

Normes OEPP EPPO Standards

Diagnostics
Diagnostic

PM 7/51



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Approval

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard. In the terms of Article II of the IPPC, EPPO Standards are Regional Standards for the members of EPPO.

Review

EPPO Standards are subject to periodic review and amendment. The next review date for this EPPO Standard is decided by the EPPO Working Party on Phytosanitary Regulations.

Amendment record

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

Distribution

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

Scope

EPPO Standards on Diagnostics are intended to be used by NPPOs in their capacity as bodies responsible for the application of phytosanitary measures. Standards on diagnostic protocols are concerned with the diagnosis of individual pests and describe different methods which can be used to detect and identify pests of phytosanitary concern for the EPPO region. General Standards on diagnostics are in preparation on: (1) the purpose of diagnostic protocols (which may differ according to the circumstances of their use); and (2) reporting and documentation of diagnoses.

In 1998, EPPO started a new programme to prepare diagnostic protocols for the regulated pests of the EPPO region (including the EU). The work is conducted by the EPPO Panel on Diagnostics and other specialist Panels. The objective of the programme is to develop an internationally agreed diagnostic protocol for each regulated pest. The protocols are based on the many years of experience of EPPO experts. The first drafts are prepared by an assigned expert author(s). They are written according to a 'common format and content of a diagnostic protocol' agreed by the Panel on Diagnostics, modified as necessary to fit individual pests. As a general rule, the protocol recommends a particular means of detection or identification which is considered to have advantages (of reliability, ease of use etc.) over other methods. Other methods may also be mentioned, giving their advantages/disadvantages. If a method not mentioned in the protocol is used, it should be justified.

The following general provisions apply to all EPPO Standards on Diagnostics:

- laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- 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
- laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

References

- EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).
- EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.
- FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).
- IPPC (1993) *Principles of plant quarantine as related to international trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).
- IPPC (2002) *Glossary of phytosanitary terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).
- OEPP/EPPO (2003) EPPO Standards PM 1/2(12): EPPO A1 and A2 lists of quarantine pests. *EPPO Standards PM1 General phytosanitary measures*, 5–17. OEPP/EPPO, Paris (FR).

Definitions

Regulated pest: a quarantine pest or regulated non-quarantine pest.
Quarantine pest: a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

Outline of requirements

EPPO Standards on Diagnostics provide all the information necessary for a named pest to be detected and positively identified by an expert (i.e. a specialist in entomologist, mycology, virology, bacteriology, etc.). Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then gives details on the detection, identification, comparison with similar species, requirements for a positive diagnosis, list of institutes or individuals where further information on that organism can be obtained, references (on the diagnosis, detection/extraction method, test methods).

Existing EPPO Standards in this series

Forty-one EPPO standards on diagnostic protocols have already been approved and published. Each standard is

numbered in the style PM 7/4 (1), meaning an EPPO Standard on Phytosanitary Measures (PM), in series no. 7 (Diagnostic Protocols), in this case standard no. 4, first version. The existing standards are:

