

Diagnostics
Diagnostic

Margarodes prieskaensis*, *Margarodes vitis*, *Margarodes vredendalensis

Specific scope

This standard describes a diagnostic protocol for *Margarodes prieskaensis*, *Margarodes vitis* and *Margarodes vredendalensis*.

Specific approval and amendment

Approved in 2007-09.

Introduction

Seven non-European species of *Margarodes* have been recorded on the roots of grapevine: *Margarodes vitis* (Philippi) in Brazil and Chile; *M. meridionalis* Morrison in California; *M. capensis* Giard, *M. greeni* Brain, *M. prieskaensis* (Jakubski), *M. trimeni* Giard and *M. vredendalensis* De Klerk in South Africa. *Margarodes prieskaensis*, *Margarodes vitis* and *Margarodes vredendalensis* are included in the EPPO A1 list of pests recommended for regulation and are covered in this diagnostic protocol. While *Margarodes prieskaensis* and *Margarodes vredendalensis* are only recorded on the roots of grapevine (*Vitis vinifera*), *Margarodes vitis* is a polyphagous pest which, in addition to its main host grapevine, is found on wild plants or weeds belonging to various families (EPPO/CABI, 1997).

Identity

Name: *Margarodes prieskaensis* (Jakubski) 1965

Synonyms: *Sphaeraspis prieskaensis* Jakubski

Taxonomic position: Hemiptera: Sternorrhyncha: Margarodidae

EPPO computer code: MARGPR

Phytosanitary categorization: EPPO A1 list, UE II/A1.

Name: *Margarodes vitis* (Philippi, 1884)

Synonyms: *Heterodera vitis* Philippi, *Margarodes vitium* Giard, *Margarodes trilobitum* Reed, *Sphaeraspis vitis* Jakubski

Taxonomic position: Hemiptera: Sternorrhyncha: Margarodidae

EPPO computer code: MARGVI

Phytosanitary categorization: EPPO A1 list, UE II/A1.

Name: *Margarodes vredendalensis* De Klerk 1980

Taxonomic position: Hemiptera: Sternorrhyncha: Margarodidae

EPPO computer code: MARGVR

Phytosanitary categorization: EPPO A1 list, UE II/A1.

Detection

Grapevines infested with *Margarodes* exhibit a gradual decline in vigour, shoots become thinner and shorter and leaves smaller (Annecke & Moran, 1982). One or more of the branches of the vine may die, followed in severe infestations by the eventual death of the whole plant. The duration of this process varies greatly. Infestations of vineyards are usually patchy. The patches increase in size, presumably because of the gradual subterranean movement of the larvae and adult females. The symptoms resemble those caused by grapevine phylloxera (*Viteus vitifoliae*) but, in the case of *Margarodes*, no root galls are formed.

All *Margarodes* have subterranean stages. They live usually at depths of 20–60 cm, but occur at depths of up to 120 cm. Nymphs attach themselves to roots and feed on them. Once feeding is complete, the nymphs are capable of secreting a protective waxy covering to form pearl-like cysts. This is the stage that is most likely to be detected. These cysts are found throughout the year, while females occur only for a month each year.

Identification

Morphological identification is a recommended method. The taxonomy of scale insects is based almost entirely on the adult female and a good slide preparation of a female is required for identification at species level; see Appendix 1 for the technical procedure. A binocular microscope is needed.

For keys of Coccoidea families see Gill (1993) Kosztarab (1996).

For a key of Margarodidae genus, see Morrison 1928, Jakubski 1965, Gill 1993 and Foldi 2005 for an updated version; for *Margarodes* genus on *Vitis*, see Table 1.

Table 1 Key to the adult females of *Margarodes* pests on vine worldwide (after Foldi, 1987)

1		
• With 7 pairs of abdominal spiracles		2
• With 6 pairs of abdominal spiracles		6
2		
• Presence of bulbous spines in posterior end of abdomen	<i>M. prieskaensis</i> (Jakubski)	
• Absence of bulbous spines in posterior end of abdomen		3
3		
• Very long ventral setae (> 390 µm); second antennal segment as long as segment 3	<i>M. vredendalensis</i> De Klerk	
• Absence of these very long ventral setae		4
4		
• Presence of spines enlarged and flattened at apices, numerous especially on terminal part of abdomen; multilocular pores with 5 macroluculi	<i>M. vitis</i> (Philippi)	
• Absence of spines enlarged and flattened at apices		5
5		
• Presence of multilocular pores with 1 central macroluculus but they are absent on the head, pro- and mesothorax; presence of spines on whole thorax	<i>M. capensis</i> Giard	
• Absence of multilocular pores with 1 central macroluculus and presence of multilocular pores with 2 macroluculi distributed on all the body	<i>M. meridionalis</i> Morrison	
6		
• Multilocular pores with 5 macroluculi without central loculus only present on abdomen; numerous spines on both dorsal and ventral surfaces	<i>M. trimeni</i> Giard	
• Multilocular pores with 5 macroluculi without central loculus present all over the body; few spines	<i>M. greeni</i> Brain	

Description of *Margarodes prieskaensis* (after Jakubski, 1965; De Klerk *et al.*, 1982)

Adult female: (Figs 1 and 2)

Adult females vary greatly in size, are yellow with dark brown claws. Body densely covered with long hair-like setae. Segmentation visible on both ventral and dorsal sides. Body oval; length 5–11 mm; width 4–8 mm; with distinct segmentation on dorsal and ventral sides of abdomen; segmentation not distinct anterior to metathorax.

Dorsal surface: Long setae 300–400 µm long; thin and hair-like, numerous, distributed on whole dorsum in marginal and median areas; distributed over whole surface of each abdominal segment. Spines club-shaped, 25–50 µm long, on anterior part of the body; occurring in marginal area from mesothorax, becoming more numerous, slightly shorter and bulbous in form toward posterior end of abdomen; occurring in median area only in bulbous form and on last 2–4 abdominal segments. Pores: Multilocular derm pores circular, 12–16 µm width, variation in diameter similar over entire body; dispersed in low density only from metathorax, their number becoming more numerous towards posterior end of abdomen, same density in median and marginal areas.

