# **National Diagnostic Protocol**

## Pulvinaria iceryi (Signoret)

Pulvinaria scale



NDP 34 V1

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Photo on cover – *Pulvinaria iceryi* female adult with white ovisac on sugar cane leaf (image by DJ Tree).

### Purpose

National Diagnostic Protocols (NDPs) are diagnostic protocols for the unambiguous taxonomic identification of plant pests. NDPs:

- are a verified information resource for plant health diagnosticians
- are consistent with ISPM No. 27 Diagnostic Protocols for Regulated Pests
- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
- are endorsed by regulatory jurisdictions for use (either within their own facilities or when commissioning from others) in a pest incursion.

Where an International Plant Protection Convention (IPPC) diagnostic protocol exists it should be used in preference to NDPs although NDPs may contain additional information to aid diagnosis. IPPC protocols are available on the IPPC website:

#### https://www.ippc.int/core-activities/standards-setting/ispms

#### Process

NDPs are facilitated and endorsed by the Subcommittee on Plant Health Diagnostics (SPHD). SPHD reports to Plant Health Committee and is Australia's peak technical and policy forum for plant health diagnostics.

NDPs are developed and endorsed according to Reference Standards developed and maintained by SPHD. Current Reference Standards are available at <u>http://plantbiosecuritydiagnostics.net.au/sphd/sphd-reference-standards/</u>

NDPs are living documents. They are updated every 5 years or before this time if required (i.e. when new techniques become available).

#### **Document status**

This version of the National Diagnostic Protocol (NDP) for *Pulvinaria iceryi* is current as at the date contained in the version control box below.

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### **Further information**

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## 1 INTRODUCTION

*Pulvinaria iceryi* (Signoret) is a species belonging to the soft scale family or Coccidae (Hemiptera: Coccoidea), and is a pest of grasses (family Poaceae), particularly sugar cane (*Saccharum officinarum*) (Mamet 1958, Williams 1978, Williams 1982, Carnegie 1997, Soma & Ganeshan 2003). See Appendix 8.3 for its full host range (Ben-Dov 1993). Shafee *et al.* (1989) lists mango as a host of *P. iceryi* in India, however this is a doubtful host record (pers. comm. PJ Gullan May 2012).

Individuals of *P. iceryi* feed on leaves using their sucking mouthparts to withdraw phloem sap and cause wilting, distortion or stunting of shoots. The sugary honeydew they produce also encourages the growth of black sooty moulds which can cover the leaves, reducing photosynthesis and eventually causing reduction or stoppage of growth of the plant (Williams 1982, Soma & Ganeshan 2003, Sallam 2011).

*Pulvinaria iceryi* is known commonly in French as "pou á poche blanche" because of the white wax ovisac that is produced from the female's ventral surface and expands as eggs are laid (Williams 1978, Soma & Ganeshan 2003).

## 2 TAXONOMIC INFORMATION

### Pulvinaria iceryi (Signoret)

Kingdom – Animal Phylum – Arthropoda Class – Insecta Order – Hemiptera Suborder – Sternorrhyncha Superfamily – Coccoidea Family – Coccidae Genus – *Pulvinaria* Targioni Tozzetti

*Synonyms.* The synonymy list is from Ben-Dov (1993) and the full details of the references for the names are available from ScaleNet (Ben-Dov *et al.* 1993).

Lecanium iceryi Guerin-Meneville, 1868: 92. Lecanium iceryi Signoret, 1869a: 857. Lecanium iceryi Signoret, 1869b: 95. Lecanium gasteralphe Signoret, 1869c: 101. Pulvinaria gasteralphe Signoret, 1869c: 101. Pulvinaria gasteralpha Signoret, 1873: 37. Pulvinaria iceryi; Fernald, 1903b: 133. Change of combination. Pulvinaria iceryi; Fernald, 1903b: 133. Incorrect citation of "Guerin-Meneville" as author. Pulvinaria lepida Brain, 1920a: 20. Pulvinaria elongata durbanensis Munro & Fouche, 1936: 94. Pulvinaria elongata; Mamet, 1949: 27. Misidentification. Pulvinaria lepida; Mamet, 1949: 28. Misidentification. Coccus iceryi; Tao et al., 1983: 87. Change of combination. Saccharipulvinaria iceryi; Tao et al., 1983: 87. Change of combination. Pulvinaria iceryi; Ben-Dov, 1993: 266. Revived combination.

