

Normes OEPP EPPO Standards

Diagnostics
Diagnostic

PM 7/54



Organisation Européenne et Méditerranéenne pour la Protection des Plantes
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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) *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.

Diagnostics¹

Diagnostic

Lopholeucaspis japonica

Specific scope

This standard describes a diagnostic protocol for *Lopholeucaspis japonica*.

Specific approval and amendment

Approved in 2004-09.

Introduction

Lopholeucaspis japonica, an armoured scale insect, originated in the Far East, but now occurs in much of the warmer parts of Asia, and locally in North and South America (EPPO/CABI, 1997). *Citrus* spp. are its main hosts of economic importance. The scale can also develop on other fruit trees (apple, pear, fig, persimmon) and various ornamental trees such as species of *Acer*, *Betula*, *Cytisus*, *Laurus*, *Magnolia*, *Rosa*, *Syringa* and *Tilia*.

Identity

Name: *Lopholeucaspis japonica* (Cockerell, 1897)

Synonyms: *Leucaspis japonica* (Fernald, 1903), *Leucaspis japonica* var. *darwinensis* (Green, 1916), *Leucodiaspis hydrangeae* (Takahashi, 1934), *Leucodiaspis japonica* (Takahashi, 1934), *Leucodiaspis japonica darwiniensis* (Takahashi, 1934), *Leucaspis hydrangeae* (Takahashi, 1934), *Lopholeucaspis japonica* (Balachowsky, 1953), *Lopholeucaspis japonica darwiniensis* (Balachowsky, 1953), *Lopholeucaspis menoni* (Borchsenius, 1964); *Lopholeucaspis darwiniensis* (Borchsenius, 1966), *Leucaspis menoni* (Takagi, 1969)

Taxonomic position: *Insecta: Hemiptera: Sternorhyncha: Diaspididae.*

EPPO computer code: LOPLJA

Phytosanitary categorization: EPPO list A2, EU Annex designation II/AI

Detection

Adults may be found in cracks in the bark and small-sized populations are then difficult to detect. Heavy infestations give

bark a greyish-white appearance. Attacks by *L. japonica* result in dieback and premature leaf fall. Large populations can cause branch dieback and even plant death.

Identification

The taxonomy of the *Coccoidea* is almost entirely based on characters of the adult female and a good slide preparation of a teneral female is required for identification to species level. See Appendix 2 of OEPP/EPPO (2005) for a short glossary of terminology on the morphology of scales.

Family Diaspididae

A key of the families of *Coccoidea* based on female structures is given by Kosztarab & Kozár (1988) and is available on http://www.sel.barc.usda.gov/scalekeys/all_Families.htm. The *Diaspididae* or armoured scales are easily recognizable by the presence of a detached scale covering the body of the insect (except in crawler and adult male) and the best diagnostic feature of 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). The *Diaspididae* is the largest family in *Coccoidea*, with about 400 genera and 2000 species (Gill, 1997).

Genus *Lopholeucaspis*

The genus *Lopholeucaspis* may be identified by using the following combination of characters for the adult female:

- body shape elongate-oval, widest at thorax
- pygidium with 2 pairs of lobes, median lobes separate never fused at their base, plates present, or second stage presenting them; macroducts commonly showing some evidence of arrangement in segmental rows or series

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

- macroducts two-barred type; fimbriate plates present, anterior spiracles commonly associated with spiracular disc pores; antennae commonly with two or more sensory setae
- body entirely enclosed within the exuvia of the preceding stage
- perivulvar disc pores present on at least one segment anterior to and in addition to the usual perivulvar disc pores
- margin of thorax and 2 first abdominal segments with a row of gland tubercles; pygidial macroducts randomly scattered across dorsum.

