, 2015):

- Body length from 1.7 to 2.2 mm
- Dorsal body surface has a weak bronze reflection
- Antennae, tibia and tarsi are yellow to pale testaceous and the 11th antennomere is slightly darkened. Femora are dark, becoming lighter towards the apex
- A less tight pronotal punctuation than in the three other species. Punctures on the pronotum with an interval twice as wide as the diameter. Ante-basal impression is significantly less deep. Anterior angles of the pronotum are weakly dentate (Fig. 5b)
- Elytral hairs are sometimes a little less abundant and less dense than in the other three species. The sutural puncture row of elytra ends at the basal third of elytra length, and with a less tight pronotal punctuation. Points are separated by a distance greater than their diameter
- The central part of the spermatheca is strongly narrowed in its centre and the ductus is relatively short (Fig. 6b)
- The apex of the median lobe of the aedeagus is regularly narrowed, lancet-shaped and its tip is very shortened (Fig. 7b).

Epitrix subcrinita (based on Seeno, 1972, Deczynski, 2014):

- Body length from 1.76 to 2.27 mm
- Dorsal body surface generally of a dark reddish–dark brown colour with a brassy metallic lustre, although it may be practically black there is always a brassy sheen visible
- Antennae yellowish brown, often darkened towards the apex. Tibia and tarsi are testaceous and all femora testaceous to piceous
- The pronotum has a shallower transverse ante-basal impression than the other three species, a more depressed form. Especially on the pronotum there is a brassy sheen visible. In the black species the ante-basal impression is distinctly sinuate and quite deep (Fig. 5c)
- Elytral hairs of are of medium density
- Spermatheca with the upper side of the receptacle concave and the lower side convex; spermathecal duct curved

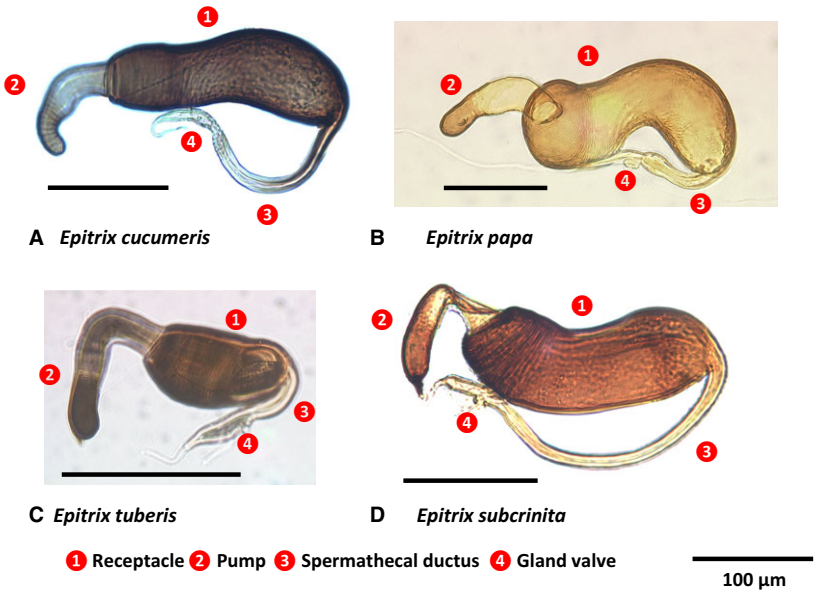

Fig. 6 Spermatheca.

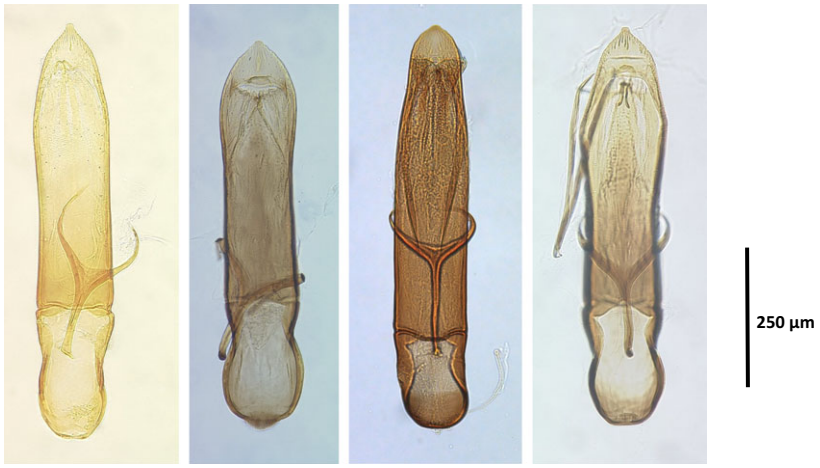

Fig. 7 Aedeagus (ventral view).