See Appendix 8.4 for a full description.

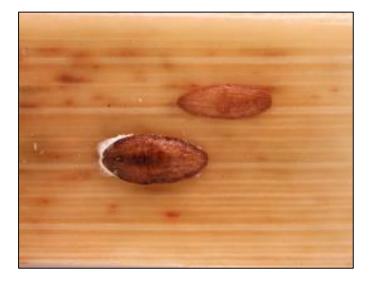
## 3 DETECTION

## 3.1 Signs and symptoms

*Pulvinaria iceryi* occurs mainly on lower leaf surfaces of the host plants (Soma & Ganeshan 2003, Sallam 2011), however Mamet (1958) reports that this species occurs on the upper as well as the lower surfaces of the leaves. On sugar cane *P. iceryi* also may occur on the leaf sheath near its junction with the leaf blade, and on grass weeds it can occur on stems as well as the rachis of the inflorescence (Williams 1980). Sugar cane can be infested by *P. iceryi* at all stages of growth, however numbers tend to increase as the cane matures (Sallam 2011). Heavy infestations result in growth reduction, chlorosis of leaves and sometimes death of shoots and stools (Carnegie 1997, Soma & Ganeshan 2003). Plants that survive heavy infestations of *P. iceryi* remain weak and can fail to ratoon after harvest (Sallam 2011).

## 3.1.1 Field description

"Adult female extremely elongate or elliptical, extremities more or less equally attenuated, dorsum convex and shiny: cephalic region flattened in mature females; eyes black, fringed with glassy filaments. Before production of ovisac, female more or less membranous and its coloration varies from pale straw-coloured to pale brownish-yellow; submarginal and submedian areas speckled with greyish, blackish or deep bordeaux-red or violet spots which sometimes occur in patches especially in the submedian zone; these spots absent from the broad median area which is straw-coloured. Beginning of formation of ovisac marked by appearance all-round the female of pure white secretionary matter on which the female rests. At oviposition time, female becomes darker throughout and is more sclerotised; posterior extremity transversely ridged, tilted upwards and rests on waxy secretions forming the ovisac. Ovisac well developed, sometimes longer than the insect, pure white in colour, with very feebly marked carinae; extending anteriorly to about the region of the eyes (Figure 1). Venter of female before oviposition membranous but afterwards becoming somewhat sclerotised" (Mamet, 1958: 70).



**Figure 1** Adult female showing the well-developed ovisac, together with an immature female. (image by DJ Tree)

## 3.1.2 Development

Typically for coccid species, females have four instars and males have five instars (Figure 2), although many species of coccids are parthenogenetic and do not produce males (Williams 1978, Williams 1980, Soma & Ganeshan 2003).

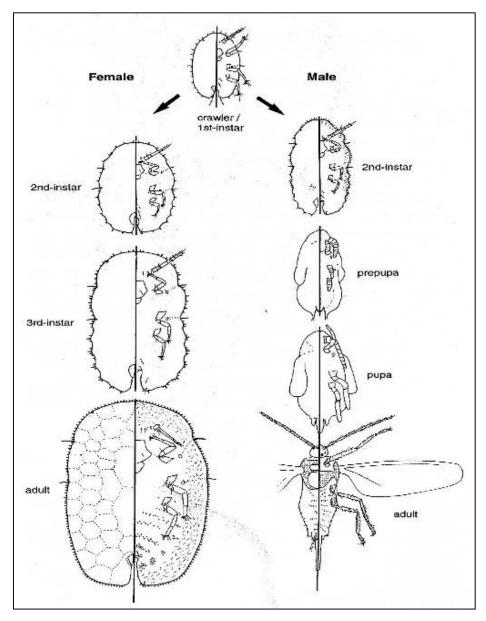


Figure 2 Typical life cycle of Coccidae (Hodgson & Henderson 2000).

*Pulvinaria iceryi* is the most damaging soft scale on sugarcane and nearly all of the literature on this species has been published in Mauritius, where major outbreaks occurred in the 1850's (Mamet 1958) and 1975/76 (Williams 1978, Williams 1980, Williams 1982, Carnegie 1997, MSIRI 2001, Soma & Ganeshan 2003, Sallam 2011). The first-instar nymph, or crawler (mobile stage), may either be carried by wind, moved by farm machinery, clothing or planting material. Once settled, the nymphs are protected by the leaf sheaths. The honeydew produced by the scale attracts ants and their presence may reduce the effectiveness of parasitoids and predators (Carnegie 1997).