- PM 7/1 (1) *Ceratocystis fagacearum*. *Bulletin OEPP/EPPO Bulletin* **31**, 41–44
- PM 7/2 (1) *Tobacco ringspot nepovirus*. *Bulletin OEPP/EPPO Bulletin* **31**, 45–51
- PM 7/3 (1) *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin* **31**, 53–60
- PM 7/4 (1) *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **31**, 61–69
- PM 7/5 (1) *Nacobbus aberrans*. *Bulletin OEPP/EPPO Bulletin* **31**, 71–77
- PM 7/6 (1) *Chrysanthemum stunt pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **32**, 245–253
- PM 7/7 (1) *Aleurocanthus spiniferus*. *Bulletin OEPP/EPPO Bulletin* **32**, 255–259
- PM 7/8 (1) *Aleurocanthus woglumi*. *Bulletin OEPP/EPPO Bulletin* **32**, 261–265
- PM 7/9 (1) *Cacoecimorpha pronubana*. *Bulletin OEPP/EPPO Bulletin* **32**, 267–275
- PM 7/10 (1) *Cacysus marshalli*. *Bulletin OEPP/EPPO Bulletin* **32**, 277–279
- PM 7/11 (1) *Frankliniella occidentalis*. *Bulletin OEPP/EPPO Bulletin* **32**, 281–292
- PM 7/12 (1) *Parasaissetia nigra*. *Bulletin OEPP/EPPO Bulletin* **32**, 293–298
- PM 7/13 (1) *Trogoderma granarium*. *Bulletin OEPP/EPPO Bulletin* **32**, 299–310
- PM 7/14 (1) *Ceratocystis fimbriata* f. sp. *platani*. *Bulletin OEPP/EPPO Bulletin* **33**, 249–256
- PM 7/15 (1) *Ciborinia camelliae*. *Bulletin OEPP/EPPO Bulletin* **33**, 257–264
- PM 7/16 (1) *Fusarium oxysporum* f. sp. *albedinis*. *Bulletin OEPP/EPPO Bulletin* **33**, 265–270
- PM 7/17 (1) *Guignardia citricarpa*. *Bulletin OEPP/EPPO Bulletin* **33**, 271–280
- PM 7/18 (1) *Monilinia fructicola*. *Bulletin OEPP/EPPO Bulletin* **33**, 281–288
- PM 7/19 (1) *Helicoverpa armigera*. *Bulletin OEPP/EPPO Bulletin* **33**, 289–296
- PM 7/20 (1) *Erwinia amylovora*. *Bulletin OEPP/EPPO Bulletin* **34**, 159–172
- PM 7/21 (1) *Ralstonia solanacearum*. *Bulletin OEPP/EPPO Bulletin* **34**, 173–178
- PM 7/22 (1) *Xanthomonas arboricola* pv. *corylina*. *Bulletin OEPP/EPPO Bulletin* **34**, 179–182
- PM 7/23 (1) *Xanthomonas axonopodis* pv. *dieffenbachiae*. *Bulletin OEPP/EPPO Bulletin* **34**, 183–186
- PM 7/24 (1) *Xylella fastidiosa*. *Bulletin OEPP/EPPO Bulletin* **34**, 187–192
- PM 7/25 (1) *Glomerella acutata*. *Bulletin OEPP/EPPO Bulletin* **34**, 193–200
- PM 7/26 (1) *Phytophthora cinnamomi*. *Bulletin OEPP/EPPO Bulletin* **34**, 201–208
- PM 7/27 (1) *Puccinia horiana*. *Bulletin OEPP/EPPO Bulletin* **34**, 209–212
- PM 7/28 (1) *Synchytrium endobioticum*. *Bulletin OEPP/EPPO Bulletin* **34**, 213–218
- PM 7/29 (1) *Tilletia indica*. *Bulletin OEPP/EPPO Bulletin* **34**, 219–228
- PM 7/30 (1) *Beet necrotic yellow vein benyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 229–238
- PM 7/31 (1) *Citrus tristeza closterovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 239–246
- PM 7/32 (1) *Plum pox potyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 247–256
- PM 7/33 (1) *Potato spindle tuber pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **34**, 257–270
- PM 7/34 (1) *Tomato spotted wilt tospovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 271–280
- PM 7/35 (1) *Bemisia tabaci*. *Bulletin OEPP/EPPO Bulletin* **34**, 281–288
- PM 7/36 (1) *Diabrotica virgifera*. *Bulletin OEPP/EPPO Bulletin* **34**, 289–294
- PM 7/37 (1) *Thaumetopoea pityocampa*. *Bulletin OEPP/EPPO Bulletin* **34**, 295–298
- PM 7/38 (1) *Unaspis citri*. *Bulletin OEPP/EPPO Bulletin* **34**, 299–302
- PM 7/39 (1) *Aphelenchoides besseyi*. *Bulletin OEPP/EPPO Bulletin* **34**, 303–308
- PM 7/40 (1) *Globodera rostochiensis* and *Globodera pallida*. *Bulletin OEPP/EPPO Bulletin* **34**, 309–314
- PM 7/41 (1) *Meloidogyne chitwoodi* and *Meloidogyne fallax*. *Bulletin OEPP/EPPO Bulletin* **34**, 315–320

Some of the Standards of the present set result from a different drafting and consultation procedure. They are the output of the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98-2252). This project involved four ‘contractor’ diagnostic laboratories (in England, Netherlands, Scotland, Spain) and 50 ‘inter-comparison’ laboratories in many European countries (within and outside the European Union), which were involved in ring-testing the draft protocols. The DIAGPRO project was set up in full knowledge of the parallel activity of the EPPO Working Party on Phytosanitary Regulations in drafting diagnostic protocols, and covered regulated pests which were for that reason not included in the EPPO programme. The DIAGPRO protocols have been approved by the Council of EPPO as EPPO Standards in series PM 7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnosics¹ Diagnostic

Aonidiella citrina

Specific scope

This standard describes a diagnostic protocol for *Aonidiella citrina*.

