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

Normes OEPP
EPPO Standards

Diagnostic protocols for regulated pests
Protocoles de diagnostic pour les organismes réglementés

PM 7/38

Organization 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 Diagnostic Protocols for Regulated Pests are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the application of phytosanitary measures to detect and identify the regulated pests of the EPPO and/or European Union lists.

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 diagnostic protocols:

- 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.

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 Diagnostic Protocols for Regulated Pests 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

Nineteen 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

Several 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 'intercomparison' 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 PM7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnostic protocols for regulated pests[1]
Protocoles de diagnostic pour les organismes réglementés

Unaspis citri

Specific scope

This standard describes a diagnostic protocol for *Unaspis citri*.

Specific approval and amendment

Approved in 2003-09.

Introduction

Unaspis citri (EPPO/CABI, 1997) is a serious pest widely distributed on citrus throughout the main citrus-growing areas of the world. It is often detected on consignments of citrus fruits in international trade. It as been reported to attack plants of 12 different genera belonging to 9 families, but it seems to prefer *Rutaceae*. Other hosts include: *Annona muricata*, *Musa paradisiaca*, *Cocos nucifera*, *Psidium guajava*, *Ananas comosus*, *Artocarpus heterophyllus* and *Tillandsia usneoides*. The origin of *U. citri* is in South-East Asia and it is now present in Australia, North, Central and South America, Pacific Islands, Egypt, Syria and China.

Identity

Name: *Unaspis citri* (Comstock), 1883.
Synonyms: *Chionaspis citri* Comstock, 1883; *Prontaspis citri* (Comstock, 1883); *Diaspis veitchi* Green & Laing, 1923.
Taxonomic position: Insecta, Hemiptera, Homoptera, *Diaspididae*.
Bayer computer code: UNASCI.
Phytosanitary categorization: EPPO A1 List: n° 226, EU Annex designation II/A1.

Detection

U. citri can be found on plants or parts (fruits, bark, leaves) of all citrus, especially orange (*C. sinensis*). In the field, symptoms of a heavy infestation are easily detected because of the snowy appearance of the plants, resulting from the presence of the numerous white scale covers of males. Infestation begins on the trunk but quickly spreads to branches and twigs, causing weakening of the tree and dieback of branches. This predisposes the tree to attack by other pests; leaves may develop yellow spots and fall.

U. citri is especially damaging on the trunk and larger limbs of older citrus trees. Infestation of the canopy (leaves and fruits) is only evident in the case of high population densities. Normal growth of the bark is prevented, making the trees become 'hidebound'. Although increase of trunk and limb diameter is resumed after a reduction of the pest population, the toughened bark may not be able to follow it, and may split, thus allowing access to borers and pathogens. The leaves show yellow areas where the scales are feeding, and heavily spotted leaves may drop prematurely. Twigs and even large limbs in the centre of the canopy may die. Infested fruits are stunted and have a pitted appearance.

Identification

Identification of armoured scales is performed firstly by studying the shape, dimension and colour of the cover. For this reason, the infested part of the plant have to be examined under a stereomicroscope. For an exact identification to species, the body of adult females should be studied, since there are no adequate keys for the separation of species based on nymphs or adult males. For identification, covers of specimens are moved with a needle to collect scale bodies. Specimens removed from under the cover have to be prepared for morphological study under a microscope. Among the several methods for preparation of scale insects, the method of Williams & Watson (1988) is convenient and effective (Appendix 1).

The female scale cover is oyster-shell shaped, brown or brown-black, with a distinct longitudinal dorsal ridge which, in some species, may be poorly developed. The female under the cover is bright orange. The length of scales is about 2–2.5 mm. The male scale covers are white and have three longitudinal ridges. The adult male is about 1 mm long, winged and light

yellow in colour. Good descriptions of the species are provided by Rao (1949), Balachowsky (1954) and Williams & Watson (1988).

The genus *Unaspis* MacGillivray belongs to the tribe Diaspidini and is characterized by the presence on the pygidium of 'two-barred' ducts, gland spines and bilobed second pygidial lobes (Web Fig. 3). Adult female with fusiform body, with the prosoma sclerotized at maturity (Web Fig. 1). Dorsal ducts numerous on pygidium, behind the VIII (median lobe) segment, 3 pairs of pygidial lobes, the median one not zygotic, the other bilobed. Gland spines absent from between median lobes but present laterally. Perivulvar pores present or absent. Pygidium with 3 pairs of prominent lobes, the median ones divergent, well developed, close together at base, slightly recessed in the pygidium. The second and third lobes rounded and bilobed. On pygidial margin single gland spines, more numerous after the second abdominal segment. There are 6 or 7 pairs of marginal macroducts. Pygidial ducts totalling 60–75. On ventral surface, perivulvar pores present or absent; if present, numbering 2–6. Presence of duct tubercles on metathorax and on first abdominal segment. Each anterior spiracle with 8–15 pores; the posterior ones with 3–5 pores.