## 3.1.3 Similar species

Field symptoms are similar to those for many other soft scales – i.e., adults exude honeydew and this encourages the growth of sooty mould.

*Pulvinaria iceryi* is most similar morphologically to *Pulvinaria elongata* Newstead; however *P. elongata* is not considered to be such a significant pest as is *P. iceryi* (Carnegie 1997). *P. elongata* has been recorded in many countries in the Afrotropical, Neartic, Neotropical and Palaearctic regions and is also known from the Australasian countries of Australia (Queensland only) and Papua New Guinea (Ben-Dov 1993.). Its host range covers mainly grasses, including sugar cane. See Ben-Dov 1993 for a complete host list for *P. elongata*.

The ovisac of mature females of *Pulvinaria iceryi* is well developed (Figure 2), sometimes longer than the body (Hodgson 1994), whereas the ovisac of *P. elongata* is very short and extends only slightly beyond the margin of the body (Mamet 1958).

Other characteristics of similar species can be identified only with microscopic examination and are outlined in section 4.

## 3.2 Sampling procedure

Photos of the live scales on plant material are useful for diagnostic purposes to show colour, size and shape of scales as well as a record of extent of damage to the plants/crops. Several pieces of infected plant material should be collected into 70% ethanol in a screw top plastic vial. It is best not try to remove scales from plant material as this may damage them; careful removal from leaves needs to be done in the laboratory under a stereomicroscope.

Specimens need to be transported as soon as possible to the diagnostic laboratory or stored in refrigerator at 5°C to prevent specimen degradation. Once the laboratory has received the sample, suitable adults should be carefully removed from plant material for slide mounting. Excess specimens can be stored in 70% ethanol and, if future DNA analysis is required, some specimens should also be stored in 100% ethanol in freezer at -20C.

Young adult females are best to process for slide mounting and identification (PJ Gullan pers. comm. July 2011). Nymphs do not possess the character states of adults and all diagnostic keys include only adult character states. The cuticle of adult females becomes more sclerotised with age, obscuring diagnostic character states, and very mature adult females also may contain many eggs or embryos, making maceration difficult and producing unclear exoskeletons, leading to problematic identification.

A series of adult female individuals (at least 5) is needed per sample for slide mounting, allowing for detection of any variation within the sample and because some character states maybe visible on one individual but unclear on another (GW Watson pers. comm. July 2011).

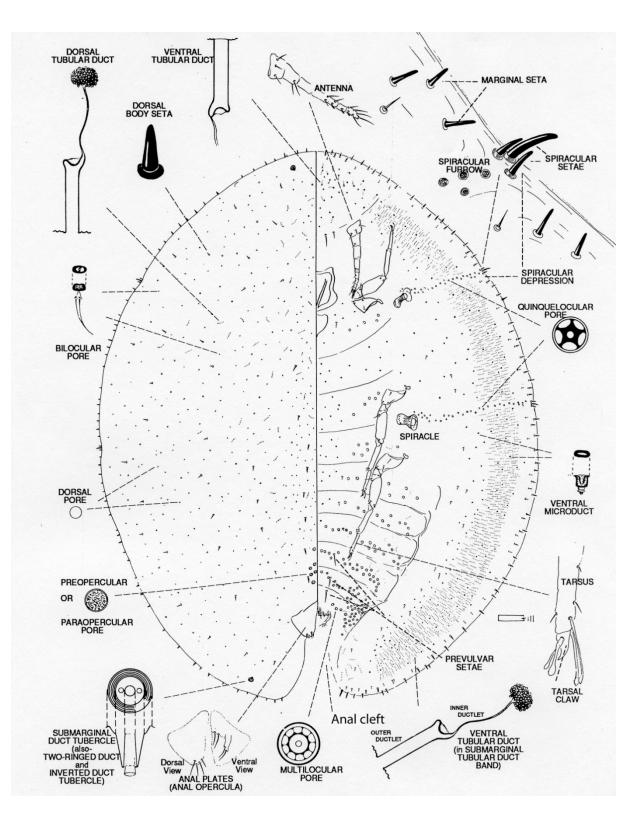
## 4 IDENTIFICATION

Proposed methodology for diagnosis:

- 1. Field identification of family: If scale cover is integral part of the body and cannot be removed, it is likely to be family Coccidae.
- 2. Prepare slides (4.1.1).
- 3. Confirm that scales are in the family Coccidae by confirming the presence of anal plates and anal cleft (Figure 3).
- 4. Identify Coccidae genera:
  - use "Key to Australian Coccidae genera known from grasses" (4.2.1). If samples key to *Pulvinaria*, proceed to 5.
  - if specimens key to another genera then samples are not *P. iceryi* and other literature will be required, such as –
  - a) Online Coccidae key (Miller *at al* 2015) available at <u>http://idtools.org/id/scales/key.php?key=soft</u>
  - b) Key to Coccidae genera of the tropical South Pacific region (Appendix 8.1)
- 5. Identify *Pulvinaria* species:
  - use Key to *Pulvinaria* species known from grasses (4.2.2), this key will determine if specimens are *P. iceryi*
  - if specimens do not match species in key then they are unlikely to be *P. iceryi* proceed to Key to *Pulvinaria* species from Australia (Appendix 8.2).

Identification is based on morphological characteristics of well-prepared slide mounts of adult female scales. Slides are viewed under a compound microscope, differential interference contrast or phase contrast is required to see fine structures such as ducts, pores and setae. A 40x objective lenses is essential along with either a 10x or 20x objective, 60x and/or 100x objective lenses are optional. Currently there are no protocols developed for molecular analysis for *Pulvinaria iceryi*.

Voucher specimens of *Pulvinaria iceryi* can be found in Queensland Primary Industries Insect Collection (QDPC), DAF, Queensland, and the Australian National Insect Collection (ANIC), CSIRO Ecosystem Sciences, A.C.T.



**Figure 5** – General morphology of Coccidae (soft scales) (modified from Gill 1988)

## 4.1 Methods based on morphological character states

## 4.1.1 Procedure for mounting scale specimens onto microscope slides

Slide-mounting method for coccoidea (P.J. Gullan), adapted from Kozarzhevskaya, E.F. (1968) Techniques for preparing slides for coccoid (Homoptera; Coccoidea) determination. *Entomol. Rev.* 47:146-149

1. Kill insects and preserve in either 70-75% ethanol, **or** in lactic acid/ethanol:

2 parts 95% ethanol + 1 part 75% lactic acid (Note that lactic acid destroys DNA)

- 2. Make a small slit on right margin OR on transversely on dorsal thorax OR cut around 3/4 of margin (to expose dorsal and ventral surfaces) while insect is venter up. Place in cold 10% KOH overnight or for up to 24 hours to clear body contents (much longer is needed if the insects were preserved in 100% ethanol) and then heat gently on a hot plate (40–50°C) for several hours to finish the clearing process.
- 3. Once the body contents have dissolved in the KOH, place specimens in water to which a drop of detergent has been added and express body contents using two very fine-tipped paint brushes.
- 4. Place specimens into stain solution. For membranous cuticles use a more concentrated stain mixture; experience will denote correct strength and time in stain.

**Stain:** Add 3 parts acid alcohol to 1 part acid fuchsin solution and dilute to required stain concentration with extra acid alcohol if necessary, i.e. light or dark pink.

Acid alcohol: 20% acetic acid plus 80% of 50% ethanol Acid fuchsin: acid fuchsin powder

0.5 g, 10% HCI 25 ml, water --- 300 ml

Leave specimen in stain for 5 minutes to 24 hours or until ready. Check by removing from stain and viewing under microscope in 95% ethanol. Required duration of staining varies from species to species and also varies with maturity of specimens.

5. Place a little 95% ethanol into a small petri dish, and then place stained specimen into petri dish and soak to remove excess stain. Arrange insect in the position for final mounting (e.g. make the legs tidy if the specimen has well developed legs); if specimen has been cut around margin then arrange so that dorsum and venter are side by side (opened out).

Change ethanol to 100% and leave for 5 to 10 mins; repeat this dehydration 2 or 3 times.

6. Change from the ethanol to 100% propanol (= propyl alcohol) and leave for 5 to 10 mins; repeat this dehydration two times.

- 7. Fill three small dishes with 100% xylene (or Histolene or anhydrous clove oil safer substitutes for xylene if you do not have access to a fume cupboard), place lids on them and put in fume cupboard. Transfer specimen from last change of 100% propanol to first dish of xylene and leave for about 10 minutes; repeat this twice more. **NOTE: Only use xylene in a fume cupboard -- it is toxic; Histolene (also called Histoclear) and clove oil can be used as safer alternatives but still use a fume cupboard.**
- 8. Place a small amount of mounting medium (Canada balsam) on a microscope slide. Transfer specimen to slide and arrange specimen as desired. Add a tiny smear of mounting medium to coverglass and drop flat onto specimen.