Specific approval and amendment

Approved in 2004-09.

Introduction

Aonidiella citrina is a highly polyphagous armoured scale insect attacking plants belonging to more than 50 genera in 32 families. The scale of the adult female is circular, 1.5–2 mm in diameter, flat, yellow-brown, composed of waxy secretions and exuviae of previous instars. The male scale is oval elongate and smaller. The fully grown adult female is reniform and sclerotized while the young adult female is circular and teneral. This species is morphologically close to *Aonidiella aurantii* (Maskell). *A. citrina* is a serious pest, mainly of citrus, causing leaf drop, twig die-back and fruit drop. It apparently originated in the Far East, from where it has spread to various tropical and subtropical regions of the world (CABI/EPPO, 1997). Its precise distribution is uncertain due to difficulties in separating it from *A. aurantii*, which is a common pest of citrus throughout the Mediterranean region and in all major citrus-growing areas of the world. In the Mediterranean basin, *A. citrina* has been recorded in southern France (Germain & Bertaux, 2002), southern Italy (Longo *et al.*, 1994), Libya and Turkey. The insect has been recorded from citrus, banana, guava, mango, olive and peach, from forest trees such as *Acacia*, *Eucalyptus* and poplars, and from ornamental woody plants.

Identity

Name: *Aonidiella citrina* (Coquillett, 1891)

Synonyms: *Aspidiotus citrinus* Coquillett (1891), *Aspidiotus aurantii* var. *citrinus* (Coquillett), *Aonidiella aurantii* var. *citrina* (Coquillett), *Chrysomphalus aurantii citrinus* (Coquillett)

Taxonomic position: *Insecta: Hemiptera: Sternorrhyncha: Diaspididae*

EPPO computer code: AONDCI

Phytosanitary categorization: EU Annex designation: II/A1

Detection

A. citrina can be detected by visual examination of fruits and leaves, but careful examination is required when the population is very low. It is rarely present on bark (compared with *A. aurantii*, which occurs on all aerial parts of the plant). Heavy infestations may result in leaf drop, dieback of apical twigs and discoloured, stunted and pitted fruits which fall prematurely or are unmarketable. *A. citrina* has been detected on consignments of citrus fruits, mainly on *Citrus sinensis*.

Because adult females are fixed onto the leaf or fruit, they should be removed from the host plant with an angled spatula under a low-power microscope.

Identification

No reliable identification of *A. citrina* can be achieved by macroscopic field examination. Though the scale of *A. citrina* is usually more yellow than that of *A. aurantii*, its colour may be affected by the background colour of the host plant, by parasitism or by maturity. Authoritative identification can only be based on a study of slide-mounted specimens (Appendix 1) examined with a high-power microscope. Important diagnostic morphological characters are the wax-producing ducts, pores, plates and setae and internal apophyses (Appendix 2).

For the identification of *Aonidiella* spp., see McKenzie (1938, 1946). A key for the identification of coccid families is available at: http://www.sel.barc.usda.gov/scalekeys/all_families.htm.

Family *Diaspididae*

A key to the families of Coccoidea is given by Kosztarab & Kozár (1988). The *Diaspididae* (armoured scales; Gill, 1997)

¹The Figures in this Standard marked 'Web Fig.' are published on the EPPO website www.eppo.org.

are easily recognizable by the presence of a detached scale covering the body of the insect (except in crawler and adult male). The feature which is most diagnostic of the adult female and larval instars is the reduction and fusion of the terminal abdominal segments into a pygidium bearing the anal opening, wax glands and various marginal appendages (lobes, plates and gland spines).

