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EPPO STANDARD ON DIAGNOSTICS

PM 7/007 (2) *Aleurocanthus citriperdus, Aleurocanthus spiniferus* and *Aleurocanthus woglumi*

Specific scope: This Standard provides guidance for the identification of *Aleurocanthus citriperdus, Aleurocanthus spiniferus* and *Aleurocanthus woglumi*.[1]
Specific approval and amendment: First approved in 2001–09 as PM 7/007 (1) *Aleurocanthus spiniferus* and PM 7/008 (1) *Aleurocanthus woglumi*. Revision approved in 2021–11.

1 | INTRODUCTION

The family Aleyrodidae is a group of minute hemipteran insects of more than 1610 described species placed in 3 extant sub-families and about 170 genera (Martin & Mound, 2007; Ouvrard & Martin, 2021; Streito & Germain, 2020). The life-cycle is characterized by six developmental stages: the egg, four pre-adult instars and the adult. The first instar is mobile (crawlers), but subsequent immature instars are fixed on the plant. The taxonomy of the group and the recognition of the species is mainly based on the morphology of the fourth instar called 'puparium'. Aleyrodidae cause damage to plants directly by sucking sap and indirectly by transmitting plant viruses, and excreting copious amounts of honeydew, which coat leaf and fruit surfaces and lead to mould infection.

The genus *Aleurocanthus* comprises 91 described species world-wide (Ouvrard & Martin, 2021), in great need of revision according to Jansen and Porcelli (2018), with species delimitation hindered by both sexual dimorphism and intraspecific morphological variability depending on the host on which the puparium has developed. Nguyen et al. (1993) report 11 *Aleurocanthus* species from Citrus, including *Aleurocanthus cheni* Young, a junior synonym of *Aleurocanthus spiniferus* (Quaintance), and Dubey and Ko (2012) consider three of them to be invasive and causing serious damage: *Aleurocanthus citriperdus* Quaintance & Baker, *A. spiniferus* and *Aleurocanthus woglumi* Ashby, which will be the main objects of this protocol. Other species reported from *Citrus* are: *Aleurocanthus cocois* Corbett; *Aleurocanthus delottoi* Cohic; *Aleurocanthus husaini* Corbett; *Aleurocanthus inceratus* Silvestri; *Aleurocanthus mackenziei* Cohic; *Aleurocanthus spinosus* (Kuwana) and *Aleurocanthus valenciae* Martin & Carver. Among these species found on *Citrus*, most of them are recorded from Asia, with the exception of two species known from Africa only (*A. delottoi* and *A. mackenziei*) and one from Australia (*A. valenciae*).

Identification keys to species of *Aleurocanthus* are available for regional faunas, e.g. Africa (Bink-Moenen, 1983), India (Dubey & Sundararaj, 2004), Taiwan (Dubey & Ko, 2012), Australia (Gillespie, 2012), but only the two latter include the three species considered in this protocol, and none will cover all species found on *Citrus*.

The aim of this protocol is to allow the recognition of puparia belonging to the *Aleurocanthus* genus, and then the identification to species for puparia found on *Citrus*. Diagnostic morphological characters are listed for all three most invasive and damaging species *A. citriperdus*, *A. spiniferus* and *A. woglumi*.

Identification of *Aleurocanthus* species is difficult and confirmation by a specialist is highly recommended in case of first identification (see Section 8).

1.1 | Species information: *Aleurocanthus citriperdus*

Aleurocanthus citriperdus Quaintance & Baker is absent from EPPO member countries. It is present in South-East Asia, Japan, China, India and Sri Lanka (Gillespie, 2012; Nguyen et al., 1993). It is also common in Hong-Kong, where it is found on citrus, guava, *Litsea* spp. and *Macaranga tanarius* (Martin & Lau, 2011). It is recorded from five *Citrus* species and coffee (Evans, 2008; Martin, 1987). This whitefly is reported as a serious pest on *Citrus* (EFSA Plant Health Panel, 2018; Nguyen et al., 1993), especially in India and Pakistan (EFSA, 2019).

[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.

1.2 | Species information: *Aleurocanthus spiniferus*

Aleurocanthus spiniferus (Quaintance) occurs throughout Asia and the Pacific, and has spread to parts of Central, Eastern and Southern Africa. Recorded from the EPPO region initially in Italy (Porcelli, 2008), where its distribution area has been growing continuously, it has since then been recorded from Croatia, Greece, Montenegro and Albania. It is a highly polyphagous insect and its major host plants include *Citrus*, *Pyrus*, *Vitis*, *Psidium*, *Diospyros* and *Rosa*. Heavy infestations of *A. spiniferus* may lead to mortality of young trees. *Aleurocanthus spiniferus* is recorded on almost 100 host plants in 37 plant families (Bubici et al., 2020; Cioffi et al., 2013; Dubey & Ko, 2012; Gillespie, 2012; Jansen, 2011; Kapantaidaki et al., 2019; Mound & Halsey, 1978; Nugnes et al., 2020; Wang et al., 2019). Details on the host range is available in EPPO Global Database (EPPO, 2022). Available mtCOI sequences (published and unpublished) suggest that *A. spiniferus* may be a complex of several different species.

1.3 | Species information: *Aleurocanthus woglumi*

Aleurocanthus woglumi (Ashby) originates from South-East Asia and has spread throughout Asia and the Pacific, Central, Eastern and Southern Africa, Central America, the Antilles, and parts of North and South America. It is absent from EPPO member countries. The species is highly polyphagous. Gillespie (2012) lists 75 host plant species belonging to 37 families, whereas Nguyen et al. (1993) group host plants in three different categories: (1) Plants heavily infested on which complete whitefly development has been observed (34 species in 7 families, including *Citrus*, coffee, mango, persimmon, pear and quince), (2) Plants occasionally infested on which complete whitefly development has been observed (74 species in 35 families, including avocado, banana, cocoa, cashew, chilli pepper, coconut, grape and plum), and (3) Plants on which the whitefly development was incomplete (73 species in 33 families). *Citrus* species are the main hosts of economic importance because they allow for large population development (Nguyen et al., 2019).

2 | IDENTITY

Name: *Aleurocanthus citriperdus* Quaintance & Baker, 1916: 459.
Other scientific names: *Aleurocanthus cameroni* Corbett, 1935: 799, synonymised by Mound and Halsey (1978): 14.
Taxonomic position: Insecta, Hemiptera: Sternorrhyncha: Aleyrodidae, Aleyrodinae.
EPPO Code: ALECCT.
Phytosanitary categorization: EU A1 Quarantine pest (Annex II/A).

Name: *Aleurocanthus spiniferus* (Quaintance, 1903) described as.
Other scientific names: *Aleurodes spinifera* Quaintance, *Aleurodes citricola* Newstead, 1911, synonymised by Silvestri (1928), *Aleurocanthus citricolus* (Newstead, 1911): Quaintance and Baker (1914), *Aleyrodes marlatti* (Quaintance, 1903): misidentification in Shiraki (1913): 104 and Maki (1915) according to Takahashi (1932), *Aleurocanthus spiniferus* var. *intermedia* (Silvestri, 1928), synonymised by Mound and Halsey (1978), *Aleurocanthus spiniferus* var. *intermedius* (Silvestri, 1928): Martin and Mound (2007), *Aleurocanthus rosae* Singh, 1931, synonymised by Takahashi (1932), *Aleurocanthus cheni* (Young, 1942), synonymised by Martin and Lau (2011).
Taxonomic position: Insecta, Hemiptera: Sternorrhyncha: Aleyrodidae, Aleyrodinae.
EPPO Code: ALECSN.
Phytosanitary categorization: EPPO A2 list n°186; EU A2 Quarantine pest (Annex II/B).

