Organisation Européenne et Méditerranéenne pour la Protection des Plantes
European and Mediterranean Plant Protection Organization

Normes OEPP
EPPO Standards

Diagnostics
Diagnostic

PM 7/55

Organisation Européenne et Méditerranéenne pour la Protection des Plantes
1, rue Le Nôtre, 75016 Paris, France

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) *Cacyreus 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.

Diagnostics[1]
Diagnostic

Rhizoecus hibisci

Specific scope

This standard describes a diagnostic protocol for *Rhizoecus hibisci*.

Specific approval and amendment

Approved in 2004-09.

Introduction

Mealybugs of the genus *Rhizoecus* are widespread all over the world (118 species listed in Ben-Dov, 1994). Most of them occur in neotropical and nearctic areas. They are hypogeal species, living on roots, and trade of pot plants has facilitated their spread outside their native areas. All species of *Rhizoecus* are regarded as potential pests (Williams, 1996).

Rhizoecus hibisci occurs in China, Japan, Taiwan and USA (Florida, Hawaii and Puerto Rico) (Beardsley, 1995; Williams, 1996). Despite regular detection in consignments imported into Europe, in the Netherlands since 1989 (Jansen, 2001) and in Italy (Pellizzari & Dalla Montà, 1997), *R. hibisci* is not established in Europe.

R. hibisci is a polyphagous species that can develop on plants of many plant families (OEPP/EPPO, 2005), both woody (e.g. *Oleaceae*, *Rhamnaceae*, *Rutaceae*, *Ulmaceae*) and herbaceous (e.g. *Crassulaceae*, *Geraniaceae*), and also monocotyledonous (e.g. *Araceae*, *Arecaceae*, *Poaceae*). It is typically associated in trade with perennial ornamental pot plants, and in particular bonsai plants.

Identity

Name: *Rhizoecus hibisci* (Kawai & Takagi, 1971)
Synonyms: *Ripersiella hibisci* (Kawai & Takagi, 1971)
Taxonomic position: *Insecta*: *Hemiptera*: *Sternorrhyncha*: *Coccoidea*: *Pseudococcidae*
EPPO computer code: RHIOHI
Phytosanitary categorization: EPPO list A1, EU Annex designation I/AII

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

Detection

All *Rhizoecus* spp. are hypogeal and feed on roots. Symptoms on the plant are difficult to detect. Slow plant growth and leaf deterioration may be signs of the presence of the pest. Plants that are slow-growing, root-bound, or under environmental or nutritional stress, are more susceptible to attack. It is mainly potted plants (especially bonsai plants) that are concerned during import inspections. The pot should be removed and roots examined for waxy secretions. In case of heavy infestations, crawlers may be observed on the soil surface.

Identification

R. hibisci has an egg stage, three nymphal stages and one adult female stage. The male has four nymphal stages (Jansen, 2001). No keys exist for nymphal stages or males. The taxonomy of the *Coccoidea* is almost entirely based on the adult female and a good slide preparation of a teneral (young) female is required for identification to species level (for the technical procedure see Appendix I). For terminology, see Appendix II.

Family *Pseudococcidae*

A key to the families of *Coccoidea* is given by Kosztarab & Kozár (1988), based on female structures. The family may be identified using the following combination of characters: body of the female normally elongate to broadly oval, usually membranous, often with a pair of anal lobes, each terminating in an apical seta. Antennae each normally 6–9 segmented, but sometimes reduced. Legs normally present, each with a single tarsal segment and a single claw. Claw with a pair of digitules at base and often a denticle on plantar surface. Translucent pores frequently present on hind legs. Ostioles normally present. Circulus present or absent. Anal ring present, normally

with at least 2 rows of cells and 6 setae. Cerarii present, sometimes absent entirely. Trilocular pores usually present and often abundant on dorsum and venter. Multilocular disc pores often present, at least on venter. Quinquelocular pores present in some genera, usually on venter, rarely on dorsum. Oral collar tubular ducts normally present on venter, less frequently on dorsum. Oral rim tubular ducts sometimes present, at least on dorsum, rarely present on venter only (Williams & Granara de Willink, 1992). The *Pseudococcidae* is the second largest family in the *Coccoidea* and contains some 288 genera, with 1947 species and subspecies (Ben-Dov, 1994).