Genus *Aonidiella*

The genus *Aonidiella* may be identified using the following combination of characters for the adult female (Web Figs 1 and 2): normally with plates or gland spines, or second stage presenting them; macroducts commonly showing some evidence of arrangement in segmental rows or series; pygidial lobes usually present, median lobes rarely united into single lobe, otherwise various. Macroducts one-barred type, second pygidial lobes never bilobulate; fringed plates normally present; gland tubercles rarely or never present; anterior spiracles normally without associated disc pores; antennae of adult female rarely with more than one seta. Never more than five groups of perivulvar pores; lobes and plates normally present, if lacking, paraphyses are present. Second exuvia smaller than and not at all enveloping. Pygidium with paraphyses arising from bases of lobes or at site of obsolete lobes (other than a mere prolongation of base of median lobes, and other than a mere sclerotization of folds about a pore or pore furrow), these sometimes small but usually clearly recognizable. At least one paraphysis arising about the centre of at least one interlobular space, ordinarily that between second and third lobes, frequently with paraphyses along margin beyond third lobe. Prosoma of mature adult female much swollen and strongly sclerotized, varying in form slightly

reniform to markedly so, lateral prosomatic lobes more or less enclosing pygidium (after Gill, 1997).

Aonidiella citrina

Description

Characteristics (adult female of *A. citrina*): scale of female circular, translucent, the yellow body of the adult female showing through, exuviae central or subcentral; scale of the male yellow-grey and elongate. Microscopical characters (slide-mounted female ducts): typically reniform, the prosomatic region heavily sclerotized; perivulvar pores lacking; prevulvar apophyses present but the prevulvar scleroses lacking; paravulvar fold usually not strongly sclerotized; three pairs of well developed pygidial lobes present, these usually somewhat more slender than in *A. aurantii* and all nearly the same size; fourth lobe usually quite strongly developed as a sclerotized point; plates not present beyond the fourth lobe; dorsal ducts of the pygidium in three quite well-defined rows, those of the median row of the same length and diameter as the others (after McKenzie, 1938). Table 1 presents a key for the identification of *Aonidiella citrina* (after McKenzie, 1938, 1946).

Diagnostic characters

The essential distinguishing characters are the presence of prevulvar apophyses and the absence of prevulvar scleroses (Web Fig. 2). About 12 adult females should be slide-mounted and observed. Additional diagnostic characters are: dorsal ducts 1-barred; plates fringed; pygidial margin with paraphyses arising from bases of lobes and in interlobular area; prosoma of

Table 1 Key for identification of *Aonidiella citrina* (after McKenzie, 1938, 1946)

1	Perivulvar pores present	2
	Perivulvar pores absent	5
2	Perivulvar pores in two groups	<i>comperei</i>
	Perivulvar pores in four or five groups	3
3(2)	Prosomatic lobes produced and broadly rounded	<i>eremocitri</i>
	Prosomatic lobes but little produced	4
4(3)	Three prepygidial abdominal segments bearing conspicuous clusters of ducts	<i>orientalis</i>
	Not more than one prepygidial abdominal segment with ducts and this with but two or three	<i>gracilis</i>
5(1)	Prosomatic lobes strongly produced and broadly rounded, typically reniform	7
	Prosomatic lobes but little produced, less reniform	6
6(5)	Three prepygidial abdominal segments with clusters of ducts	<i>andersonii</i>
	Prepygidial abdominal segments each with not more than one or two ducts	<i>settsu</i>
7(5)	Prevulvar apophyses present	8
	Prevulvar apophyses absent	9
8(7)	Prevulvar scleroses and apophyses present	<i>aurantii</i>
	Prevulvar apophyses alone present	<i>citrina</i>
9(7)	Pygidial lobes all large and approximately of the same size and shaped, thoracic tubercle present	<i>taxus</i>
	Pygidial lobes variable but the second and third lobes always smaller than the median pair, thoracic tubercle lacking	<i>inornata*</i>

*Williams & Watson (1988) have remarked that *A. inornata* McKenzie may be a synonym of *A. comperei* or *A. eremocitri* McKenzie and that the group is in need of revision.

mature female expanded postero-laterally almost enclosing pygidium, giving the body a reniform shape; plates lateral to third lobe fringed; perivulvar pores absent.

A. citrina is one of a group of several similar species composed of *A. aurantii* (Maskell), *A. comperei* McKenzie, *A. eremocitri* McKenzie, *A. inornata* McKenzie, *A. orientalis* (Newstead) and *A. taxus* Leonardi, with a strongly reniform prosoma, heavily sclerotized at maturity. *A. citrina* is morphologically closely related to *A. aurantii*, from which it differs by the presence ventrally of only the basal apophyses like a slender inverted 'V' on the pygidium (Web Figs 1 and 2); in *A. aurantii*, each basal apophysis is accompanied by 2 submedian transversal scleroses (Web Fig. 2) (McKenzie, 1937). Although absence of these scleroses is an important distinguishing character, there is intraspecific variation in the shape of the pygidial sclerosis (Web Fig. 2) and in the colour of the living insect. Living adult females of *A. citrina* are bright lemon-yellow, with scales yellow-brown, while females of *A. aurantii* are reddish-brown, with scales orange-red.