Name: *Aleurocanthus woglumi* Ashby, 1915.
Other scientific names: *Aleurocanthus punjabensis* Corbett, 1935, synonymised by Husain and Khan (1945): 1–2.
Aleurocanthus woglumi var. *formosana* Takahashi, 1935, synonymised by Mound and Halsey (1978): 24.
Taxonomic position: Insecta, Hemiptera: Sternorrhyncha: Aleyrodidae, Aleyrodinae.
EPPO Code: ALECWO.
Phytosanitary categorization: EPPO A1 list n°103; EU A1 Quarantine pest (Annex II/A).

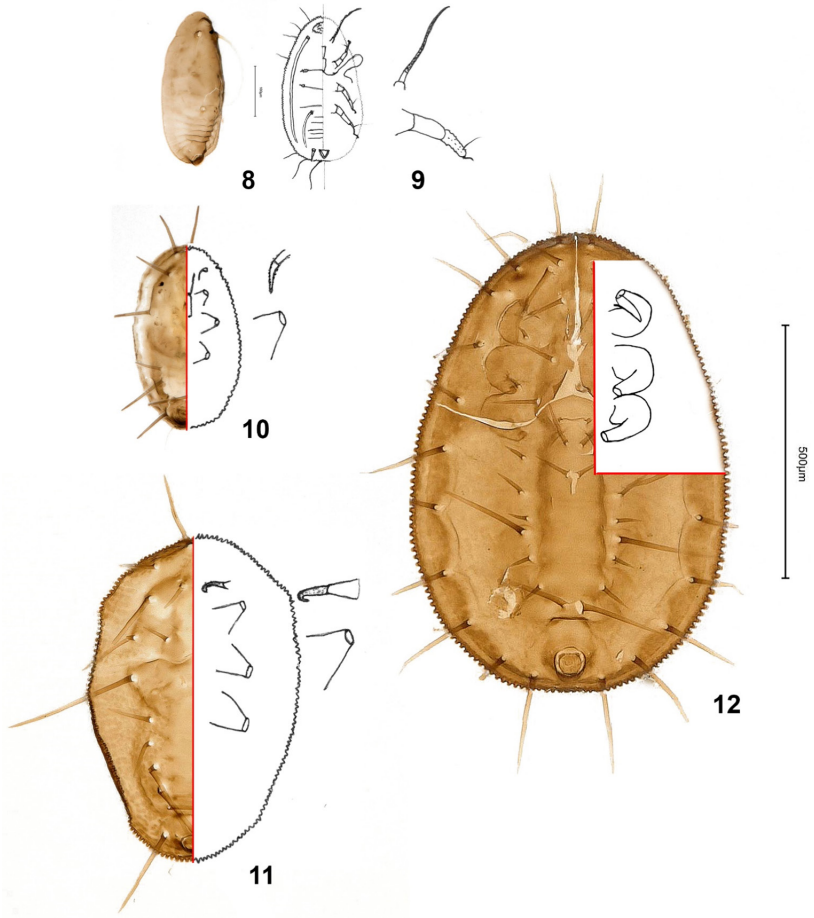
3 | DETECTION

Figures 1–7.

The presence of *Aleurocanthus* populations is often associated with the presence of sooty mould covering the leaves (Figures 1–3).

The three main species of the genus *Aleurocanthus* studied in this protocol are most readily recognisable in the field by the fact that immature stages are dark brown to black with a fringe of short wax filaments and carry the exuviae of previous instars (Figures 6–7, 15), and by the presence of conspicuous glandular spines on the submargin and the submedian area of the dorsum of immatures (Carver, 1991). They are gregarious at the immature stages and can be found as colonies on the underside of leaves. Spiral or semi-circular patterns of eggs may be sometimes visible (Figure 4). Contrary to the vast majority of whitefly species which are white, adults of these *Aleurocanthus* species have blue-grey wings with white markings (Figure 5).

FIGURES 1-7 1. *Aleurocanthus spiniferus*, puparia on underside of *Parthenocissus quinquefolia* leaves (Courtesy Maja Pintar, Centre for Plant Protection, Croatian Agency for Agriculture and Food). 2. *Aleurocanthus spiniferus*, infested *Citrus aurantium* plants (Courtesy Francesco Porcelli, Università di Bari, Italy). 3. *Aleurocanthus spiniferus*, underside of citrus leaves with numerous puparia (Courtesy Francesco Porcelli, Università di Bari, Italy). 4. *Aleurocanthus* sp., colony on *Citrus limon* showing eggs (Courtesy Jean-Claude Streito, INRAE, France). 5. *Aleurocanthus spiniferus*, adults and eggs on underside of a *Citrus reticulata* leaf (Courtesy Mladen Šimala, Centre for Plant Protection, Croatian Agency for Agriculture and Food, Croatia). 6. *Aleurocanthus* sp., colony on *Cinnamomum* sp. (Courtesy Jean-Claude Streito, INRAE, France). 7. *Aleurocanthus camelliae*, showing exuviae 2 and 3 attached to puparium (Courtesy Jean-Claude Streito, INRAE, France from sample courtesy of Maurice Jansen, the Netherlands)



FIGURES 8-12 8. *Aleurocanthus spiniferus*, first larval stage (crawler), lateral view. 9. *Aleurocanthus spiniferus*, first larval stage, (left: dorsal side; right: ventral side and detail of antenna and leg). 10. *Aleurocanthus woglumi*, second larval stage, (left: dorsal side; right: ventral side and detail of antenna and leg). 11. *Aleurocanthus woglumi*, third larval stage, (left: dorsal side; right: ventral side and detail of antenna and leg). 12. *Aleurocanthus spiniferus*, fourth larval stage (puparium) (right quarter up: detail of antennae and legs)

Only *A. spiniferus* has been recorded from the EPPO region. Once a population has been detected on *Citrus*, the identification of the puparia will need slide mounting and use of the key provided in this protocol. An example of puparium preparation for microscopic examination is available in Appendix 1.

In the field, pre-adult stages of the whitefly may be confused with the adult females of the diaspid scale insect *Parlatoria ziziphi* (Lucas) (Hemiptera: Diaspididae) (Figure 14) which is a similar size and colour, stacking exuviae and also possesses a margin of white wax (Jendoubi et al., 2021). However, the scale is more elongate than the whitefly.

Species in the genera *Cerataphis* Lichtenstein and *Aleurodaphis* van der Goot (Hemiptera: Aphididae: Hormaphidinae) resembles a whitefly or scale insect and both genera are distributed throughout tropical regions of the world. Apterae of these two genera have short legs which are hidden under the body which is 1–2 mm long, slightly convex to flat, oval-shaped and dark brown with a shiny white waxy fringe. A species which is occasionally imported into Europe is the palm aphid *Cerataphis brasiliensis* (Hempel) (Figure 13).