Kozár & Konczné Benedicty (2002) give a key to the genera of the tribe *Rhizoecini* & Williams (1998) a key of the subfamily *Rhizoecinae*.

Genus *Rhizoecus*

Adult females of the genus *Rhizoecus* (as defined by Williams & Watson (1988) and Williams & Granara de Willink (1992)) can be recognized by the following characters:

- legs present
- hind coxae normally same size as anterior and median coxae, or no more than twice the size
- anal lobe only moderately produced, even if well posterior to level of anal ring, or inconspicuous, sometimes posterior end of body rounded
- recognizable cerarii absent from anal lobe; if cerarian setae are present they are flagellate, either elongate resembling apical setae, or short
- bitubular or tritubular pores present
- spiracles each with normal, slender apodeme. No crescentic area around each spiracular opening. Legs well developed
- body setae pointed, usually flagellate
- body at maturity remaining membranous. Large ducts absent. If ducts are present they are narrower than the trilocular pores, normally with ends not cupped
- circulus if present, round, not divided by intersegmental line. Oral rim tubular ducts absent. Anal lobe bars absent
- anal ring normal, in dorsal position, cellular. Antennae each with 5 or 6 segments, strongly geniculate
- body setae normally abundant. Tubular ducts if present with circular orifices
- anus symmetrical, usually circular, with six setae and usually with large, conspicuous, often elongate cells.

Rhizoecus hibisci

No key exists to identify all *Rhizoecus* spp. The information is scattered, and a revision of the genus is needed. The following literature, which includes identification keys, concerns the species occurring in glasshouses and detected during import inspection in Europe: Hambleton (1976); Jansen (2003), Marotta (1995); Tang (1992) and Williams (1962, 1996).

In the EPPO area, the genus *Rhizoecus* is represented by nearly 26 species (Ben-Dov, 1994; Foldi, 2001; Longo *et al*., 1995). *R. hibisci* is close to the polyphagous *R. cacticans* (Hambleton), *R. dianthi* (Green), *R. falcifer* Künckel d'Herculais and *R. americanus* (Hambleton). The last has only been recorded from Italy. A key for the identification of *R. hibisci* and the most common polyphagous species present in Europe, adapted from Pellizzari & Pavan (1994) and Williams (1996), is presented in Table 1. A new species (*R. maasbachi*) described by Jansen (2003) has been detected on penjing plants of *Sageretia* spp. ('bonsai' from China). *R. hibisci* and *R. maasbachi* are the only two species regularly detected on Chinese penjing/bonsai and could be confused with one another. In *R. maasbachi*, eyes are present and the antennae are 6-segmented, one circulus is present, tubular ducts and dorsal multilocular pores absent, antennae relatively slender, about five times longer than wide (Jansen, 2003). In *R. hibisci*, the eyes are absent and antennae are 5-segmented.

Table 1 Key for identification of *Rhizoecus hibisci* and the most common polyphagous species present in Europe, adapted from Pellizzari & Pavan (1994) and Williams (1996)

1	Tritubular pore present	2
	Bitubular pores present	3
2	Antenna-5 segmented	*falcifer*
	Antenna-6 segmented	4
3	Small bitubular pores present on dorsum	*hibisci*
	Small bitubular pores absent from dorsum	*saintpauliae*
4	Multilocular disc pores present	5
	Multilocular disc pores absent	*cacticans*
5	Few multilocular disc pores present only on the last abdominal segment, ventral side	*dianthi*
	Multilocular disc pores present, ventral side, on abdominal, thoracic and head segments	*americanus*

Description of *Rhizoecus hibisci*

Adult female (Web Fig. 1): appearance fleshy to pinkish colour. Body with a layer of greyish powdery, mealy secretion. Body of field collected adult female is elongate oval, flattened ventrally and convex dorsally, length 1.2–2.35 mm.