## 4.2 Keys

### 4.2.1 Key to the Australian Coccidae genera known from grasses

(adapted from Williams & Watson 1990 and Hodgson 1993)

1 Dorsal surface with polygonal or tessellated pattern (Figure 4) <i>Parasaissetia</i> Takahashi - Dorsal surface without polygonal or tessellated pattern
<ul> <li>2 Stigmatic cleft (or spiracular furrow) absent and stigmatic setae not differentiated from marginal setae (Figure 5)</li> <li>- Stigmatic cleft present and stigmatic setae different from marginal setae</li> </ul>
3 Stigmatic setae numbering 2 of equal length (Figure 6)

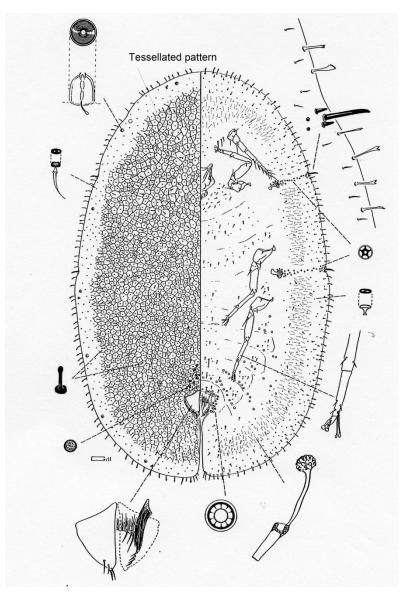
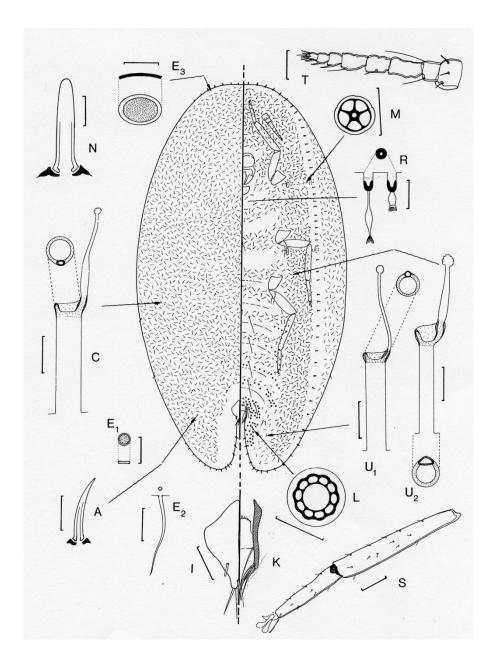
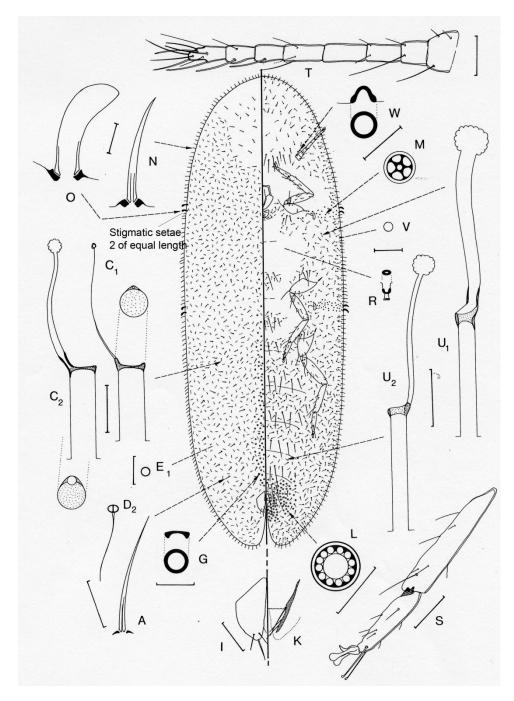


Figure 4 - Parasaissetia nigra (modified from Hodgson 1993)



**Legend** – A = dorsal setae, C = dorsal tubular ducts, E = dorsal pores other than microducts, K = anogenital fold, L = pregenital disc-pores, M = spiracular disc-pores, N = marginal setae, R = ventral microducts, S = legs, T = antenna, U = ventral tubular ducts.