Ventral surface: Long setae, 280–390 µm long thin and hair-like; all areas of body are as densely covered as the dorsum; evenly distributed over whole surface of each abdominal segment. Short setae, 70–130 µm long; occurring in low numbers amongst the long setae in marginal areas of whole ventral surface; more numerous in median area of abdomen. Spines club-shaped; 30–50 µm long, on anterior part of the body; occurring in marginal from mesothorax, becoming more numerous, slightly shorter (24–36 µm) and bulbous in form

towards posterior end of abdomen; occurring in median area from metathorax, becoming more numerous, bulbous in form but of same length (34–62 µm) towards posterior end of abdomen. Pores: multilocular derm pores circular (12–16 µm) in diameter; variation in diameter similar over entire body; dispersed in low density only from metathorax, their numbers becoming more numerous towards end of abdomen; density in median and marginal areas the same.

Antennae: Eight-segmented; 486–893 µm long. Segment I twice as wide as long, with 4–5 min setae placed in a row at the base. Segment II half as long as segment III, with 3–6 long setae and 1–2 sensory pores at distal end. Segment III–VII all of the same length but diminishing gradually in width; each with variable numbers of long-hair-like setae and short bluntly pointed, cylindrical setae distributed around distal end of each segment. Segment VIII longer and narrower than segment VII, rounded at apex with 5–6 long-hair like setae, 4–9 shorter setae and 7–12 short cylindrical setae placed apically.

Fore legs much larger than middle and hind legs. Coxa with sclerotized apodeme; with long and shorter setae. Trochanter also with long and short setae. Femur 556–1080 µm long; 472–918 µm wide with numerous long setae (196–236 µm) on ventral side and short rigid setae on anterior and posterior sides; dorsal side almost completely bare. Tibia short, 15–25 long setae on each anterior and posterior side. Tarsus with 5–10 long setae on each of the posterior and anterior sides and 4 dorsal pores grouped together proximally. Claw slightly curved, smooth on inner surface; heavily sclerotized except basally with 1 long seta-like digitule on each posterior and anterior side.

Middle and hind legs: Both pairs similar in size and shape. Coxa twice as wide as long with sclerotized apodeme; 7–13 long setae on ventral side, posterior side with 6–16 long and

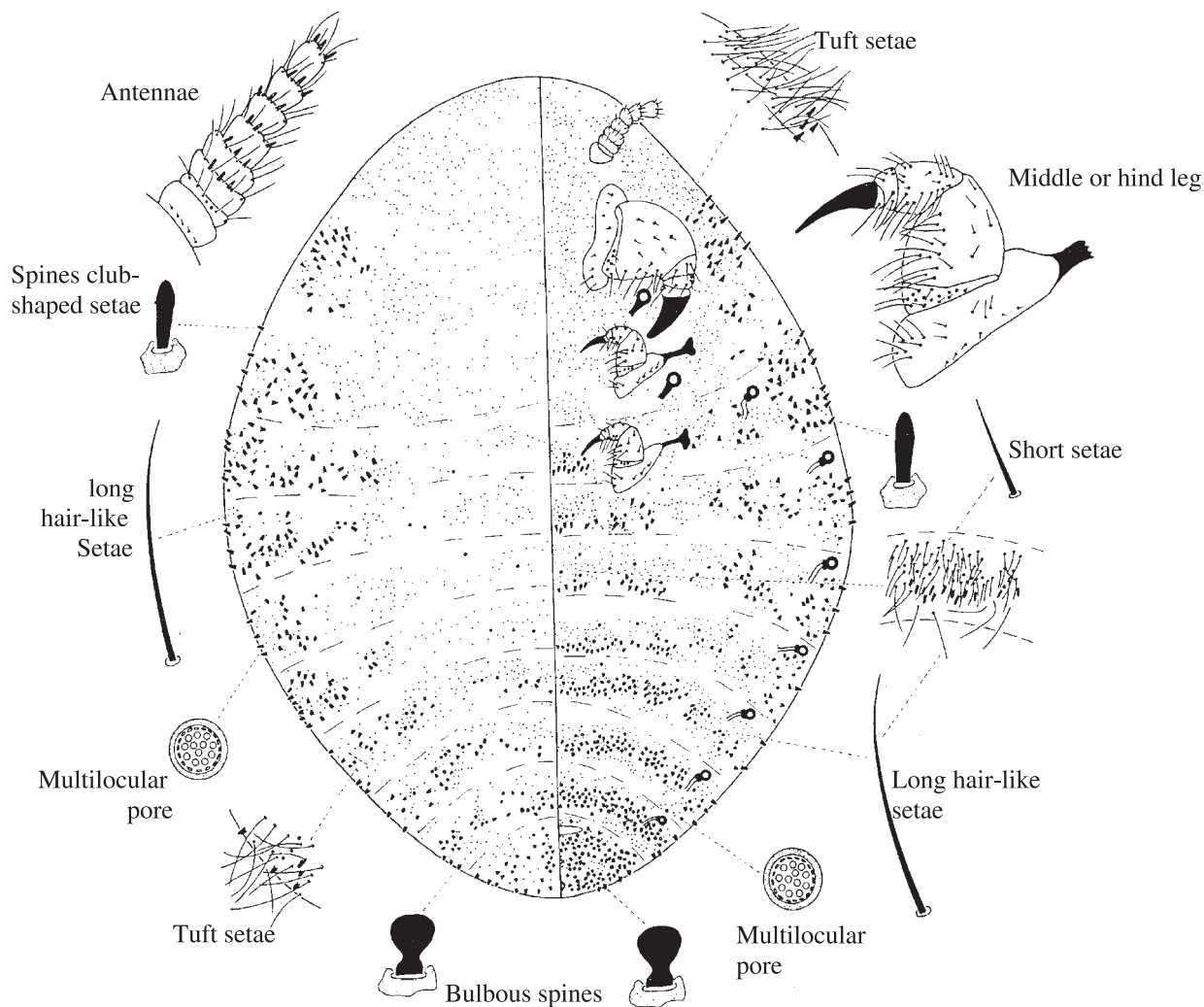


Fig. 1 *Margarodes prienskaensis* (after De Klerk) adult female.