4 | IDENTIFICATION

4.1 | Morphological identification

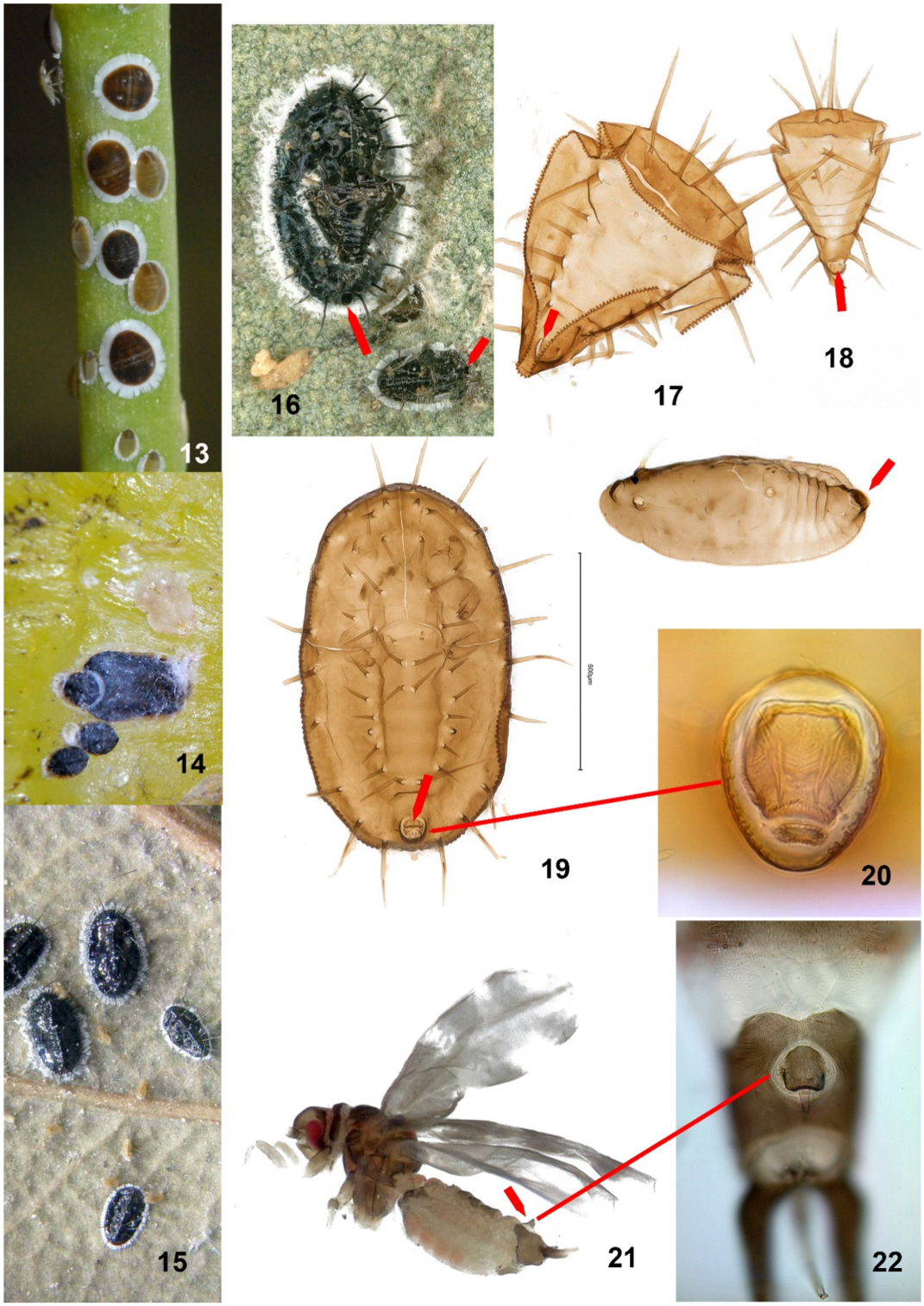
4.1.1 | Recognizing the puparial stage

Figures 8–12.

The first stage is mobile and named crawler, other stages are fixed on the host leaf. From the second larval stage onwards, moulting occurs at the same place on the plant. In the genus *Aleurocanthus* the exuviae are piled up, remaining attached to the next instar. The puparium (which is the fourth and last larval stage) therefore has two exuviae on its upper surface, those of the 3rd and 2nd stages (Figure 7).

The first larval stage is mobile and characterized by a small size (0.29–0.32 mm measured on 3 individuals of *A. spiniferus*) and well developed and functional three segmented legs ended by a claw (Figures 8–9). Antennae are two segmented, with the apical segment elongated (Figure 9).

The second larval stage (Figure 10) is 0.42–0.45 mm long (measured on 3 individuals of *A. woglumi*). Legs and



FIGURES 13-22 13. *Cerataphis* sp. (Aphididae). 14. *Parlatoria ziziphi* (Diaspididae). 15. *Aleurocanthus* sp. (Aleyrodidae). 16. *Aleurocanthus camelliae*, arrows showing vasiform orifices (top on puparium, bottom on younger larva). 17. *Aleurocanthus spiniferus*, arrow showing the vasiform orifice of 3rd instar larva exuvia. 18. *Aleurocanthus spiniferus*, arrow showing the vasiform orifice of 2nd instar larva exuvia. 19. *Aleurocanthus spiniferus*, arrow showing the vasiform orifice of puparium. 20. *Aleurocanthus spiniferus*, vasiform orifice of puparium. 21. *Aleurocanthus spiniferus*, arrow showing vasiform orifice of an adult male. 22. *Aleurocanthus spiniferus*, vasiform orifice of an adult male. Figures 13-22 courtesy Jean-Claude Streito, INRAE, France, Figure 16 from sample courtesy of Maurice Jansen, the Netherlands

antennae are reduced: antennae are straight and not overlapping the legs; legs are triangular with apex pointing outwards, ended by a pad (a trait shared by all Aleyrodinae).

The third larval stage (Figure 11) is 0.57–0.65 mm long (measured on 5 individuals of *A. woglumi*). Legs and antennae are reduced: antennae are hooked at the

end. Legs are triangular, with apex pointing outwards, ended by a pad.

The fourth larval stage named 'puparium' (Figure 12) is 0.72 (male of *A. woglumi*) to 1.13 (female of *A. spiniferus*) mm long (Dubey & Ko, 2012). Legs and antennae are reduced: antennae are straight, terminated by a granular "finger" and overlapping the legs. Legs are curved inwards, ended by a pad. This is the only stage allowing identification to species.

Adults have six functional segmented legs and four wings.

4.1.2 | Recognizing Aleyrodidae

Figures 13–22.

In the laboratory, Aleyrodidae can be readily separated from any other organisms by the presence of a vasiform orifice. This family-specific structure is present on all larval stages including the first instar and in adults of both sexes. According to Martin (2000), this structure comprises the anus, a lingula which ejects excreta (including honeydew), and an operculum which partially or wholly covers the orifice itself (Figures 16–22). The vasiform orifice can be detected with a stereomicroscope and is better observed with a compound microscope on mounted slides.