Microscopic characters of slide-mounted females (Web Fig. 2): anal lobes moderately developed, each with a distinct sclerotized area and 3 or 4 long setae. Antennae each 5-segmented, with the apical segment wider than preceding segment, about twice as long as wide, with 4 falcate setae (it is often necessary to examine a few specimens to determine reliably the correct number of antennal segments; asymmetry, secondary annulation and fusion of antennal segments in some specimens may be confusing). Legs well developed, fairly stout, claws with short, setose digitules. Eyes absent. Cephalic plate present, triangular, but sometimes not discernible. 0–2 circuli. Ostioles present, with inner edges of lips sclerotized. Anal ring about 60 µm wide, its 6 setae slightly longer than diameter of the ring, with 12 cells in outer row. Trilocular pores and body setae evenly distributed. Bitubular pores of 2 sizes: a large type, each with stout ducts, present on dorsum, and a smaller type present on both sides of abdomen. Tubular ducts absent. Multilocular disc pores numerous, present on dorsum across most abdominal

segments and occasionally on thorax, more numerous on venter on abdominal segments and thorax (Williams & Granara de Willink, 1992).

A more complete description can be found in Kawai & Takagi (1971) and in Williams (1996). For information on the life cycle of *R. hibisci* and description of nymphal instars, see Jansen (2001).

Reference material

Kawai & Takagi (1971). Syntype females, Japan, Tokyo, Tachikawa and Kyushu, Kagoshima, on 10 species of plants.

Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM7/– (in preparation).

Further information

Further information on this organism can be obtained from:

M. G. M. Jansen, Plant Protection Service, Entomology Section, PO Box 9102, 6700 HC Wageningen (Netherlands)

J.-F. Germain, LNPV-Unité d'entomologie. Zoologie. 2, place Viala F-34060 Montpellier Cedex 1 (France).

Acknowledgements

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

References

Beardsley JW (1995) Notes on two *Rhizoecus* species new to the Hawaiian islands, with a revised key to Hawaiian hypogaeic mealybugs (Homoptera: Pseudococcidae: Rhizoecinae). *Bishop Museum Occasional Papers* **42**, 28–29.

Ben-Dov Y (1994) *A Systematic Catalogue of the Mealybugs of the World*. Intercept, Andover (GB).

Ben-Dov Y & Hodgson CJ (1997) *World Crop Pests 7A Soft Scale Insects Their Biology, Natural Enemies and Control*, pp. 389–395. Elsevier, Amsterdam (NL).

Foldi I (2001) Liste des cochenilles de France (Hemiptera, Coccoidea). *Bulletin de la Société Entomologique de France* **106**, 303–308.

Gordh G & Headrick DH (2001) *A Dictionary of Entomology*. CAB International, Wallingford (GB).

Hambleton EJ (1976) A revision of the New World mealybugs of the genus *Rhizoecus*. *Technical Bulletin United States Department of Agriculture* **1522**, 1–188.

Jansen MGM (2001) Instar identification and some notes about the life cycle of *Rhizoecus hibisci*. *Bollettino di Zoologia Agraria e di Bachicoltura Series* **II** (33), 53–66.

Jansen MGM (2003) A new species of *Rhizoecus* on bonsai trees. *Tijdschrift voor Entomologie* **146**, 297–300.

Kawai S & Takagi K (1971) Descriptions of three economically important species of root-feeding mealybugs in Japan (Homoptera: Pseudococcidae). *Applied Entomology and Zoology* **6**, 175–182.

Kosztarab M & Kozár F (1988) *Scale Insects of Central Europe*. Akadémiai Kiadó, Budapest (HU).

Kozár F & Konczné Benedicty Z (2002) Description of the *Marottarhizoecus issisi* gen. et sp. nova (Homoptera, Coccoidea, Pseudococcidae, Rhizoecinae) from Africa with a review and key of the subfamily. *Bollettino di Zoologia Agraria e di Bacchicoltura Series* **II** (34), 213–218.

Longo S, Marotta S, Pellizzari G, Russo A & Tranfaglia A (1995) An annoted list of the scale insects (Homoptera: Coccoidea) of Italy. *Israel Journal of Entomology* **29**, 113–130.

Marotta S (1995) [Two *Rhizoecus* species new to the Italian fauna]. *Bollettino di Zoologia Agraria e di Bachicoltura* **27**, 117–121 (in Italian).