short setae; anterior side with 10–14 long and short setae. Trochanter with 2–5 long setae on ventral side; 3–5 min, 2–4 long setae and 7–16 sensory pores on posterior side; 3–4 min, 2–3 long setae and 4–8 sensory pores on anterior side. Femur 204–463 μm ; 204–456 μm wide with long ventral (143–233 μm) and short rigid setae on posterior and anterior sides. Tibia 143–278 μm long; 109–204 μm wide with 5–6 long ventral setae on posterior side; 11–18 long and 3–5 min setae on anterior side. Tarsus with 2 long ventral setae; 3–5 long setae on each of posterior and anterior sides; 3–6 dorsal pores grouped together proximally. Claws 175–426 μm long; 24–60 μm wide; almost straight, smooth on inner surface; heavily sclerotized except basally; with 1 long seta-like digitules on each of posterior and anterior sides.

Thoracic spiracles: Two pairs with circular or elongated openings; 3–5 small pores next to opening on posterior side. Atrium 86–133 μm wide; with 8–11 large multilocular pores arranged in one or two circles on the peritreme surface just

inwards of the atrium; 8–20 simple pores. Abdominal spiracles: Seven pairs; first two pairs slightly smaller than thoracic spiracles; spiracles diminishing slightly in size towards posterior part of the abdomen; first pair situated more marginally. Atrium of first two pairs 56–93 μm wide; with about 10 multilocular pores arranged in a circle on the peritreme surface, just inwards of the atrium.

Mouthparts absent. Folds occur in body at the place where mouthparts occur in other stages.

Genital opening: a transverse fissure, situated anteriorly near anus, with indistinct lips, with or without setae.

Anal opening circular to oval, sclerotized, situated apically or sub-apically on the ventral surface in middle of small bare area.

Nymph of the cyst stage: almost spherical and up to 6 mm in diameter. Cyst wall thick and very hard. Outer surface rough and gives the impression of a tortoise shell; amber yellow in color.

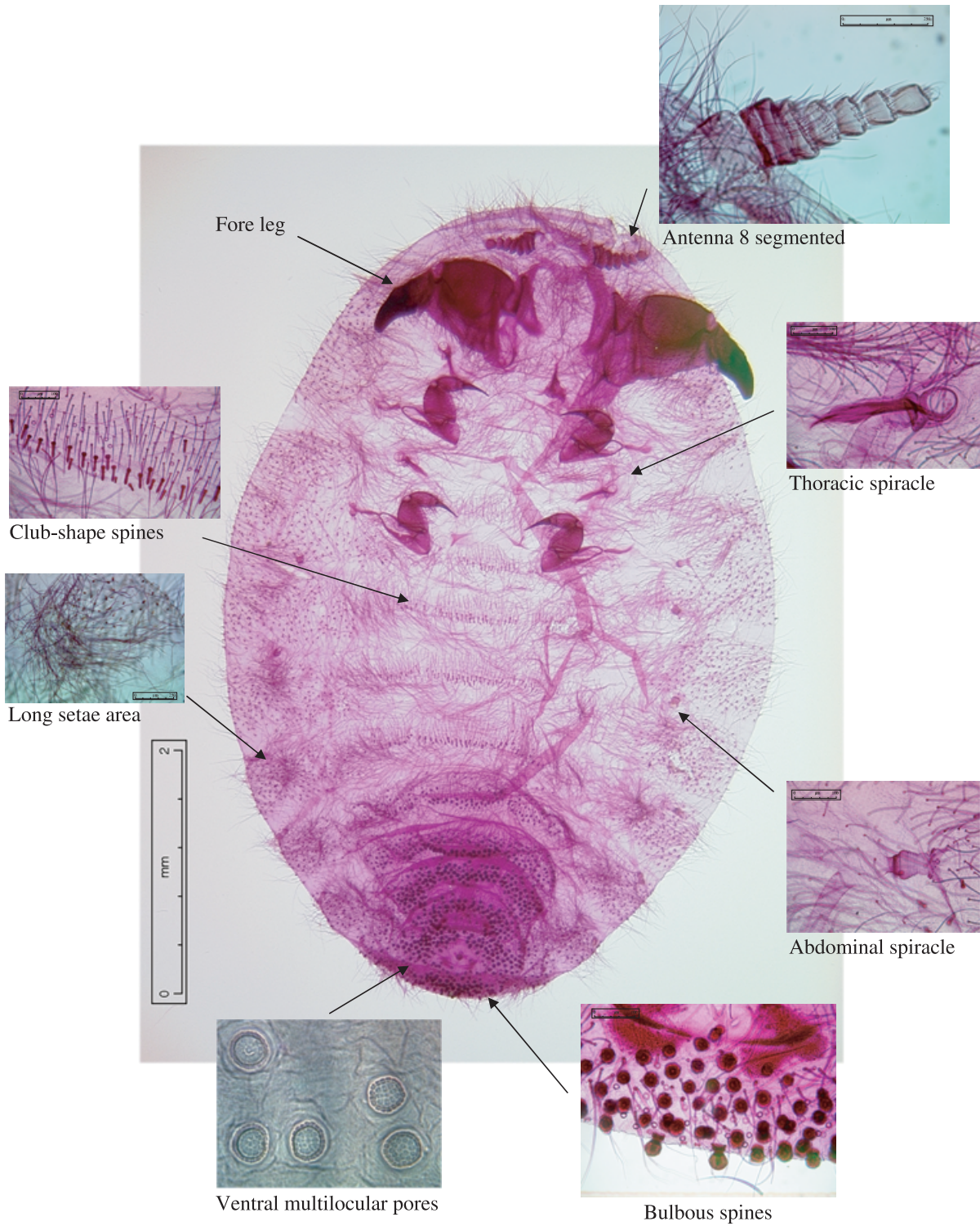


Fig. 2 *Margarodes prieskaensis* (Jakubski) adult female.