Identification of adult female can be carried out with keys. Table 1 provides a simplified key which includes the other species commonly associated with citrus:

Comparison with similar species

U. citri can be confused with other species of armoured scale insects living on citrus. It differs from *Lepidosaphes* spp. in the colour of the male cover which is white in *U. citri* and brown in *Lepidosaphes* spp. Moreover, the adult female-cover usually has a median ridge that is not present on the cover of *Lepidosaphes*. In *Lepidosaphes* spp., gland spines, even if minute, are present between median lobes, whereas they are not present in *Unaspis*. Other similar species belong to the genus *Pinnaspis*, but they can be separated from *Unaspis* because of the presence in *Pinnaspis* spp. of medial lobes joined at the base. The arrowhead scale, *Unaspis yanonensis* (Kuwana) is already present in the EPPO region (Web Fig. 2) and it is very similar to *U. citri*. The two species can be separated using microscopic characters such as position of median lobes and number of pygidial macroducts, as described in Table 1. Moreover, *U. citri* is less often present on fruits and leaves than *U. yanonensis*.

Requirements for a positive diagnosis

The procedures for detection and identification described in this protocol should have been followed. The characters of the genus *Unaspis* should have been determined to be present. The characters of *U. citri* should have been determined to be present.

Report on the diagnosis

A report on the execution of the protocol should include:

- information and documentation on the origin of the infested material (if possible)
- measurements, drawings or photographs (as relevant) of the morphological features required for a positive diagnosis of the species
- an indication of the level of the infestation (number of individual pests found; entity of damaged tissue)
- comments as appropriate on the certainty or uncertainty the identification

Preserved specimens of the pest should be retained.

Further information

Further information on this organism can be obtained from: CAB, International Institute of Entomology, Wallingford, Oxon OX10 8DE (UK): Dipartimento di Scienze e Tecnologie fitosanitarie, Sez. Di Entomologia agraria, Via Valdisavoia, 5, 95120 Catania (IT); LNPV, Unité d'entomologie, 2 place Viala, 34060 Montpellier Cedex 01 (FR).

Table 1 Simplified key to diaspid scales on citrus in the EPPO region

1	Dorsal ducts 1-barred. Second and third lobes never bilobed. Plates present, fringed; gland spines absent	Subfamily Aspidiotinae
	Dorsal ducts 2-barred. Second and third lobes, when present, often bilobed. Gland spines present, but sometimes replaced by plates	2
2	Plates present. Gland spines absent	Subfamily Parlatorinae
	Plates absent. Gland spines present	3
3	Gland spines present between median lobes, sometime minute	*Lepidosaphes* Shimer
	Gland spines absent from between median lobes	4
4	Median lobes joined at base by an internal sclerosis	*Pinnaspis* Cockerell
	Median lobes separated at base, although at times the bases may be close together	5
5	Widely separated median lobes and numerous dorsal pygidial macroducts (about 125, Web Fig. 2)	*Unaspis yanonensis* (Kuwana)
	Median lobes close together and fewer dorsal pygidial macroducts (about 70)	*Unaspis citri* (Comstock)

Acknowledgements

This protocol was originally drafted by: A. Russo & S. Longo, Università degli Studi di Catania (IT).

References

Balachowsky AS (1954) *Les Cochenilles Paléarctiques de la Tribu de Diaspidini*. Institut Pasteur, Paris (FR).

EPPO/CABI (1997) *Unaspis citri*. *Quarantine Pests for Europe* (2nd edn), *pp*., pp. 560–564. CAB International, Wallingford (GB).

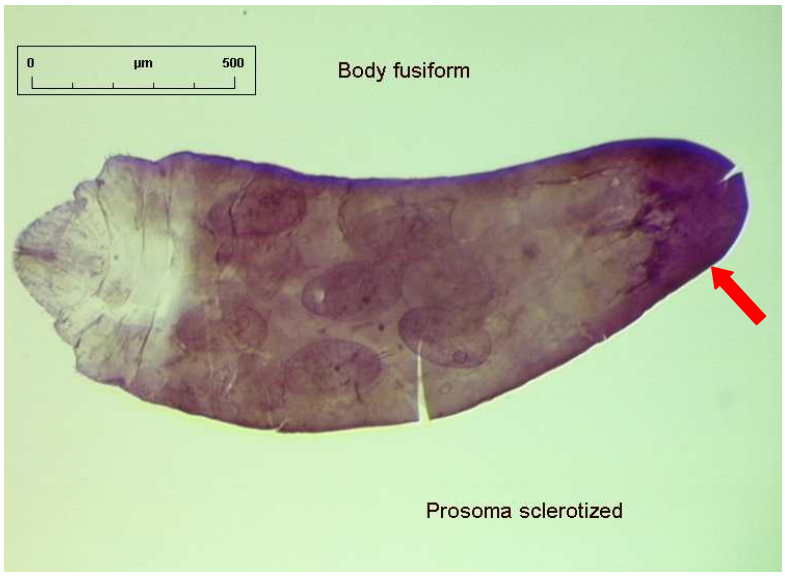
Rao VP (1949) The genus *Unaspis* MacGillivray (Homoptera: Coccoidea: Diaspididae). *Microentomology* **14**, 59–72.