OEPP/EPPO (2005) Data sheets on quarantine pests: *Rhizoecus hibisci*. *Bulletin OEPP/EPPO Bulletin* **35** (3) (in press).

Pellizzari G & Dalla Montà L (1997) [Phytophagous insects introduced into Italy from 1945 to 1995.] *Informatore Fitopatologico* no. 10, 4–12 (in Italian).

Pellizzari G & Pavan F (1994) [Detection of *Rhizoecus saintpauliae* on bonsai plants imported from Japan.]. *Bollettino di Zoologia Agraria e di Bacchicoltura Series* **II** (26), 275–278 (in Italian).

Seguy E (1967) *Dictionnaire des termes d'entomologie*. P. Lechevalier, Paris (FR).

Tang FT (1992) [*The Pseudococcidae of China*.] Chinese Agricultural. Science Technology Press, Beijing (CN) (in Chinese).

Watson GW & Chandler LR (1999) *Identification of Mealybugs Important in the Caribbean Region*. CAB International, Wallingford (GB).

Williams DJ (1962) The British Pseudococcidae (Homoptera: Coccoidea). *Bulletin of the British Museum (Natural History) Entomology* **12**, 1–79.

Williams DJ (1996) Four related species of root mealybugs of the genus *Rhizoecus* from east and southeast Asia of importance at quarantine inspection (Hemiptera: Coccoidea: Pseudococcidae). *Journal of Natural History* **30**, 1391–1403.

Williams DJ (1998) Mealybugs of the genera *Eumyrmococcus* and *Xenococcus* associated with the ant genus *Acropyga* and a review of the subfamily Rhizoecinae (Hemiptera, Coccoidea, Pseudococcidae). *Bulletin of the Natural History Museum Entomology Series* **67**, 1–64.

Williams DJ & Granara de Willink MC (1992) *Mealybugs of Central and South America*. CAB International, Wallingford (GB).

Williams DJ & Watson GW (1988) *The Scale Insects of the Tropical South Pacific Region. Part 2 Mealybugs*. CAB International, Wallingford (GB).

Appendix I

Preparation of mealybugs

General information about the preparation of mealybugs, the steps in slide-making, recipes for slide-making reagents, storage of microscopic slide mounts for reference and use and care of microscopes is given by Watson & Chandler (1999). The following procedure is adapted from Williams & Granara de Willink (1992) and Ben-Dov & Hodgson (1997):

Heat specimen gently in 90% alcohol for a few minutes. Transfer to a 10% solution of KOH, heat for about 20 min (never exceeding 40°C). Timing depends of the size and maturity of the specimen. Make a dorso-lateral incision to speed up the maceration of internal tissues. Expel the body contents by gentle pressure with a spatula (use a mandrel with flattened fishing thread). Clean in distilled water. Retain in cleaning solution for 20 min. Stain in acid fuchsin-saturated Aman lactophenol for 1 h. Wash in glacial acetic acid to eliminate excess stain for 1 h, changing the acid once. Transfer to lavender oil for at

least 1 h. Mount 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. Compare the slide preparation with a voucher specimen if possible.

Appendix II

Glossary

Circulus: ventral structure usually situated between abdominal segments 3 and 4. It is probably an adhesive organ.

Ostioles: these are slit-like organs whose function is probably defensive.

Cerarii: these structures give rise to the lateral wax filaments.