Reference material

Coquillett (1891), USDA Division of Entomology Bulletin 23, 29. Type: United States National Museum (NH), Beltsville (US).

Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM7/- (in preparation).

Further information

Further information on this organism can be obtained from:
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Acknowledgements

This protocol was originally drafted by J.-F. Germain, LNPV, Montpellier (FR).

References

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Appendix I

Preparation of scales

There are several procedure for preparing scales. The one described here is adapted from Williams & Watson (1988) and Ben-Dov & Hodgson (1997).

Heat specimen gently in 10% KOH solution for ~20 min (not exceeding 40°C). The timing depends of the size and maturity of the specimen. The internal tissues will dissolve more readily if a dorso-lateral incision is made. Flatten the body contents by gentle pressure with a spatula (use a mandrel with flattened fishing thread). Clean specimen by placing in distilled water for 20 min. Stain for 1 h in acid fuchsin-saturated lactophenol. Wash in glacial acetic acid for 1 h to stabilize the staining. Transfer to lavender oil for at least 1 h. To mount the specimen, place in a drop of Canada balsam on a slide and cover with a cover-slip. Label and hold at 40°C (and no more) for 4–6 weeks to cure.

Appendix II

Glossary of terms relating to scales

- Apophyses: sclerotized areas.
 1-barred ducts: ducts with inner end formed by a single bar.
 2-barred ducts: ducts with inner end formed by two bars.
 Crawler: vernacular name for the ambulatory stage (first nymphal instar).
 Exuviae: the portion of the integument of a larva or nymph that is shed from the body and incorporated into the scale cover.
 Gland spines: spine-like structures, each having a microduct opening at its apex.
 Gland tubercles: glands looking like small rounded protuberances.
 Lobes: the rounded, tooth like processes on the margin of the pygidium.

Macroducts (macropores): gland with a channel with a large diameter.

Microducts (micropores): gland with a channel with a narrow diameter.

Paraphyses: elongate sclerotized structures arising from the dorsal angles of the lobes and other parts of the margins.

Plates: fringed processes projecting from the pygidium, may or may not possess a microduct.

Prosoma: cephalothorax.