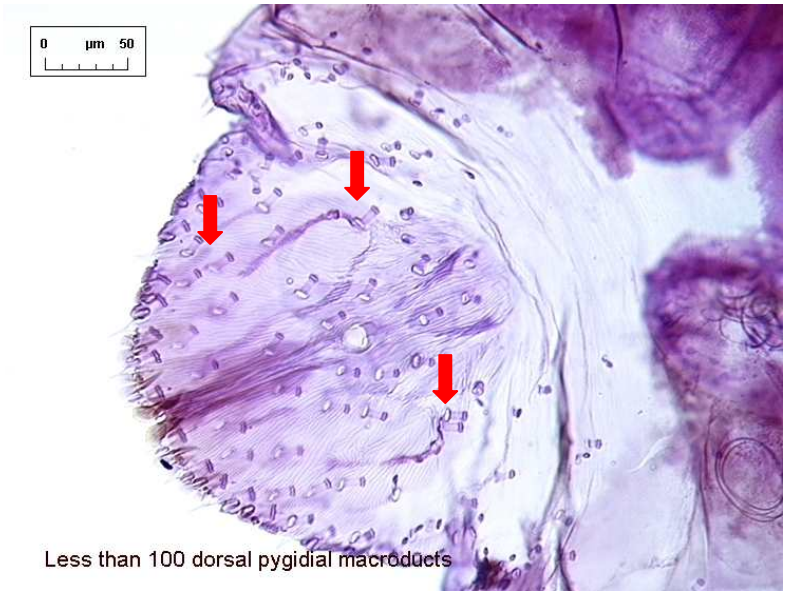
Williams DJ & Watson GW (1988) *The Armoured Scales (Diaspididae). The Scale Insects of the Tropical South Pacific Region*. CAB International, Wallingford (GB).

Appendix 1 Preparation of specimens

Place the specimen in a 10% solution of KOH. Heat for about 10 min but do not allow to boil. Remove to distilled water and, if necessary, expel the body contents by gentle pressure. Transfer from water to an acid alcohol bath made up of 20 parts glacial acetic acid and 80 parts of 50% alcohol. Leave for a few minutes. Stain for about 1 h in a staining solution of acid fuchsin (0.5 g), 10% HCl (25 mL), distilled water (300 mL). Transfer from the stain to 95% alcohol for a very few minutes to remove surplus stain; then transfer to absolute alcohol. Transfer to clove oil for 20 min. Place the specimen on a slide and remove surplus clove oil by means of fine filter paper. Put a drop of Canada balsam on the specimen and gently lower the cover slip by its own weight.

Fig 1. *Unaspis citri* (Comstock) on *Citrus*, Kindia Guinea, M.N.H.N., Paris: whole body, pygidium, ducts (Courtesy J.F. Germain, LNPV, Montpellier, FR).