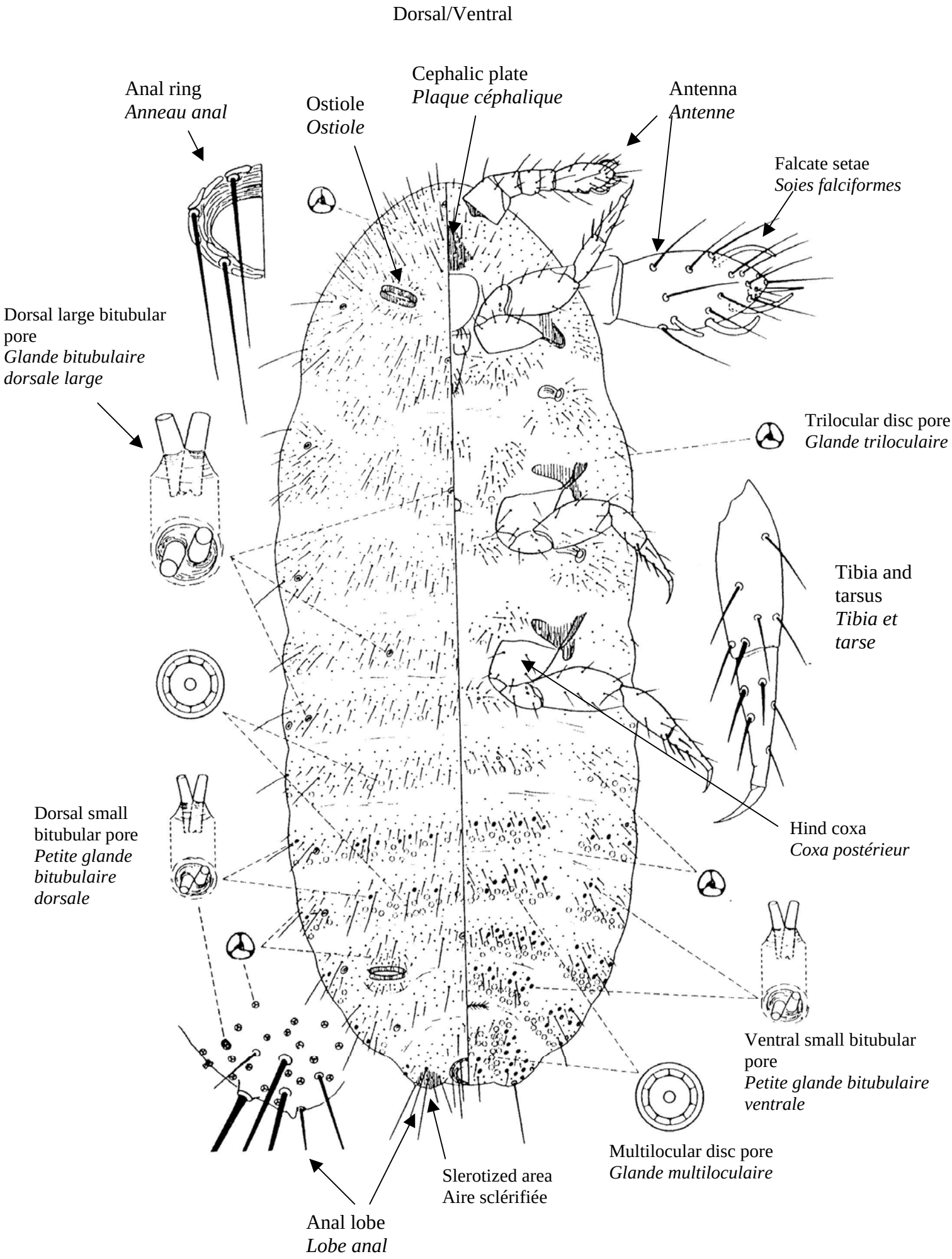
Digitules: seta-like structures near the bases of the claws.

Denticle: small teeth on the plantar surface of the claw.

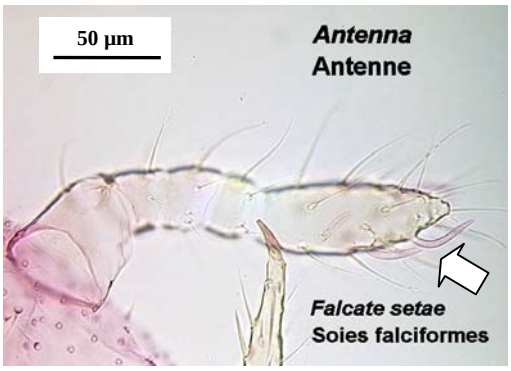
Translucent pores: structures often present on the posterior surface of the coxa, femur and tibia. Their function is unknown.