Antennae: small rounded protrusion in deep pit with minute, bluntly pointed setae at distal end.

Mouthparts: sclerotized clypeo-labral complex situated opposite thoracic spiracles. Stylets forming an oblong loop; approximately as long as body when extended. Labium sclerotized; with about 10 short setae.

Pores: 7–29 multilocular derm pores situated around labium; each with numerous microluculi in an outer circle and 3 to 4 macroluculi in the centre.

Thoracic spiracles: Two pairs with circular opening; 2–4 small pores grouped together next to opening on posterior side. Atrium 35–140 µm in diameter with about 20 multilocular pores. Abdominal spiracles: seven pairs; first pair approximately the same size as thoracic spiracles and situated more marginally, becoming smaller towards posterior end of abdomen. Atrium of first pair 32–137 µm in diameter with 8 multilocular pores.

Anal opening circular, situated sub-apically on ventral surface with 3–7 cicatrices arranged in a line on each side of anus. Cicatrices occur between anus and fifth pair of abdominal spiracles.

Genital scar: a transverse line situated anteriorly near anal opening.

Description of *Margarodes vitis* (after Jakubski, 1965; Foldi and Soria 1989)

Adult female: (Figs 3 and 4)

Adult female varies greatly in size, white-yellowish. Body densely covered with long hair-like setae. Segmentation plainly visible on both ventral and dorsal sides. Body oval, length 6–8 mm; width 3–5 mm.

Dorsal surface: long and fine tegumentary setae (300 µm) on the head, the prothorax, the median and sub-median areas of the body. Shorter setae (25 and 150 µm) on the marginal areas. Spines absent on the head and the prothorax, on the medio- and submedio-dorsal surface of the first 4 abdominal segments. Numerous spines of several types: curved spines and straight spines with widened points on the marginal and submarginal areas; short bulbous spines with strongly extended and split up points on the last 4 segments. The spines with widened points are characteristic of *M. vitis* and facilitate distinguishing it from other *Margarodes*. Multilocular pores on the last 4 segments, in several longitudinal lines.

Ventral surface: Long setae, 240–260 µm, on the head, the prothorax and marginal areas of the body; shorter setae, 100–150 µm, distributed on all the body. Numerous spines; straight spines on the medio-abdominal surface of the first 5 segments; spines with points widened and split up various sizes and forms from the 3rd segment, where they are very rare, becoming increasingly numerous, segment after segment, until 8th. Multilocular pores starting from the 3rd abdominal segment, where they are very rare, their number increasing from one segment to another towards the posterior part. Multilocular pores, 10–12 µm wide, with 14–16 loculi in outer ring and 4–7 (5) larger loculi in inner ring. Larger multilocular

pores, 14–16 µm in diameter, with 18 loculi in outer ring, 8 larger loculi in intermediate ring and two large loculi in inner ring.

Antenna of 7, sometimes 8 segments, 750 µm, tapering gradually from base to apex, with numerous setae. The first segment is delicate with one short seta only; the second one is not fully developed and has 8 setae and 2 sensillae. Other segments are ring-shaped, gradually becoming narrower and longer, with setae and numerous shorter digitules. The last segment is oval in profile and has 4 longer setae and numerous digitules.

Fore legs much larger than middle and hind legs; coxa and trochanter very small; coxa with 8 long setae, femur very developed (500 µm) as long as broad, with numerous ventral setae and apically covered with sparse, somewhat long, stiff setae. 200 µm; shorter and thinner tibia (100–150 µm) with 3 and 2 paradorsal setae, one pair of lateral sensillae and one seta below; paraventrally with one pair of long setae. Tarsae short, laterally with 3 pairs of long setae, dorsal side fused with tibia but ventral side well separated, with claw slightly curved, 250 µm long, with 2 pairs of digitules.

Middle and hind legs: coxa with 4 long setae in the mid-line of the ventral area; trochanter well demarcated from the femur, with groups of sensillae and 2 long setae in front of each group of sensillae. Femur (250 µm long and 200 µm broad) with 4 and 6 very long ventral setae and some shorter stiff setae on the other sides. Tibia with 2 pairs of paradorsal setae, one lateral sensilla and one seta behind or below, and two ventral, long setae. Tarsus with 3 dorsal sensillae, 2 paradorsal setae in front of them and 2 pairs of ventral setae.

Thoracic spiracles with peritreme 100 µm wide, with a distinct smaller outer opening, a short curved apodeme. About 15–20 multilocular pores in atrium, each with about 12 loculi in outer ring and 3 loculi in inner ring. About 2 perispiracular sensilla on a small plate. Peristigmatic pores absent. Abdominal spiracles: 7 pairs identical, smaller than thoracic spiracles: with peritreme 20 µm wide, with about 8–10 multilocular pores in atrium.

Eyes and mouthparts absent.

Nymph of the cyst stage

Cyst wall thick, 400 µm, and very hard, red brown in colour, 2–7 mm long and 4–5 mm wide, scaly rough surface.

The larvae is apodous and yellow in colour.

Antennae: small rounded protrusion with 4 long setae, 50 µm.

Mouthparts: labium with about 4–6 short setae.

Thoracic spiracles: two pairs with circular opening, 80 µm width. Atrium with about 35–38 multilocular pores and one central macroluculus.

Abdominal spiracles: 7 pairs. Atrium with pores with 1 central macroluculus, about 25–27.

Anal opening in the centre of a small sclerotized area with 12–14 cicatrices: 6 in a transversal row 6 in front of the genital line and 3 on each side of the anus, some doubled; sometimes 1 or 2 near the last spiracles.