Table 1. Key to species of North American *Epitrix* developing on *Solanum tuberosum* including Western Palaearctic species[1]

1 Both sides of the spermatheca receptacle convex Aedeagus apex lancet	*Epitrix hirtipennis* (Melsheimer)
1′ Both sides of the spermatheca receptacle concave	2
1″ Upper side of the spermathecal receptacle concave, lower side convex	4
1‴ Both sides of the spermathecal receptacle straight	6
1⁗ Upper side straight/lower side convex The apex of the median lobe of the aedeagus has its greatest width in the upper third, its tip has a rectangular aspect	*Epitrix tuberis* Gentner
2 Ductus as long as receptacle, distal part of the ductus a quarter of its length The median lobe of the aedeagus is distinctly narrowed at the apex; its tip is spatulate	*Epitrix cucumeris* (Harris)
2′ Ductus shorter than receptacle	3
3 Distal part of ductus half of its length, pump no longer than the diameter of the receptacle in its largest part The apex of the median lobe of the aedeagus is regularly narrowed, lancet-shaped, its tip is very shortened	*Epitrix papa* Orlova-Bienkowskaja
3′ Distal part of the ductus a third of its length, pump longer than the diameter of the receptacle in its largest part Aedeagus apex stretched tip	*Epitrix similaris* Gentner
4 Ductus shorter than spermatheca receptacle Aedeagus in tapered tip	*Epitrix atropae* Foudras
4′ Ductus longer than spermatheca receptacle Aedeagus with a slight constriction at its ¼ apical part, barely marked tip truncated tab	*Epitrix subcrinita* (LeConte)
4″ Ductus almost as long as the spermatheca receptacle	5
5 Pump with a short neck Aedeagus with a blunt apex	*Epitrix intermedia* Foudras
5′ Pump with a long neck Aedeagus abruptly round apically to small rounded projection	*Epitrix pubescens* (Koch)
6 Pump almost as long as receptacle which looks quite compact Aedeagus slender, subparallel sides and sharp apex	*Epitrix caucasica* (Heikertinger)
6′ Pump shorter than receptacle which appears tapered	7
7 Receptacle larger at the pump side Aedeagus slender, sharp apex, slightly narrowed at mid-length	*Epitrix abeillei* (Bauduer)
7′ Sides of the receptacle parallel or almost parallel Aedeagus short and broad	*Epitrix dieckmanni* (Mohr)

[1]Two other species which are present in the non-European part of the EPPO region, *Epitrix allardi* and *Epitrix priesneri*, are not covered in this diagnostic protocol. For these species see drawings in Warchalowski (2003).