**Figure 5** – *Symonicoccus stipae* (modified from Hodgson 1993), showing the lack of spiracular furrow/stigmatic cleft. *Symonicoccus* genus is restricted to Poaceae in Australia.



**Legend** – A = dorsal setae, C = dorsal tubular ducts, E = dorsal pores other than microducts, K = anogenital fold, I = anal plates, L = pregenital disc-pores, M = spiracular disc-pores, N = marginal setae, O = stigmatic setae, R = ventral microducts, S = legs, T = antenna, U = ventral tubular ducts, V = ventral pores other than ventral microducts.

**Figure 6** – *Luzulaspis luzulae* (modified from Hodgson 1993), showing the 2 stigmatic setae of equal length

## 4.2.2 Key to *Pulvinaria* species known from grasses

Reproduced from Williams (1982).

1	Ventral tubular ducts numerous, forming a submarginal zone that extends medially to be almost continuous with the ducts across the abdominal segments (Figure 7) <i>iceryi</i> (Signoret) Ventral tubular ducts forming a submarginal zone that is much narrower and distinct from the medial abdominal ducts
2	Dorsum with tubular ducts (Figure 8)
3	Claw digitules of one size (Figure 9)
4	Majority of dorsal setae conical (Figure 10)tenuivalvata (Newstead) Majority of dorsal setae lanceolate (Figure 11)

### See Appendix 8 for additional keys -

#### 8.1. Key to Coccidae genera of the tropical South Pacific region

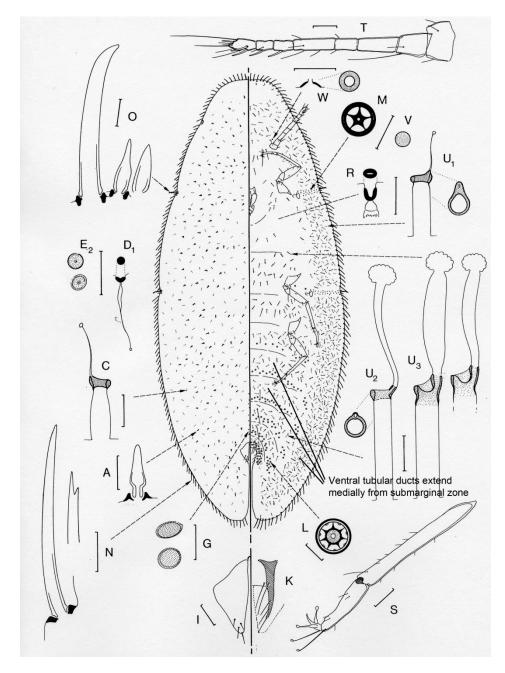
8.2. Key to *Pulvinaria* species from Australia.

## 4.3 Description of *Pulvinaria iceryi*

Figure 7:

- Marginal setae tapering to a point or frayed end
- Dorsal setae small and lanceolate
- Anal plates quadrate with outer margins subequal; with 3 small subapical setae and 1 small apical seta
- Three stigmatic setae with middle setae longer than the lateral setae
- Antennae 8-segmented
- Ventral tubular ducts numerous in submarginal band which extend medially, almost forming continuous bands across abdominal segments
- Legs well developed with tibio-tarsal articulation and articulatory sclerosis; claw without denticle
- Pregenital disc-pores abundant around genital opening, reducing in number across preceding abdominal segments and almost absent entirely from thoracic segments with 1 or 2 present near metathoracic spiracle.

For a complete taxonomic description see Appendix 7.4.



**Legend** – A = dorsal setae, C = dorsal tubular ducts, E = dorsal pores other than microducts, K = anogenital fold, I = anal plates, L = pregenital disc-pores, M = spiracular disc-pores, N = marginal setae, O = stigmatic setae, R = ventral microducts, S = legs, T = antenna, U = ventral tubular ducts, V = ventral pores other than ventral microducts, W = pre-antennal pores.