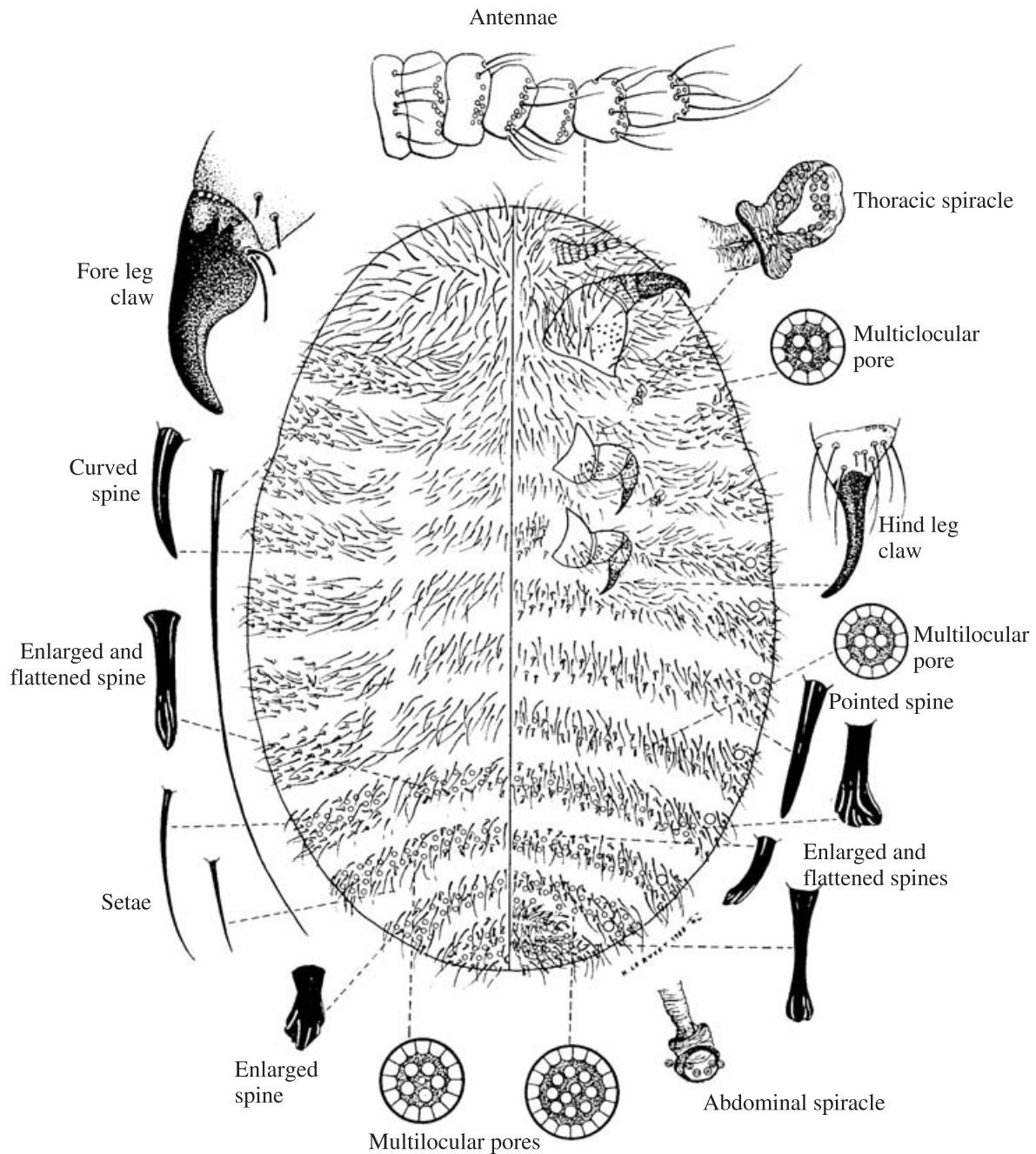


Fig. 3 *Margarodes vitis* (in Foldi & Soria, 1989) adult female.

Description of *Margarodes vredendalensis* (after De Klerk, 1982)

Adult female: (Figs 5 and 6)

Adult female varies greatly in size, yellow in colour with dark brown claws. Body densely covered with long hair-like setae. Segmentation plainly visible on both ventral and dorsal sides. Body oval; length 5.2–8.75 mm; with distinct segmentation on

dorsal and ventral surfaces of abdomen; segmentation not distinct anterior to metathorax.

Dorsal surface: Long setae 307–509 µm; thin and hair-like; numerous; distributed on whole dorsum in marginal and median areas; distributed over whole surface of each abdominal segment. Short setae 55–97 µm long; rigid; occurring in low numbers amongst the long setae and distributed as the latter. Medium setae vary in length between short and long setae;