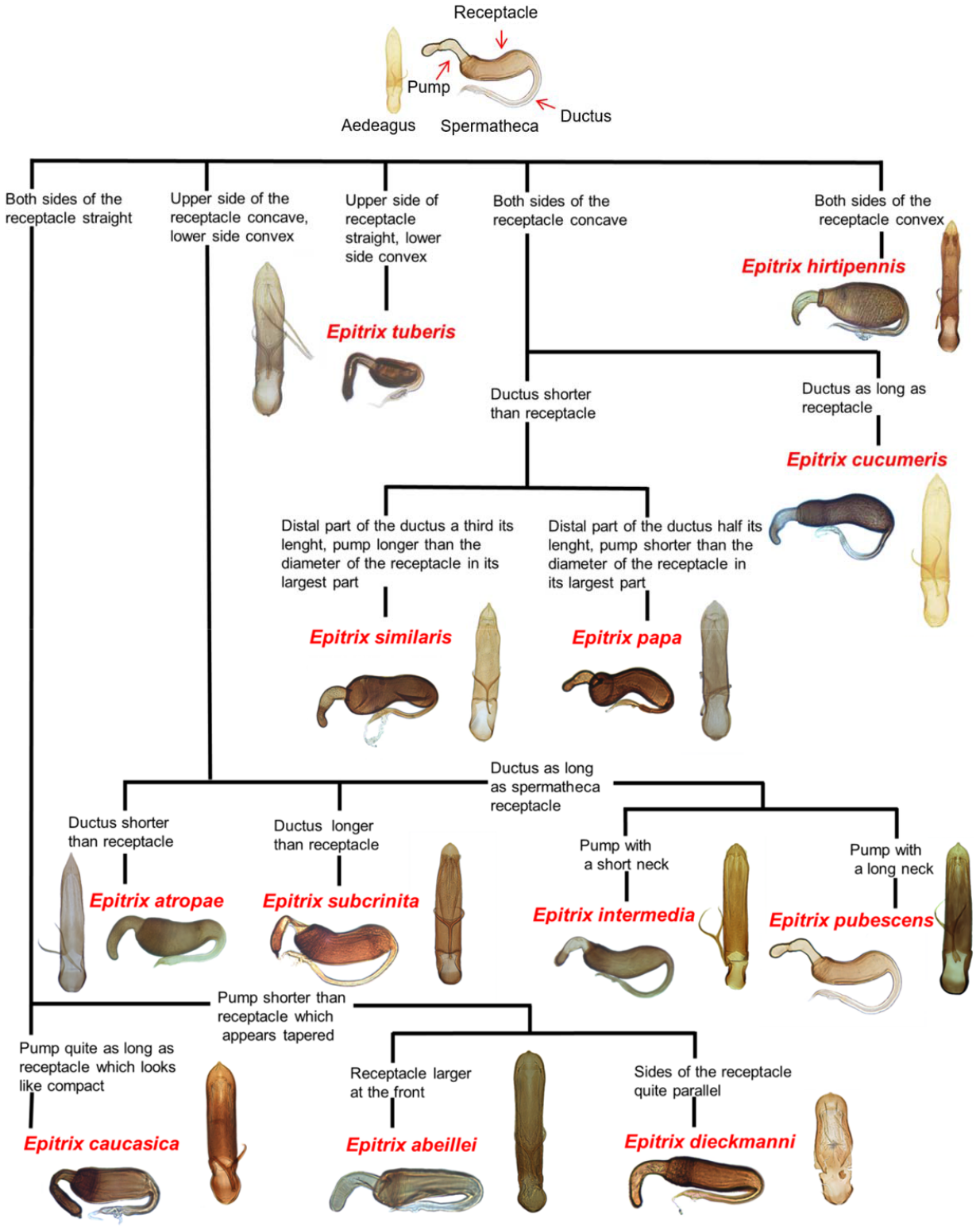

Fig. 8 Pictorial key.

and at least as long as the receptacle. The distal end of the pump has a small appendix (Fig. 6c)
- The aedeagus has a slight constriction at its ¼ apical part, barely marked tip truncated tab (Fig. 7c).

Epitrix tuberis (based on Gentner, 1944, Seeno, 1972):
- Body length from 1.60 to 2.04 mm
- The upper side is less shiny than in the other three species
- Antennae are brownish yellow near the base, becoming darker near the apex. Legs are reddish brown becoming lighter towards the tarsi
- The elytral pubescens is thick
- The pronotum presents a very marked, deep, coarser punctuation, each point separated by a distance less than its diameter, anterior sides angular, clearly dentate. The ante-basal impression is deep and winding, also with a coarse punctuation (Fig. 5d)
- The spermatheca presents an oval without any constriction; the pump is longer than the receptacle (Fig. 6d)
- The apex of the median lobe of the aedeagus has its greatest width in the upper third and its tip has a rectangular aspect (Fig. 7d).

A key to species of North American *Epitrix* developing on *Solanum tuberosum* including Western Palaearctic species (based on female spermatheca and male aedeagus observation) [external morphology can be confirmed with Seeno & Andrews (1972), Doeberl (2000), Doguet (2009), Warchalowski (2003), Bieńkowski & Orlova-Bienkowskaja (2016), Deczynski (2016)] is presented in Table 1 and should be used with the pictorial key presented in Fig. 8.

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 allows the identification of *E cucumeris, E. papa, E. subcrinita* and *E. tuberis.* Sequences are available in databases including Q-bank (http://www.q-bank.eu/arthropods/). A PCR-restriction fragment length polymorphism (RFLP) test has been described (Germain *et al.*, 2013); however, this test does not cover *E. subcrinita. In silico* testing on COI sequences of *E. subcrinita* shows that the test would not allow the distinction between *E. subcrinita* and *E. cucumeris*.

5. Reference material

Anses-LSV, Unité Entomologie et Plantes Invasives, CBGP, 755 Avenue du Campus Agropolis, CS 30016, FR-34988 Montferrier-sur-Lez (FR).

6. Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 (1) *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 to consult this database 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 these organisms can be obtained from:

J.-F. Germain/R. Mouttet. Anses-LSV, Unité Entomologie et Plantes Invasives, CBGP, 755 Avenue du Campus Agropolis, CS 30016, FR-34988 Montferrier-sur-Lez (FR).

jean-francois.germain@anses.fr/raphaelle.mouttet@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.

11. Acknowledgements

This protocol was originally drafted by: J. F. Germain and R. Mouttet. Anses-LSV, Unité Entomologie et Plantes Invasives, Montferrier-sur-Lez (FR).

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The Protocol was reviewed by the EPPO Panel on Diagnostics in Entomology.

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Appendix 1 – Preparation of male genitalia and female spermatheca for study under compound microscope (×200).

If the specimen is dry soften it in warm water for approximately 30 min until the abdomen can be moved. Remove the whole abdomen with forceps under a stereomicroscope and place it in warm potassium hydroxide solution (approximately 10%) for approximately 20 min to macerate the muscles and fat tissues. Rinse and dissect the abdomen in distilled water under a stereomicroscope (×20), opening the abdomen along one side with fine scissors or pins. Clean out the remaining tissues and carefully sever the genitalia from the apical segments. Rinse the abdomen and genitalia in distilled water or ethanol and place into glycerine on a cavity slide for study and temporary storage. For permanent storage, place the genitalia (aedeagus or spermatheca) in a drop of Canada balsam on a slide.