4.1.3 | Identification at the genus level (*Aleurocanthus*)

Figures 23–55.

Like most Aleyrodidae, the genus *Aleurocanthus* has been described based on the puparium. The generic characters of the genus *Aleurocanthus* are given in Martin (1999) and the generic diagnosis has been revised in Dubey and Sundararaj (2004), Dubey and Ko (2012) and Kanmiya et al. (2011).

Martin (1999) gives the following characters to define the genus *Aleurocanthus*: puparia with cuticle usually dark (but some species are pale), margin regularly toothed, not deflexed; dorsum with stout glandular spines; cephalic, eighth abdominal and caudal setae present; first abdominal setae present but usually thickened, sometimes similar to glandular spines; vasiform orifice subcircular to subcordate, often elevated, mostly occupied by operculum which obscures lingula. Often dimorphic, with male puparia much smaller than female (Dubey & Ko, 2012), sometimes with different number and arrangement of spines.

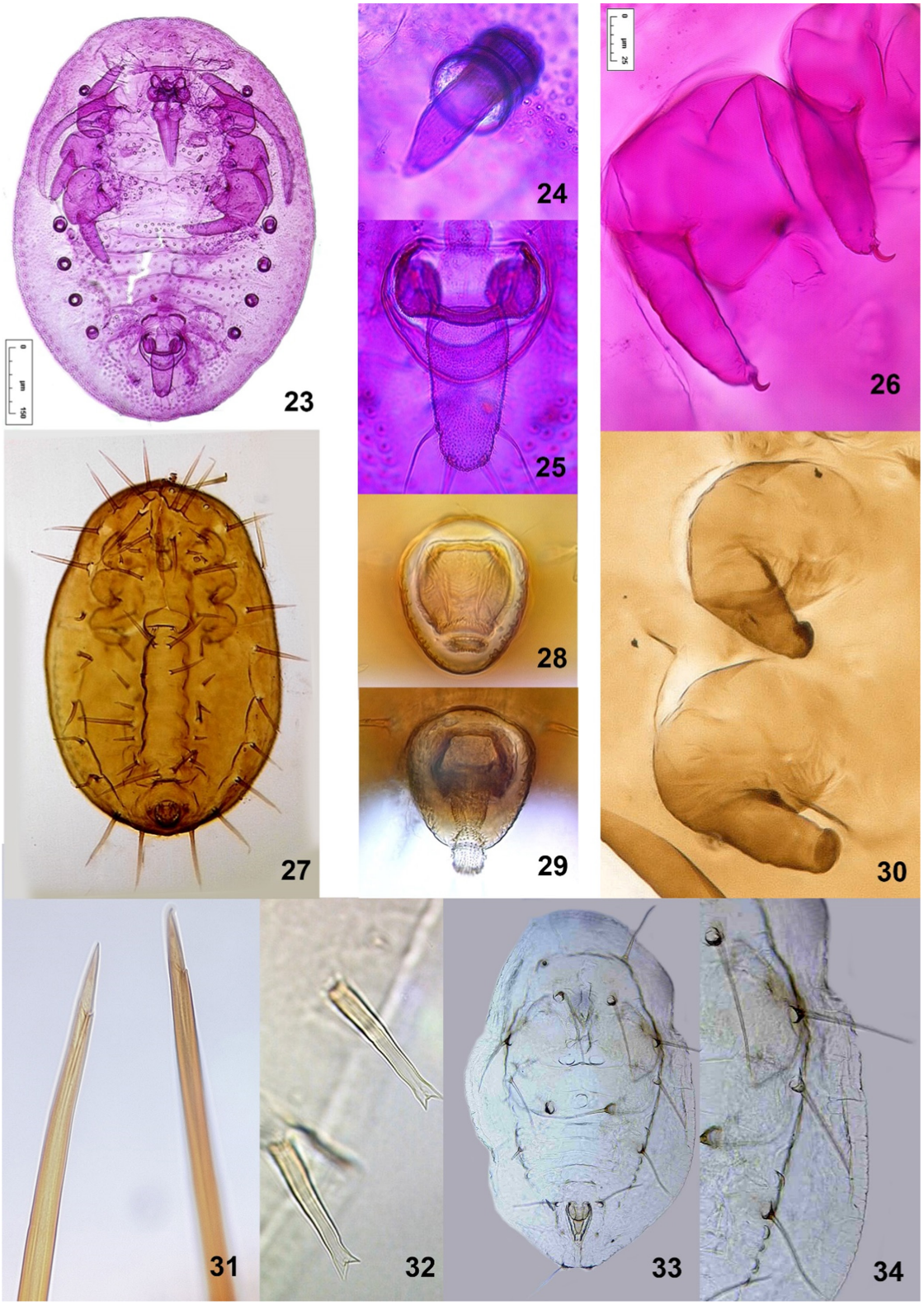
As shown by Carver (1991), the glandular spines show different kinds of apexes, with either a subapical or apical opening (Figure 37), sometimes with droplets of secreted fluid at their apex. The shape, length, position and number of the spines are used for diagnostic purposes including the recognition of species. Martin (1999) stated that the puparia of some species have their glandular spines reduced and located on tubercles; these species were included in *Aleurocanthus* based on the presence of larger spines in third-instar larvae.

These stout dorsal glandular spines should not be confused with the robust dorsal disc setae present in other whiteflies, or with tubular siphons that are not acute, especially in the genera *Siphoninus* (Figure 32), *Aleurotuba* (Figure 44) and some species of *Xenaleyrodes* (Figure 40).