Figure 7 Pulvinaria iceryi (modified from Hodgson 1994)

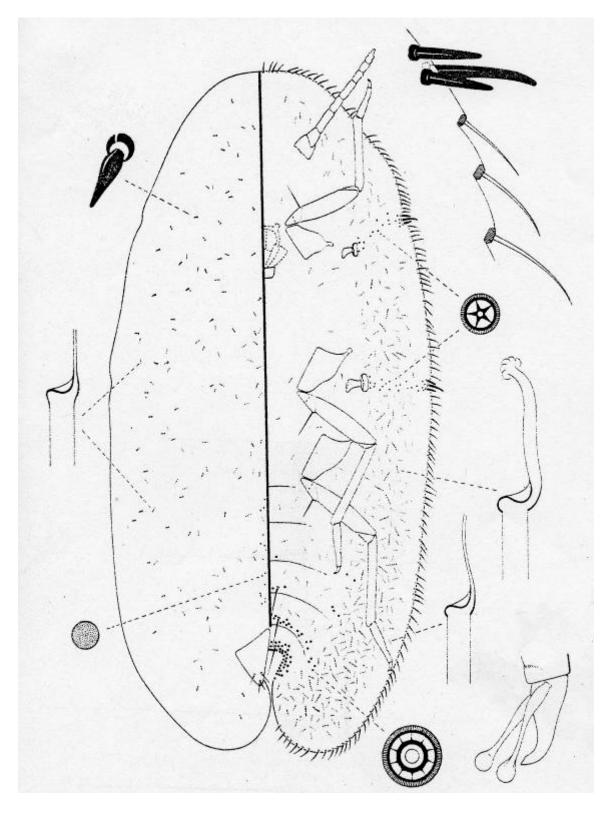
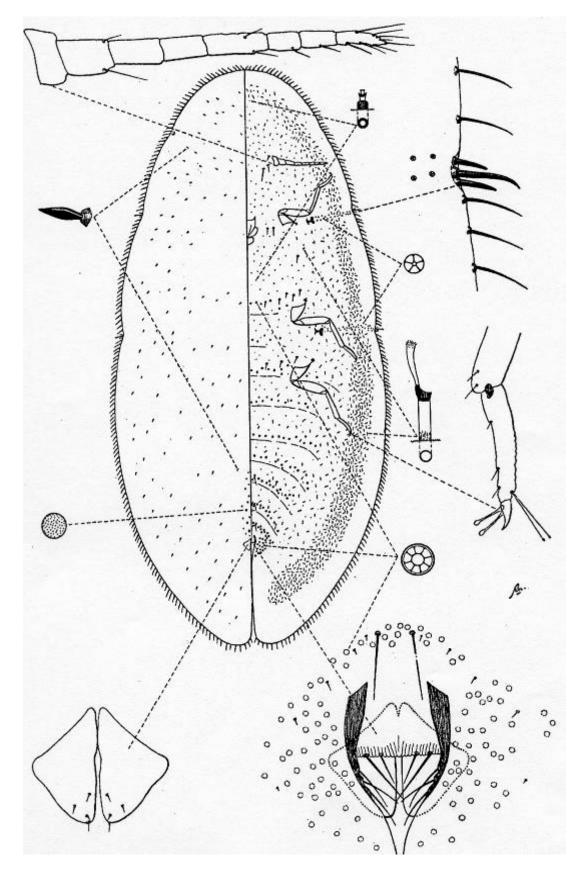
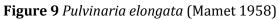


Figure 8 Pulvinaria sorghicola (De Lotto 1979)





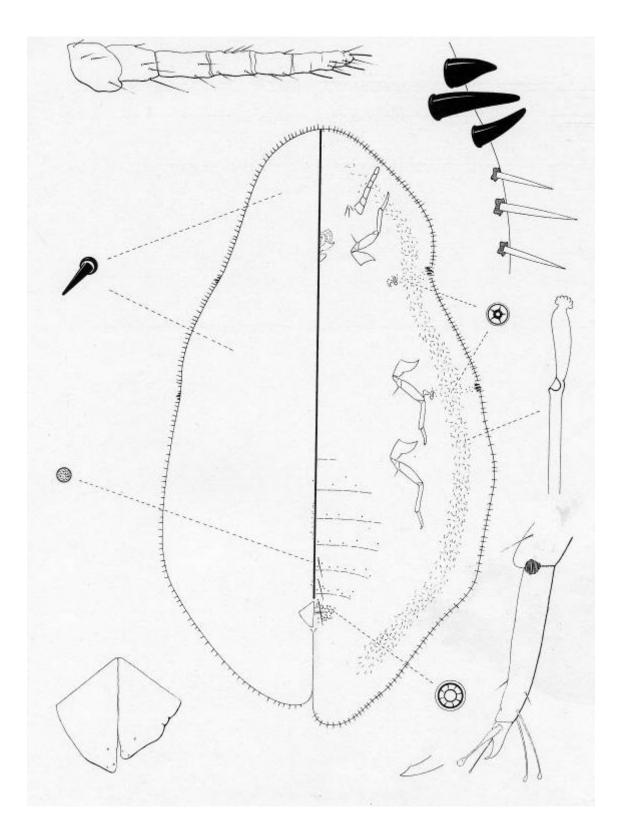


Figure 10 Pulvinaria tenuivalvata (De Lotto 1965)