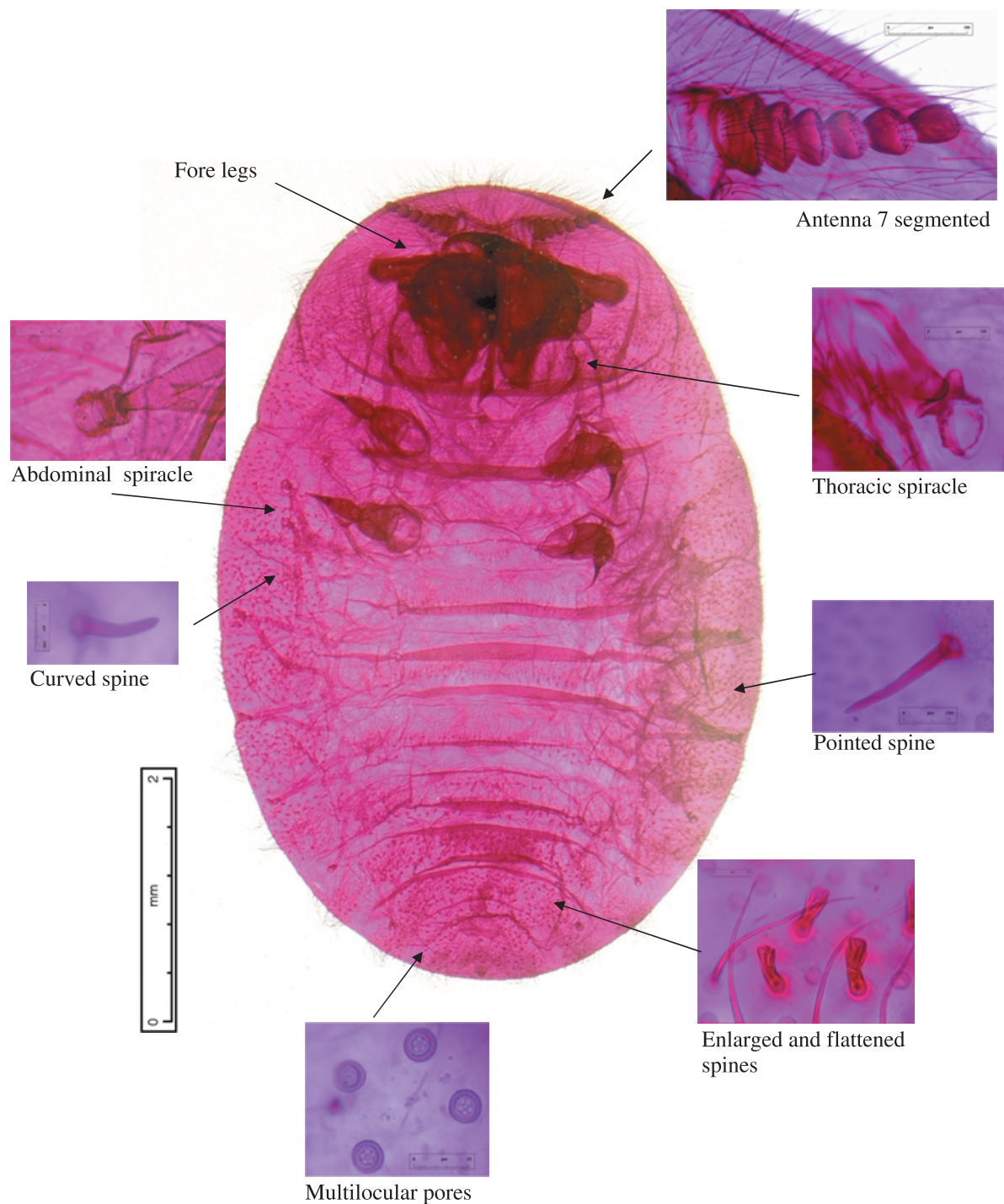


Fig. 4 *Margarodes vitis* (Philippi) adult female.

hair-like; distributed densely amongst the long setae on whole dorsum in marginal and median areas. Spines bluntly pointed; 29–39 μm long on anterior part of the body; occurring in low numbers in marginal area of thorax, becoming less numerous but of the same length towards posterior part of abdomen or even absent on last three abdominal segments; absent in

median areas of whole dorsum. Pores: circular multilocular derm pores, 12–15 μm in diameter; variation in diameter similar over entire body; irregularly dispersed only from metathorax, becoming more numerous towards posterior end of abdomen; same density in median and marginal areas.

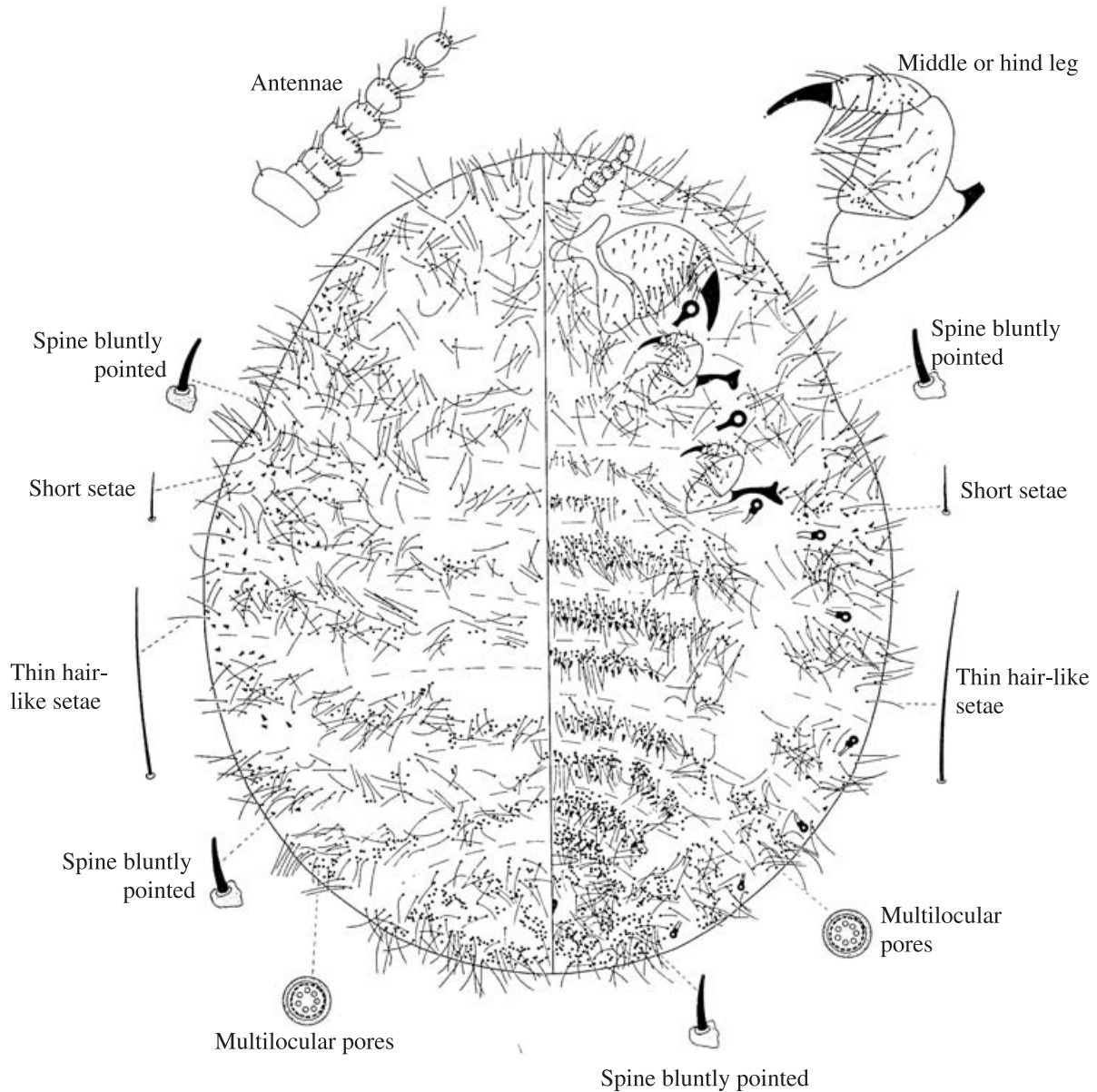


Fig. 5 *Margarodes vrendendalensis* (after De Klerk) adult female.