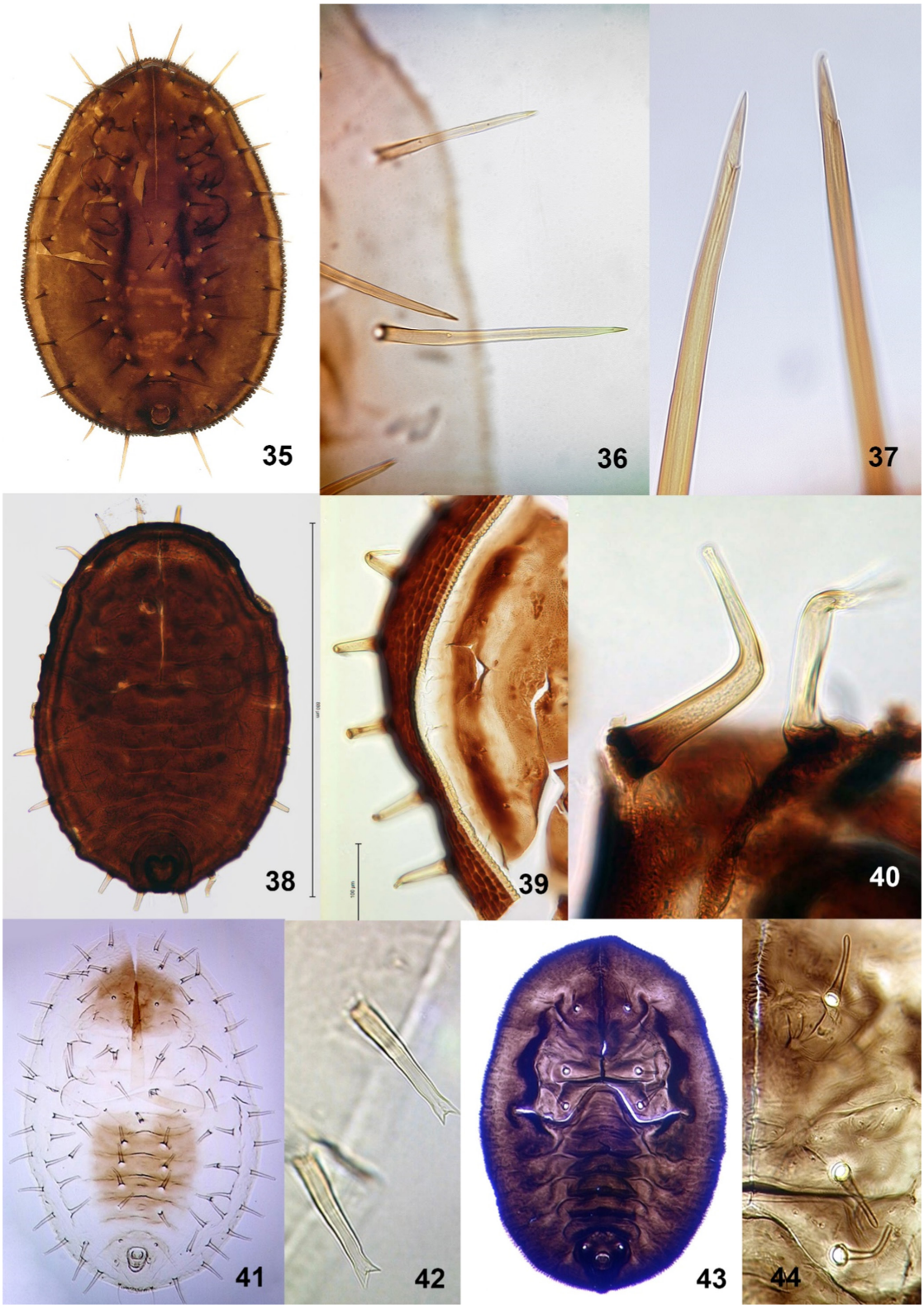
The most important puparial characters used in the following keys are illustrated in Appendix 2.

Key to the puparia of genera morphologically related to *Aleurocanthus*:

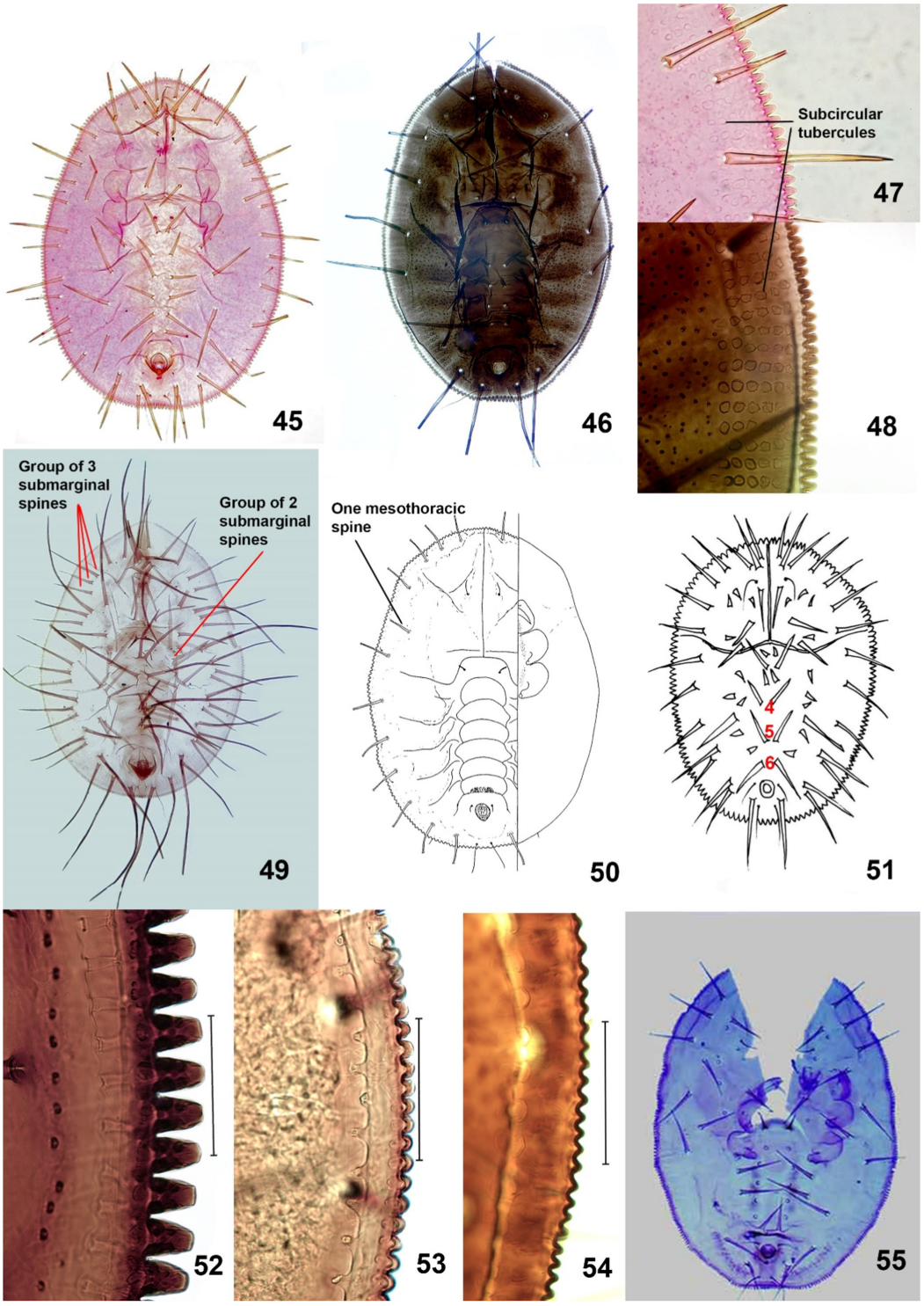
1	Subdorsal area with compound pores (Figure 23), each which may bear a central process (Figure 24); each leg with one apical claw (Figure 26); lingula large, exposed with four stout setae (Figure 25)	Aleurodicinae
	No compound pores on subdorsal area (Figure 27) but simple pores/papillae variable in size and form can be present; legs without claw, ended by a rounded pad (Figure 30); lingula variable, often hidden by operculum	2 Aleyrodinae (including *Aleurocanthus*)
2	Dorsum with many long acute glandular spines or siphons variously expanded (exceptions: a few Australian species) (Figures 31–32, 35–44)	3
	Dorsal area without such spines or setae but stout normal setae often present on dorsal disk or submarginal area (Figures 33–34)	other genera of Aleyrodinae
3	Operculum not covering lingula (Figure 41). Dorsal area with siphons expanded apically (Figure 42). Margin smooth and not deflexed	*Siphoninus*
	Operculum covering lingula (Figures 28, 56, 58, 60) (but lingula may be exceptionally exposed: see Figure 29)	4
4	Dorsal area with only four pairs of blunt siphons: one pair on head, one pair on mesothorax, one pair on metathorax and one pair on the eighth abdominal segment (Figures 43–44)	*Aleurotuba*
	Dorsum with more than 4 pairs of glandular spines acute or expanded	5
5	Margin deflexed, of complex structure, apparent margin ventral smooth to irregular; glandular spines almost always in a single submarginal row only, their apices always expanded (Figures 38–40) [Australasia]	*Xenaleyrodes*
	Margin not deflexed, regularly crenulate or toothed (Figures 52–54); glandular spines usually paired in submargin, subdorsum and submedially (Figures 45–46, 49–51), occasionally only in submargin or subdorsum (exceptions: only tubercules on puparium, but typical glandular spines in third instar larva, in a few Australian species)	*Aleurocanthus*