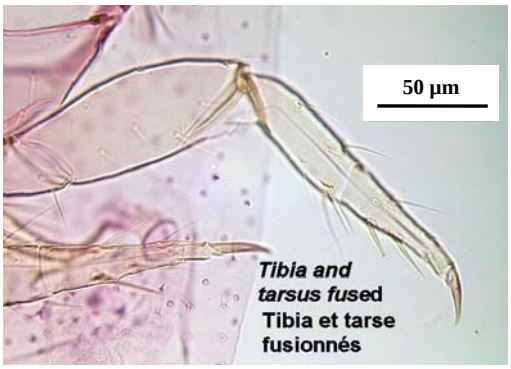
Tubular ducts: internal structures that normally produce filaments of wax that are incorporated into the formation of the ovisac.

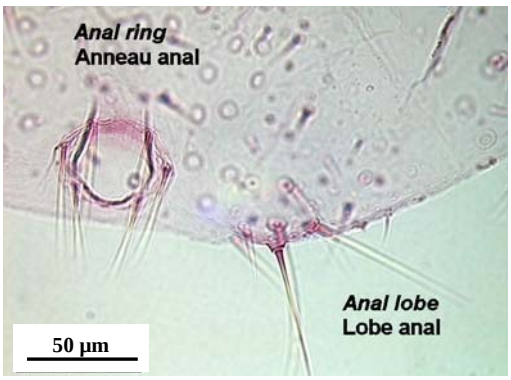
For further information, see entomological dictionaries such as Gordh & Headrick (2001), Seguy (1967).

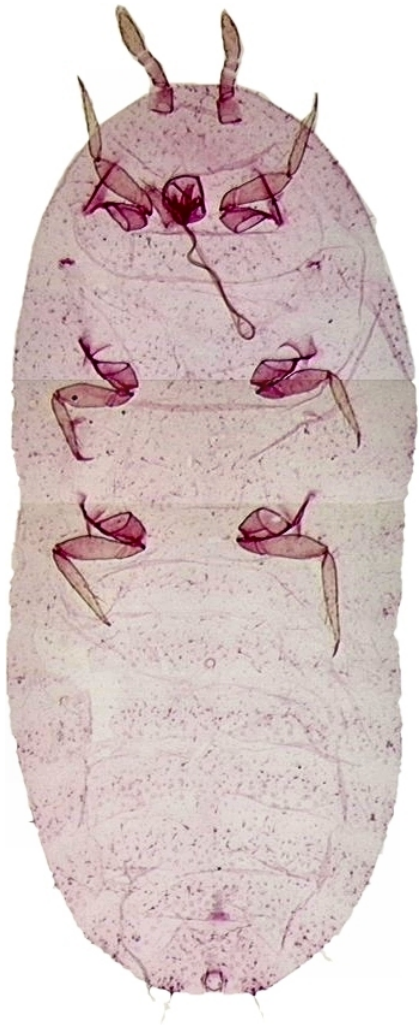


Web Fig. 1 Morphological characters of *Rhizoecus hibisci*

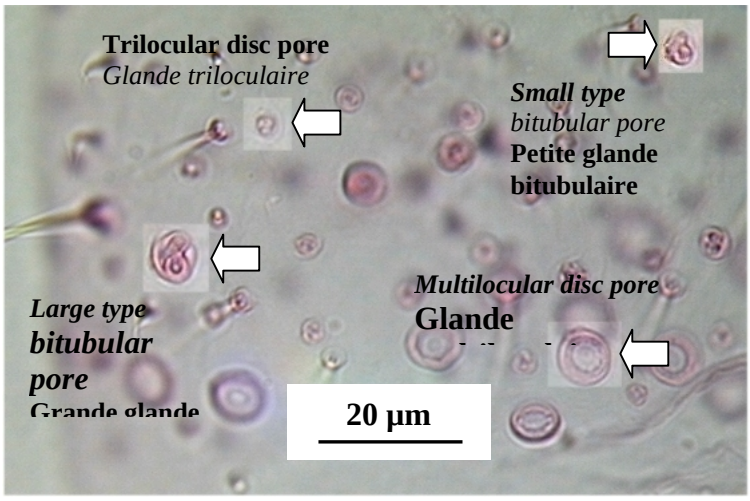








General aspect
Aspect général



***Dorsal view*/Face dorsale**

Web Fig. 2 *Rhizoecus hibisci*, slide mounted observations