Ventral surface: Long setae 380–487 μm ; thin and hair-like; all areas of body as densely covered as dorsum except a narrow area between median and marginal areas of abdomen where they are less numerous or even absent; equally distributed over whole surface of each abdominal segment. Short setae 71–123 μm long; rigid; occurring in low number amongst the long setae in marginal areas of whole ventral surface; more numerous in median area of abdomen. Medium setae vary in length between short and long setae; distributed amongst the long setae on whole ventral surface in median and marginal areas. Spines bluntly pointed, 26–44 μm long on anterior part of the body; in marginal areas occurring in low numbers on thorax, becoming less numerous but of the same length towards

posterior end of abdomen or even absent on last three abdominal segments; in median areas occurring from metathorax, becoming slightly more numerous towards posterior end of abdomen. Denser in median areas than in marginal areas. Absent on a narrow area between median and marginal areas of abdomen.

Pores: circular multilocular derm pores, 12–15 μm width; variation in diameter similar over entire body; dispersed irregularly only from metathorax, their numbers becoming more numerous towards posterior end of abdomen; same density in median and marginal areas.

Antennae: eight-segmented; 612–843 μm long. Segment I: 1.5 times as wide as long, with one long seta on inner side and dorsally with 4–7 min setae grouped together. Segment II: as

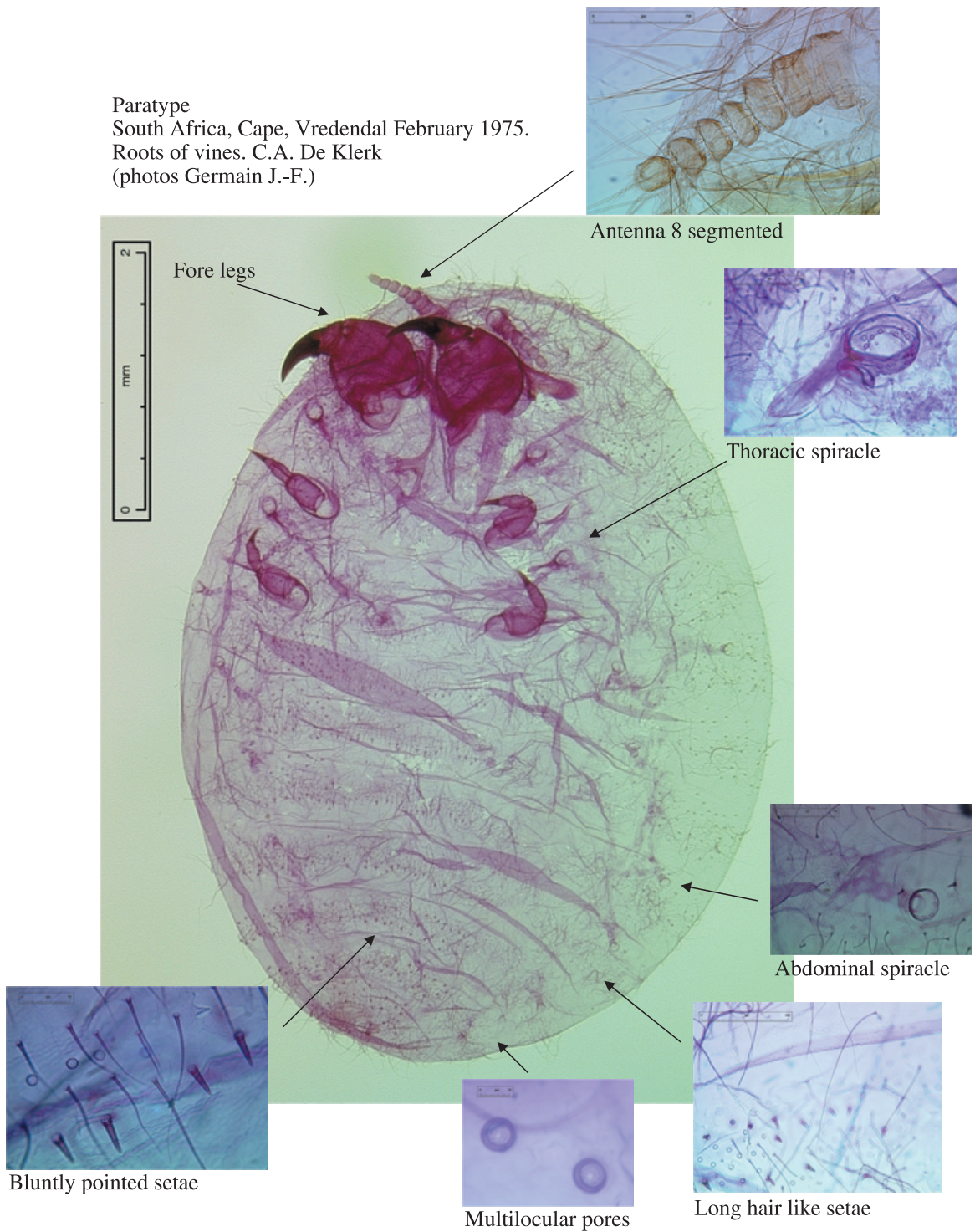


Fig. 6 *Margarodes vredendalensis* (De Klerk) adult female.

long as segment III, with 5–9 long setae and 4–5 small sensory pores at distal end. Segment III to VII: all about the same length but diminishing gradually in width; each with variable numbers of long hair-like setae and short bluntly pointed setae and sometimes with short, sharply pointed setae distributed around

distal end of each segment. Segment VIII longer and narrower than segment VII, rounded at apex with 2–4 long hair-like setae, 5–9 shorter setae and 7–13 short, cylindrical setae placed apically.