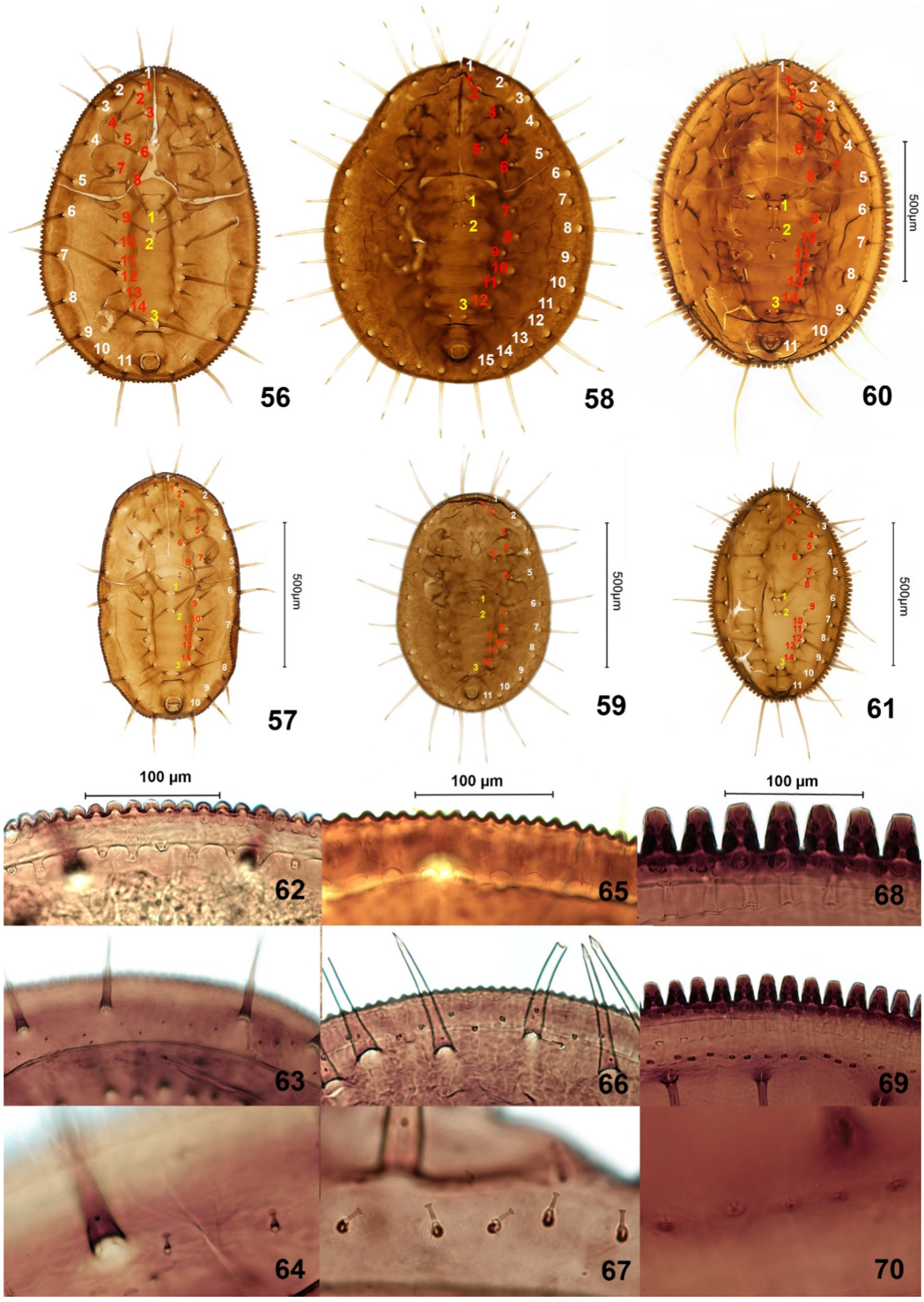
FIGURES 23-34 23. *Aleurodicus dispersus* (Aleurodicinae), puparium showing compound pores, legs and lingula. 24. *Aleurodicus dispersus* (Aleurodicinae), detail of a compound pore. 25. *Aleurodicus dispersus* (Aleurodicinae), detail of lingula. 26. *Aleurodicus dispersus* (Aleurodicinae), median and posterior legs showing claws. 27. *Aleurocanthus spiniferus*, puparium. 28. *Aleurocanthus spiniferus*, detail of vasiform orifice lingula hidden by operculum. 29. *Aleurocanthus spiniferus*, detail of vasiform orifice lingula exposed. 30. *Aleurocanthus spiniferus*, anterior and middle legs ended by a rounded pad. 31. *Aleurocanthus spiniferus*, detail of glandular spine with duct and opening. 32. *Siphoninus immaculatus*, detail of siphon like spines. 33. *Bemisia tabaci*, puparium with long setae. 34. *Bemisia tabaci*, detail of setae (not glandular, without duct and opening) Figures 23-34 Courtesy Jean-Claude Streito, INRAE, France



FIGURES 35-44 35. *Aleurocanthus spiniferus*, puparium with glandular spines. 36. *Aleurocanthus spiniferus*, detail of glandular spines. 37. *Aleurocanthus spiniferus*, detail of glandular spines with duct and opening. 38. *Xenaleyrodes* sp., puparium with one row of glandular spines. 39. *Xenaleyrodes* sp., margin deflexed, apparent margin ventral, smooth, real margin crenulate. 40. *Xenaleyrodes* sp., glandular spines geniculate. 41. *Siphoninus immaculatus*, puparium with siphons. 42. *Siphoninus immaculatus*, siphons. 43. *Aleurotuba jelinekii*, puparium. 44. *Aleurotuba jelinekii*, detail of blunt siphons (one cephalic and two thoracic pairs. Figures 35-44 Courtesy Jean-Claude Streito, INRAE, France