An alternative description of the characters of the genus is provided by Kosztarab (1996):

- second stage with well-developed lobes and large macroducts
- species pupillarial, thus test of female made of second instar exuviae and wax cover, elongate, white or grey, with only 1st exuviae at apex
- body of female spindle shaped; with two barred ducts, with a continuous row of gland tubercles along lateral margins of prosoma and 2 anterior abdominal segments
- pygidium: with lobes widely separated; median lobes (L1) not yoked (or united by sclerosis), projecting, elongate to conical. Second lobes (L2) non bilobed similar to median lobes, but smaller
- plates long, fimbriate, 2 between median lobes and between median and second lobes
- dorsal ducts few, small and scattered, marginal and other ducts similar in size
- perivulvar pores in 4 or 5 groups, but supplementary submarginal groups may be present on at least one anterior segment anterior to regular perivulvar pore groups
- anus round, located on anterior half of pygidium (Kosztarab, 1996).

Lopholeucaspis japonica

Adult female and test

Species pupillarial. Cover of female elongate oystershell-shaped, distinctly convex; made up of 2nd exuviae, and covered with grayish-white wax coating, often of same colour as bark; 1.5–2.0 mm long, 0.5 mm broad. Exuviae of first instar apical yellow-brown. Body of female elongate, spindle-shaped, tapering toward anterior end, length 0.9–1.1 mm. Body colour wine-red. Male puparium: much smaller, usually not curved towards the cephalic end, otherwise as the female scale.

Slide-mounted female: female enclosed within the enlarged and sclerotized exuviae of 2nd instar. A continuous row of gland tubercles along the lateral margins of prosoma and the anterior abdominal segments. Pygidium: mostly membranous. Two pairs of sclerotized, relatively large and acute lobes; each often notched on both sides. Second pair smaller than median ones. Two slender fimbriate plates between median lobes, also between median and second lobes. Beyond second lobes 2 plates similar to median plates and a series of short, broad, apically fimbriate plates, most with ducts at their base. The row of broad plates continues in a row of gland tubercles which extend

almost to anterior end of body. Dorsal ducts relatively small, numerous, scattered. Ventral ducts common on submedial and submarginal areas of abdomen. Vulva located at about middle of pygidium. Perivulvar pores in an almost continuous arch, supplemented by small clusters of pores on submargin of 2 prepygidial segments. Anus large, basal, surrounded by a sclerotized area. Some other small and weakly sclerotized areas common on dorsal pygidium (Bodenheimer, 1953; Takagi, 1969; Kosztarab, 1996). Illustrations of the first and second instar male are given by Takagi (2002). See Web Figs 1 and 2. The following Web sites may also usefully be consulted: <http://www.sel.barc.usda.gov/scalenet/query.htm>; <http://www.sel.barc.usda.gov/catalogs/diaspidi/Lopholeucaspisjaponica.htm>.

Identification has to be based almost entirely on characters of the adult female only. A procedure for preparing the scale insect is given in Appendix I of OEPP/EPP (2005). A good slide preparation of a teneral female is required for identification to the species level. The characters of the genus *Lopholeucaspis* and that distinguish *L. japonica* described in this section should have been determined to be present. The preparation should be compared with a voucher specimen if possible.

L. japonica can be separated from the related but less important *Lopholeucaspis cockerelli* (Grandpré & Charmoy) (Balachowsky, 1953) as follows. *L. cockerelli* has the row of gland tubercles on ventral margin of thorax and abdomen running over the segments IV and V of the pygidium; absence of ventral microducts in transversal row at the base of the pygidium in the prevulvar area and on the last prepygidial segments. *L. japonica* has the row of gland tubercles on the ventral margin of the thorax and abdomen never running over the pygidium, breaking off on the margin of segment III; presence of transversal row of microducts in the prevulvar area and on the three last prepygidial segments.

Four other minor species of *Lopholeucaspis* may be found: *Lopholeucaspis baluanensis* Williams & Watson, only present in Papua New Guinea on *Citrus* spp. (Williams & Watson, 1988); *Lopholeucaspis excoercariae* Borchsenius on *Excoercaria agallocha* (Euphorbiaceae) in India; *Lopholeucaspis limoniae* (Rutherford) only in Sri Lanka on *Limonia alata* (Rutaceae) (Rutherford, 1915); *Lopholeucaspis massoniae* Tang on *Pinus massoniana* in China (Fujian) (Tang, 1980). There is no key dealing with all these species and the aforementioned references should be consulted.