Fore legs much larger than middle and hind legs. Coxa with sclerotized apodeme; with long and short setae; Trochanter

with long and short setae as well as sensory pores. Femur 641–1029 µm long; 634–978 µm wide with long setae (194–341 µm) on ventral side and short setae on posterior and anterior sides; dorsal side almost completely bare. Tibia short, with 9–11 long setae and 2–4 min setae on posterior side; 10–18 long setae and 2–5 min setae on anterior side. Tarsus with 3–6 long setae on both posterior and anterior sides; with 4 dorsal pores grouped together proximally. Claw slightly curved; smooth on inner surface; heavily sclerotized except basally; with 2 long seta-like digitules on each posterior and anterior sides.

Middle and hind legs. Both pairs similar in size and shape. Coxa twice as wide as long with sclerotized apodeme; 5–9 long setae on ventral side; 9–15 short setae on posterior side and 8–14 on anterior side. Trochanter with 5–9 long setae on ventral side; 1–3 min setae, 0–4 long setae and 7–10 sensory pores on posterior side; 1–4 min seta, 1–5 long seta and 5–12 sensory pores on anterior side. Femur 278–444 µm long; 226–313 µm wide, with long ventral setae (140–237 µm) and short rigid setae on posterior and anterior sides. Tibia 153–215 µm long, 107–155 µm wide, with 4–6 long ventral setae (109–199 µm); 4–8 long setae and 4–7 min setae on posterior side; 4–8 long setae and 3–6 min setae on anterior side. Tarsus with 2 long ventral setae; 2–3 long setae on posterior side and 2 on anterior side; 3 dorsal pores grouped together proximally. Claw 185–272 µm long, 23–43 µm wide; slightly curved; smooth on inner surface; heavily sclerotized except basally; with one long seta-like digitule on each posterior and anterior side.

Thoracic spiracles: two pairs with circular or elongated openings; 3–4 small pores next to opening on posterior side. Atrium 125–197 µm width; with 11 to 18 large multilocular pores arranged in one or two circles on the peritreme surface, just inwards to the atrium; 18–30 simple pores.

Abdominal spiracles: seven pairs; first two pairs slightly smaller than thoracic spiracles; spiracles becoming smaller towards posterior part of abdomen; first pair closer to margin. Atrium of first two pairs 112–171 µm in diameter; with 11–18 multilocular pores arranged in one or two circles on the peritreme wall, just inwards to the atrium, 11–30 simple pores.

Mouthparts absent. Folds occur at the place where mouthparts occurs in other stages.

Genital opening: a transverse fissure situated anteriorly near anus with distinct lips; with or without setae.

Anal opening circular to oval; sclerotized; situated apically or subapically on ventral surface in middle of a small naked area.

Nymph of cyst stage: spherical, up to 8.6 mm in diameter. Cyst wall thick and very hard. Outer surface rough, like the surface of a wart; light to dark brown but bright yellow when the outer layers are removed.

Antennae: small rounded protrusion in deep pit with minute, bluntly pointed setae at distal end.

Mouthparts: Sclerotized clypeo-labral complex situated opposite thoracic spiracles. Stylets forming an oblong loop;

approximately as long as body when extended. Labium sclerotized; with about 12 short setae.

Pores: 5–8 multilocular derm pores situated around labium. Micro- and macroloculi could not be observed.

Thoracic spiracles: two pairs with circular openings; 3–4 small pores grouped together next to opening on posterior side. Atrium 93–102 µm in diameter with about 30 multilocular pores.

Abdominal spiracles: seven pairs, first pair about the same size as thoracic spiracles and situated more marginally; becoming smaller towards posterior end of abdomen. Atrium of first pair 93–102 µm in diameter with about 30 multilocular pores.

Anal opening: circular, situated sub-apically on ventral surface with 13–16 cicatrices, arranged in a line of 2 vertical pairs at each side of anus as well as 5–8 scattered around anal opening. Cicatrices occur only between anus and last pair of abdominal spiracles.

This species closely looks like *M. prieskaensis* (Jakubski, 1965), from which it differs mainly in the absence of bulbous spines.

Reference material

For *Margarodes prieskaensis* and *M. vredendalensis*: Biosystematics Division Plant Protection Research Institute. Private Bag X134 Queenswood, Pretoria 0121 (ZA). For *Margarodes vitis*: Muséum National d'Histoire Naturelle, Entomologie, 45 rue Buffon, 75005 Paris (FR).

Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

Further information

Further information on this organism can be obtained from: Ian Millar, Biosystematics Division Plant Protection Research Institute. Private Bag X134 Queenswood, Pretoria 0121 South Africa.

Acknowledgements

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Appendix 1

Among several procedures for preparing scale insects the following is purposed. See Kosztarab (1996) and Ben-Dov & Hodgson (1997) for alternatives.

- 1 Heat specimen gently in 10% solution of KOH for nearly 20 mins (never exceed 40°C). Timing depends on the size and maturity of the specimen.
 - 2 Clean specimen in heat distilled water. Retain in cleaning solution for 20 mins.
 - 3 Stain specimen in a solution of lactophenol of Aman with acid fuchsin. Retain in staining solution for 1 h.
 - 4 Wash specimen in glacial acetic acid to stabilize staining for 1 h.
 - 5 Transfer the specimen to lavender oil for at least 1 h.
 - 6 To mount, place the specimen in a drop of Canada balsam on a slide and cover with a cover-glass.
 - 7 Label slide.
- Keep slide in a dryer at 40°C, for a maximum of 4–6 weeks.