FIGURES 45-55 45. *Aleurocanthus cocois* puparium (photo Ken Walker, Padil). 46. *Aleurocanthus delottoi*, puparium; holotype from the Muséum national d'Histoire naturelle, Paris, France (Courtesy Jean-Claude Streito, INRAE, France). 47. *Aleurocanthus cocois* margin and ventral structures (Courtesy Ken Walker, Padil). 48. *Aleurocanthus delottoi*, margin and ventral structures, holotype from the Muséum national d'Histoire naturelle, Paris, France (Courtesy Jean-Claude Streito, INRAE, France). 49. *Aleurocanthus mackenziei*, puparium; collection Cohic from the Muséum national d'Histoire naturelle, Paris, France (Courtesy Jean-Claude Streito, INRAE, France). 50. *Aleurocanthus valenciae*, puparium (from Martin, 1999). 51. *Aleurocanthus inceratus*, puparium (from Wang et al., 2016). Numbers show positions of the 4th, 5th and 6th abdominal segments and corresponding submedian glandular spines. 52. *Aleurocanthus woglumi*, margin (Courtesy David Ouvrard, Anses, France). Scale bar = 0.1 mm.53. *Aleurocanthus spiniferus*, margin (Courtesy David Ouvrard, Anses, France). Scale bar = 0.1 mm. 54. *Aleurocanthus citriperdus*, margin (Courtesy David Ouvrard, Anses, France). Scale bar = 0.1 mm. 55. *Aleurocanthus spinosus*, puparium (Courtesy John Dooley, USDA)



FIGURES 56-70 56. *Aleurocanthus spiniferus*, puparium female. Median glandular spines are numbered in yellow, submedian glandular spines are numbered in red and marginal glandular spines are numbered in white. 57. *Aleurocanthus spiniferus*, puparium male. Median glandular spines are numbered in yellow, submedian glandular spines are numbered in red and marginal glandular spines are numbered in white. 58. *Aleurocanthus citriperdus*, puparium female. Median glandular spines are numbered in yellow, submedian glandular spines are numbered in red and marginal glandular spines are numbered in white. 59. *Aleurocanthus citriperdus*, puparium male. Median glandular spines are numbered in yellow, submedian glandular spines are numbered in red and marginal glandular spines are numbered in white. 60. *Aleurocanthus woglumi*, puparium female. Median glandular spines are numbered in yellow, submedian glandular spines are numbered in red and marginal glandular spines are numbered in white. 61. *Aleurocanthus woglumi*, puparium male. Median glandular spines are numbered in yellow, submedian glandular spines are numbered in red and marginal glandular spines are numbered in white. 62. *Aleurocanthus spiniferus,* margin. 63–64. *Aleurocanthus spiniferus,* knobbed setae on submargin (Courtesy Jean-Claude Streito, INRAE, France). 65. *Aleurocanthus citriperdus,* margin. 66–67. *Aleurocanthus citriperdus,* knobbed setae on submargin. 68. *Aleurocanthus woglumi,* margin. 69–70. *Aleurocanthus woglumi,* knobbed setae on submargin. Figures 56-70 Courtesy Jean-Claude Streito, INRAE, France.

4.1.4 | Identification at the species level

All three *Aleurocanthus* species treated in this protocol show sexual dimorphism, and it may also be the case in some or all other *Aleurocanthus* species feeding on *Citrus*. Dubey and Ko (2012) have shown that male puparia are usually much smaller than female puparia (Figures 56–61).

Among the 91 known *Aleurocanthus* species, some may be very close morphologically to the species listed in the key below, but they will not develop on *Citrus*. For instance, *Aleurocanthus camelliae* Kanmiya & Kasai, 2011 shows only very few and slight morphological differences with *A. spiniferus* (Jansen & Porcelli, 2018; Kanmiya et al., 2011), but does not develop on Rutaceae.

Key to the species of *Aleurocanthus* known from *Citrus*:

1	Puparium pale or dark brown, never black before bleaching; venter with a submarginal band of shallow, subcircular (sometimes indistinct) tubercules (Figures 47–48)	2
	Puparium black before bleaching; venter smooth with at most a single submarginal row of elliptical structures (Figures 52–53)	3
2	Puparium pale; abdomen normally with 7 submedian and 4 subdorsal pairs of stout spines (Figure 45)	*A. cocois*
	Puparium brown; abdomen with normally submedian pairs of spines on segment I and III-VI; cuticle of outer submargin paler than remainder of pupal case (Figure 46) [Afrotropical]	*A. delottoi*
3	Dorsal spines grouped in clusters of 3 on the submarginal area and in clusters of 2 on the subdorsal area (Figure 49) [Afrotropical]	*A. mackenziei*
	Dorsal spines arrangement different	4
4	One pair of mesothoracic subdorsal spines (Figure 50) [Australasian]	*A. valenciae*
	More than one pair of mesothoracic subdorsal spines	5
5	Submedian glandular spines present on abdominal segments 4, 5 and 6 (Figure 51)	*A. inceratus*
	Submedian glandular spines absent medially from abdominal segments 4, 5 and 6 (Figures 56–61)	6
6	Number of marginal teeth >6 per 0.1 mm (Figure 55)	7
	3.5–5 marginal teeth per 0.1 mm (Figure 52)	8
7	26 marginal teeth per 0.1 mm	*A. spinosus*
	Number of marginal teeth per 0.1 mm different	9
8	Certain dorsal disc spines longer; one pair of spines on 1st abdominal segment	*A. husaini**
	Dorsal disc spines uniformly long; one pair of bristles on 1st abdominal segment	*A. woglumi**
9	Margin crenulate (Figure 54, 66); submarginal area with 15 or 16 (female) or 11 (male) pairs of spines, of which six pairs on cephalothorax (Figure 58–59); two pairs of submarginal spines may be doubled at posterior abdominal area	*A. citriperdus*
	Margin toothed (Figures 53, 62); submarginal area with 11 (female) or 10 (male) pairs of spines, of which five pairs on cephalothorax (Figures 56–57); none or only one pair of the submarginal spines may be doubled at posterior abdominal area	*A. spiniferus*

* Rathod et al. (2013) failed in demonstrating morphological differences between puparia of *A. husaini* and *A. woglumi*, and Martin (2005) does not provide any morphological evidence when re-establishing *A. husaini* as a valid species (from synonymy with *A. woglumi*). Discriminating character states listed in the present key are from the original description by Corbett (1939).

Morphological characters useful to discriminate between *A. citriperdus*, *A. spiniferus* and *A. woglumi* are summarised in Table 1 and visible in Figures 56–70. Important characters are the size and arrangement of the minute knobbed setae on the submargin, the length of the longest glandular spines and the size and number of marginal teeth.

4.2 | Molecular identification

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, 2021).

The international GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Bold System (https://www.boldsystems.org/) databases contain many *Aleurocanthus* sequences. However, many of these relate to other parts of the genome than the standard COI barcode (5′ end) and many are contaminations (bacteria, Hymenoptera and other non whitefly organisms). Once these sequences have been eliminated, there are few usable barcodes left.

Aleurocanthus citriperdus: only one sequence of the mtCOI (5′ end) gene is available in GenBank (accession number HM150620.1), but it has been impossible to test its validity in the absence of other sequences linked to reference material.