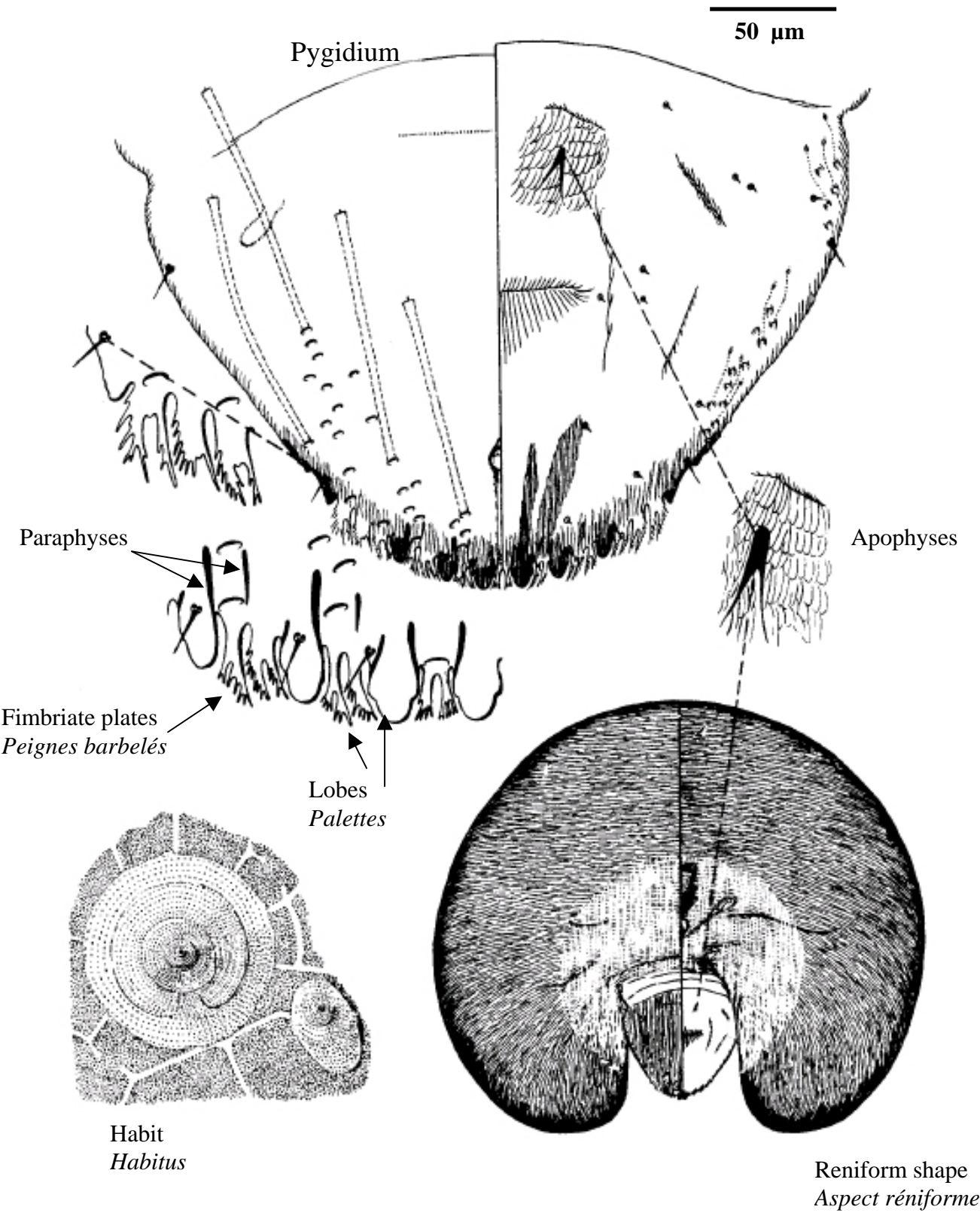
Pupillarial: adult females are enclosed in the nymphal exuviae.

Pygidium: a strongly sclerotized unsegmented region terminating the abdomen of the adult female.

Scleroses: additional apophyses.

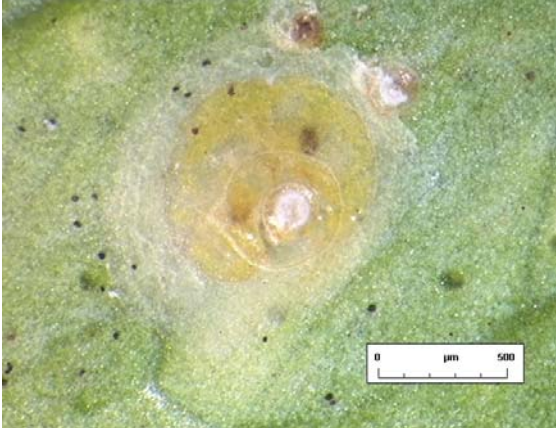
For further information, see entomological dictionaries such as Gordh & Headrick (2001) and Seguy (1967).

Web Fig. 1 Morphological description of *Aonidiella citrina* (Coquillett)



Web Fig. 2 Morphological differences between *Aonidiella citrina* and *Aonidiella aurantii*

Aonidiella citrina



Habit/Habitus

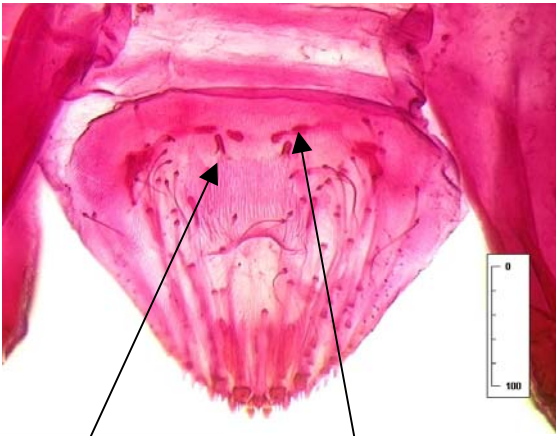
Aonidiella aurantii



Habit/Habitus



Preulvar apophyses
Apophyses préulvaires



Preulvar apophyses and sclerites
Apophyses préulvaires et scléroses