Reference material

Leucaspis japonicus Cockerell (1897): 53. Type data: Japanese material detected on *Cytisus* at import into San Francisco (US) in 1896–12, by A. Craw.

Unknown type status female. Described: female.

Reporting and documentation

Guidelines on reporting and documentation are given in EPP Standard PM7/– (in preparation).

Further information

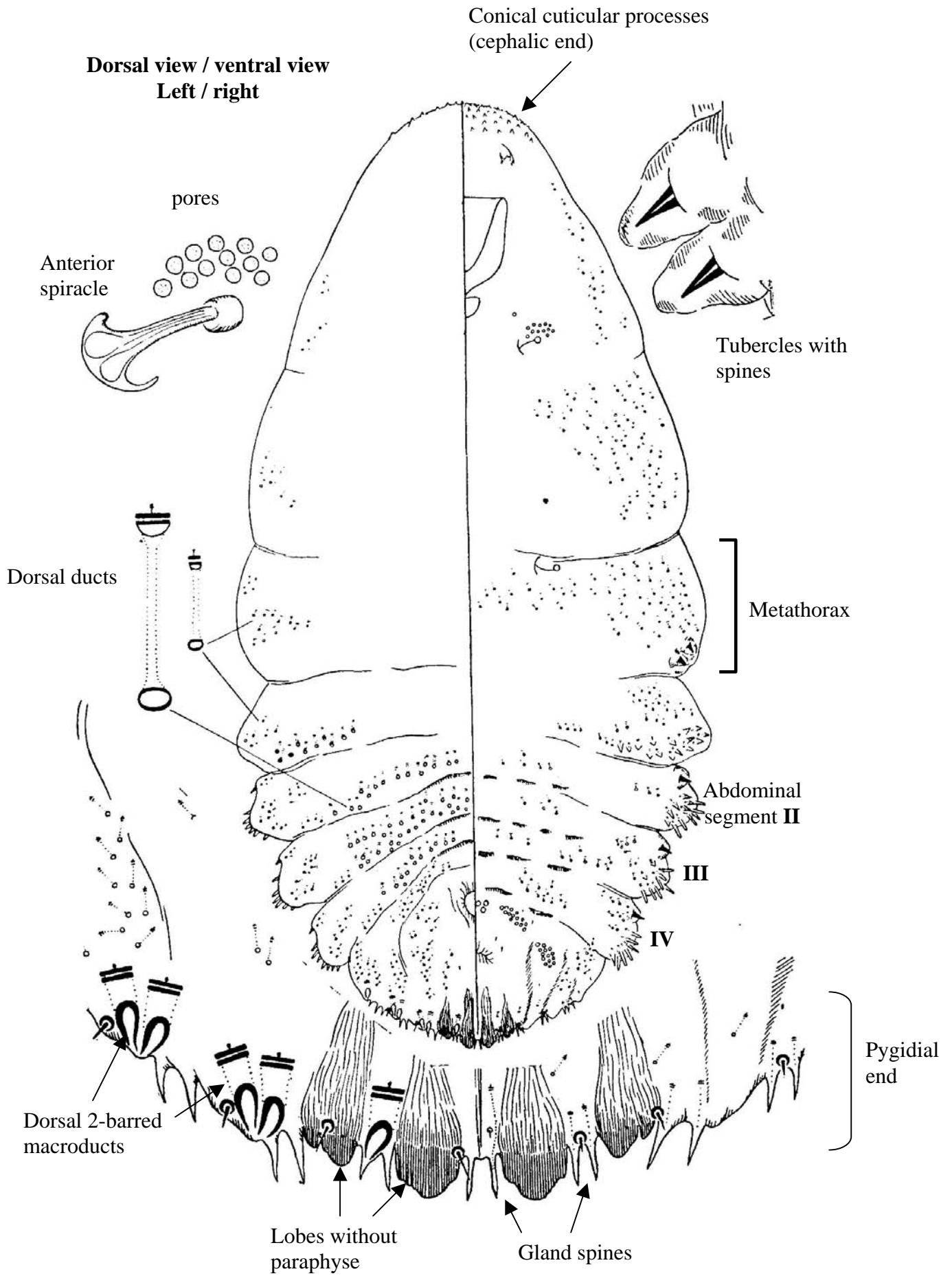
Further information on this organism can be obtained from:
 M.G.M. Jansen, Plant Protection Service, Section of Entomology,
 PO Box 9102, 6700 HC Wageningen (Netherlands)
 J.-F. Germain, LNPV-Unité d'entomologie Zoologie, 2 place
 Viala, F-34060 Montpellier Cedex 01 (France).