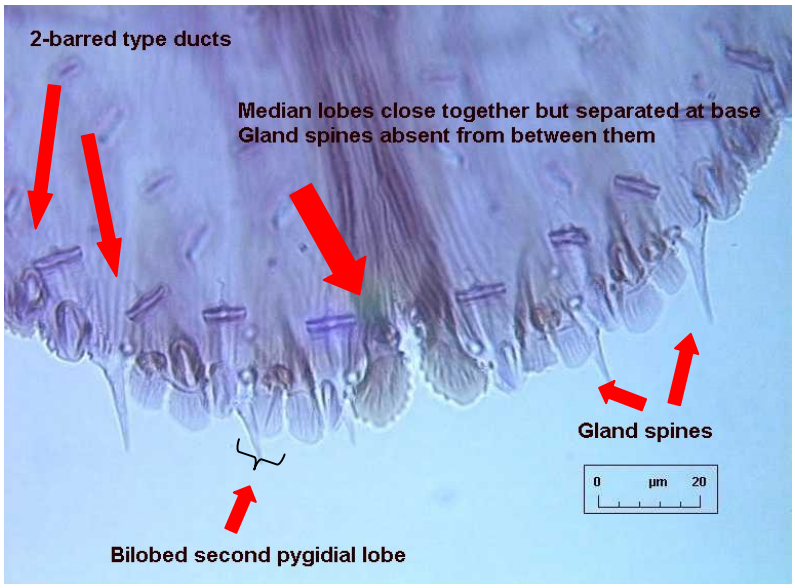
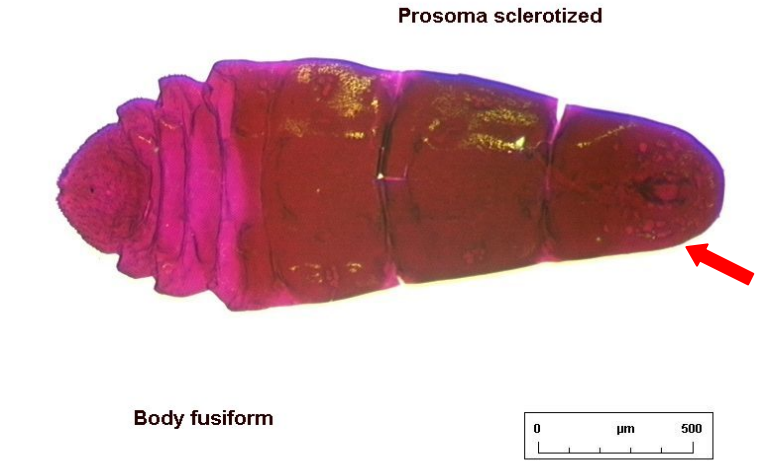
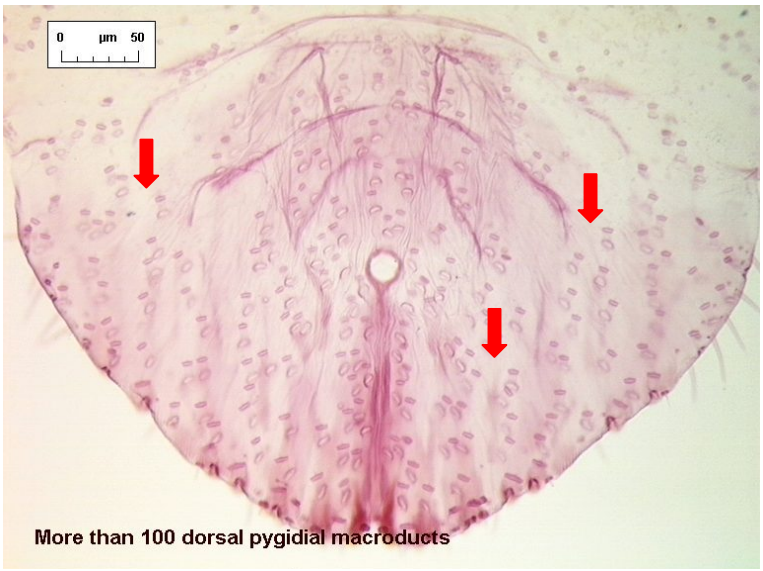
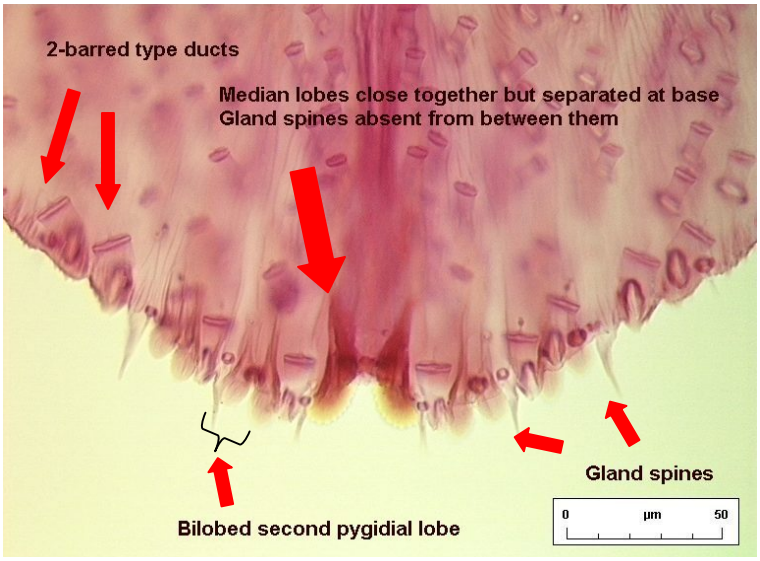


Fig. 2. *Unaspis yanonensis* (Kuwana) on *Citrus*, Antibes, France: whole body, pygidium, ducts (Courtesy J.F. Germain, LNPV, Montpellier, FR).







Habitus *Unaspis yanonensis*