Aleurocanthus spiniferus: many mtCOI sequences from both ends of the gene are available in GenBank, but they should be used with caution. Preliminary unpublished analyses of aligned sequences show multiple haplotypes which may reflect a complex of several

TABLE 1 Microscopic differences between puparia of *A. citriperdus*, *A. spiniferus* and *A. woglumi*

	Aleurocanthus spiniferus		*Aleurocanthus citriperdus*		*Aleurocanthus woglumi*	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Puparium length (μm) (Dubey & Ko, 2012)	750–770	1130–1270	770–780	1050–1220	720–800	1050–1100
Marginal teeth	moderately large, rounded, 6–12 per 0.1 mm		crenulations, not isolated teeth, 8–12 per 0.1 mm		very large, blunt, only 3.5–5 (rarely 6) per 0.1 mm	
Knobbed seta pits on submargin	very small, on more than one row		large, on more than one row		large, on one row	
Number of cephalothoracic subdorsal spines (pairs)	8–9		6		8	
Number of glandular spines on submargin (pairs)	10	11	11	15–16	10	
Length and shape of the longest glandular spines (measurements: M. Jansen)	Short and stout usually straight. Length-width ratio 1:9–1:17		Same as *A. spiniferus*		Relatively long and narrow often curved. Length-width ratio 1:28–1:48	

species. The EPPO-Q-bank database (https://qbank.eppo.int/) contains 12 mtCOI sequences (5′ end) from Italian specimens.

Aleurocanthus woglumi: two sequences are available in Genbank, one for each end of the mtCOI gene, under accession numbers MT479168.1 (5′ end) and JX281760.1 (3′ end) (Pandey et al., 2013). The former matches with other unpublished barcode 5′ sequences hosted at CBGP (Montferrier-sur-Lez, France). EPPO-Q-bank contains 16 mtCOI (5′ end) from Costa Rican and Malaysian specimens.

5 | REFERENCE MATERIAL

Reference material of *A. spiniferus* and *A. woglumi* is hosted by the CBGP, Centre de Biologie pour la Gestion des Populations, 755 Avenue du Campus Agropolis, CS 30016, 34988 Montferrier-sur-Lez Cedex, France. Reference material of *A. citriperdus* is hosted by the Natural History Museum, Cromwell Road, London, SW7 5BD, GB.

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 CHARACTERISTICS

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:

JC Streito, CBGP, 755 Avenue du Campus Agropolis, CS 30016, 34988 Montferrier-sur-Lez Cedex, France.

D Ouvrard, Anses-LSV, 755 Avenue du Campus Agropolis, CS 30016, 34988 Montferrier-sur-Lez Cedex, France.

MGM Jansen, National Plant Protection Organization (NVWA), Geertjesweg 15, 6706 EA Wageningen, The Netherlands.

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.

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APPENDIX 1 - PREPARATION OF IMMATURE WHITEFLIES FOR MICROSCOPIC EXAMINATION (ADAPTED FROM PM 7/35(1))

Specimens usually need to be macerated, de-waxed, dehydrated, cleared and bleached, before mounting on microscope slides. Post-emergence pupal cases and early larval instars are particularly suitable for temporary quick-mounting. The permanent preparation technique given below is modified from Martin (1987). The procedures are not rigid and can be readily modified to suit particular samples. Specimens are manipulated and mounted on microscope slides with the aid of a binocular dissecting microscope. Heat is supplied, where necessary, by a heating block. Square-based watch glasses and glass slides should be accurately labelled with a waterproof marker throughout the procedure. The permanent preparation technique requires a minimum time of just over 1 h.

Materials

Ammonium solution 35%; Canada balsam; chloral phenol (160 g chloral hydrate crystals; 20 mL glucose syrup 50% w/w; 160 g phenol crystals); clove oil; distilled water; ethanol 70%–95%; glacial acetic acid; hydrogen peroxide 30-volume; potassium hydroxide (KOH) 10%.

Procedures

Permanent slide preparation

The best mounts are usually made from 'pupal cases' from which the adults have recently emerged although good results can be obtained from puparia with adequate maceration. Parasitized specimens should be avoided as they are often morphologically atypical. Parasitism can cause the puparium to become melanic, induce morphological variation, damage the puparium with the parasitoid exit hole and obscure diagnostic characters with the black fragments of ecdysed parasitoid larval cuticle. Gently remove specimens from the leaf surface using a mounted blunt needle taking care not to puncture the specimen. Place about 10 specimens into 70%–90% ethanol in a watch glass, cover with a glass square and heat gently to around 80°C for 5–10 min. Fixation in hot alcohol hardens specimens and makes them less fragile, so they lose fewer setae during mounting. Add a few drops of cold 10% KOH to cool the alcohol. Pipette off the alcohol and KOH using a fine glass teat pipette, taking care not to accidentally suck up the specimens. Add approximately 1 mL of KOH and heat to around 80°C for 5–10 min, or until the specimens lose most of their colour. The length of time required varies considerably depending on the species, body size, wax secretions, how long the specimens have been preserved in alcohol, the particular instar and maturity. Pupal cases require little maceration. Puparia require longer and the process is helped by making a small ventral incision using a mounted needle. Examine the specimens under a binocular microscope. Where necessary, tease away the wax from the specimens using fine needles. With puparia, expel the liquefied body contents through the ventral incision using two fine spatulas. If the adult is well formed within the puparium it is often necessary to tease the body out. Parasitoid larvae and pupal cases and fungal hyphae are also removed. Parasitoid larvae are retained with the host specimens. Pipette off the excess macerant. Soak the specimens in about 2 mL of cold distilled water or 70% ethanol for a minimum of 10 min. This rinses out the KOH. Pipette off the liquid. Rinse the specimens in about 2 mL cold glacial acetic acid (this neutralizes any remaining KOH) which is then pipetted off. Add a few drops of liquid chloral phenol, a wax solvent, to the watch glass. Gently heat for 5–10 min, depending on how waxy the specimens are. Waxier specimens require longer. The wax interferes with staining if not adequately removed. Pipette off the chloral phenol. Rinse the specimens in glacial acetic acid to remove the chloral phenol. Pipette off the liquid. Black puparia require partial bleaching. Rinse specimens with a few drops of 95% ethanol. Decant the ethanol and add a few drops of cold ammonium solution. Add an equal number of drops of hydrogen peroxide 30-volume and watch the puparia carefully. When the puparia have become pale, decant the bleaching solution. Alternatively, the bleaching process may be stopped rapidly by adding a few drops of water-soluble acid. Add a few drops of clove oil, enough to allow the specimens to float freely, and leave for at least 10 min while the specimens clear. Using a fine spatula transfer a single specimen onto a clean glass slide, with the dorsal surface upwards. Parasitoid larvae are usually mounted with their host. Always mount specimens separately unless certain that they are the same species, in which case, mount up to six individuals on the same slide. Space the specimens evenly on the slide to prevent the coverslip from tilting when mounted. Absorb excess clove oil with the rolled corner of a tissue. Take care not to leave fibres from the tissue on the slide. Carefully apply a drop of dilute Canada balsam to the specimens on the slide. Rest one edge of a 16 or 18 mm diameter coverslip on the slide holding the opposite edge with a needle. Gently lower the coverslip with the needle onto the droplet of balsam covering the specimen. Take care to ensure that air is excluded and that the meniscus spreads outwards to the edge of the coverslip. Allow the coverslip to settle under its own weight. Label using Bristol board squares before placing in the collection to dry. Drying can take two months or more to complete. When dry scrape off excess balsam that has spread out from beneath the coverslip using a razor blade.

APPENDIX 2 - THE MOST IMPORTANT PUPARIAL CHARACTERS USED IN WHITEFLY IDENTIFICATION