Acknowledgements

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

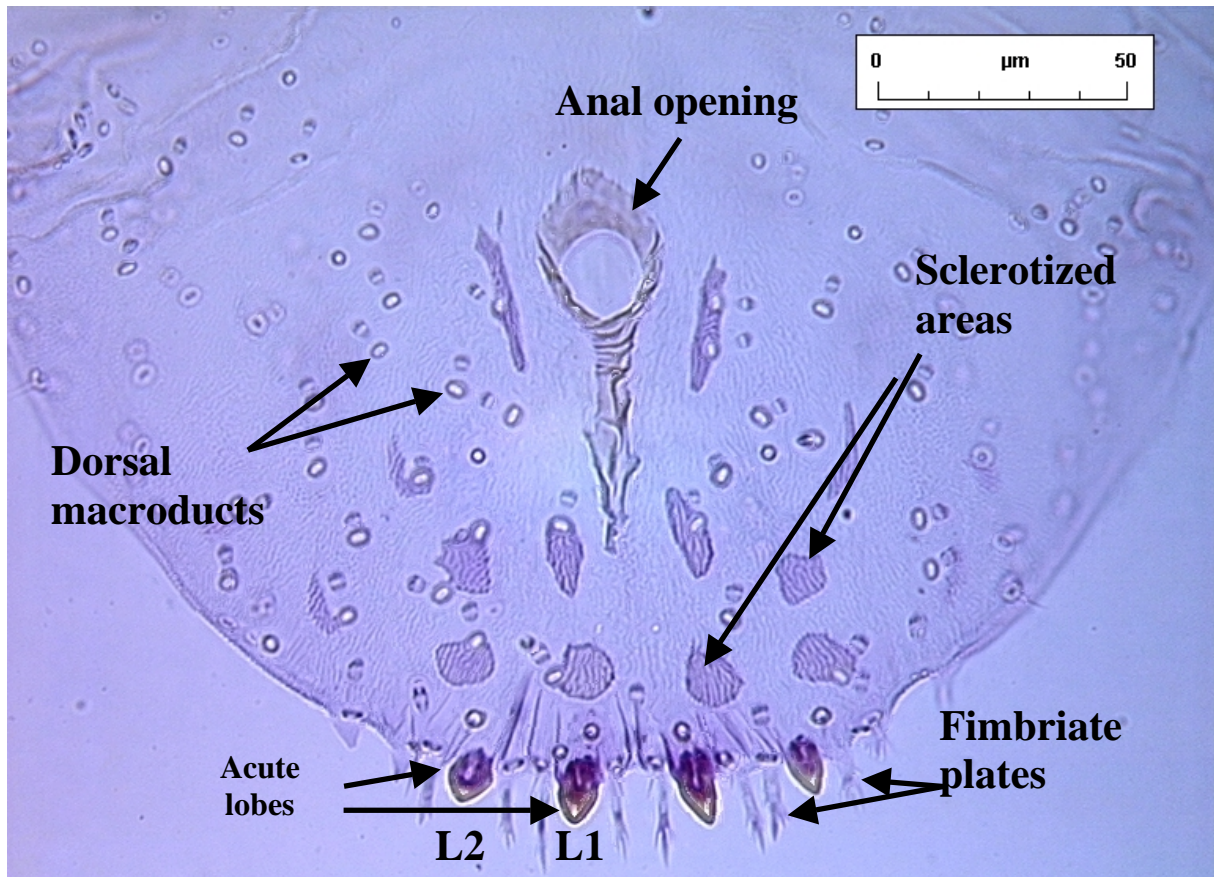
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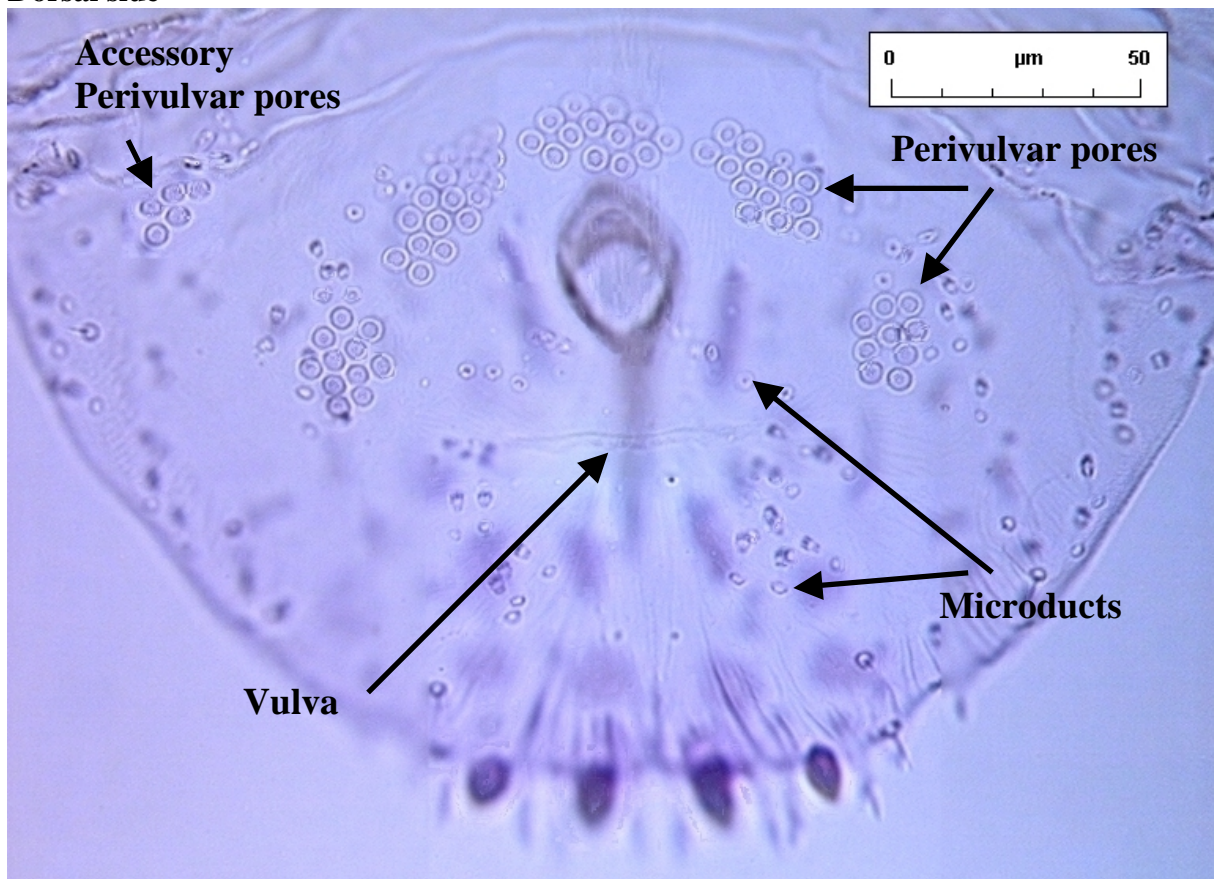


Web Fig. 1 Morphological description of *Lopholeucaspis japonica* (after Ferris, 1938)

Web Fig. 2 *Lopholeucaspis japonica*, pygidium



Dorsal side



Ventral side