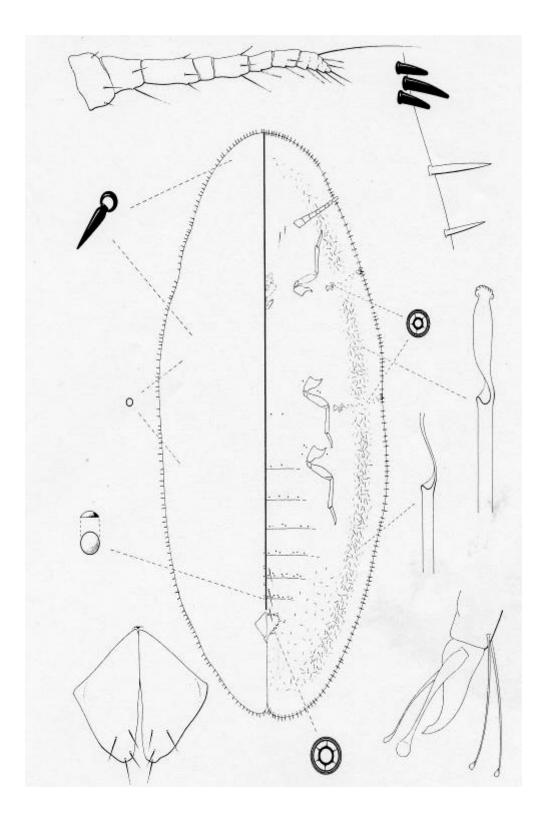


Figure 11 Pulvinaria saccharia (De Lotto 1966)

## 5 CONTACTS FOR FURTHER INFORMATION

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The protocol was reviewed and verified by Dr Jamie Davies, Department of Primary Industries, Parks, Water and Environment, Tasmania.

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#### Useful website:

#### http://scalenet.info/

ScaleNet is a tool for finding information about scale insects: their taxonomic diversity, nomenclatural history, biogeography, ecological associations, and economic importance. ScaleNet models the scale insect literature. Currently it contains data from **23476** references pertaining to **8195** valid species names.

## 8 APPENDIX

## 8.1 Key to Coccidae genera of the tropical South Pacific region

Reproduced from Williams and Watson (1990).

1	Marginal setae fan-shaped, overlapping, forming a continuous fringe <i>Paralecanium</i> Cockerell Marginal setae, if present, flagellate or spine-like
2	Second and third pairs of legs larger than first pair of legs, the second and third pairs of coxae conspicuously larger than first coxae
3	Anal plates situated at centre or at tip of sclerotized caudal process
4	Long interantennal setae numbering 1 or 2 pairs. Some dorsal pores with 3 or more loculi 
	Long interantennal setae numbering 7–10 pairs. Dorsal pores never with more than 2 loculi 
5	Stigmatic clefts each with lobes containing a few fleshy protuberances. Dorsal pores distinctly flower- shaped
6	Ventral tubular ducts numerous, present in an obvious submarginal zone
7	Stigmatic setae opposite each spiracle single, conspicuously long <i>Drepanococcus</i> gen. n. Stigmatic setae opposite each spiracle present in groups of 3 or more
8	Anal plates together at least 3.5 times longer than wide; anterior end of anal plates lying over mesothorax <i>Protopulvinaria</i> Cockerell Anal plates together 1–1.5 times longer than wide; anterior end of anal plates lying over posterior abdominal segments
9	Discal seta present on each anal plate Discal seta absent from each anal plate10
10	Tibia and tarsus distinct but not articulated, tibio-tarsal articulatory scleroses absent, tarsus straight. Dorsal setae cylindrical or slightly clavate apically
11	Stigmatic setae present in groups of more than 5 setae. Marginal setae stout, truncate, each with bidentate tip

12	Legs absent or reduced to minute tubercles13	
	Legs well developed, although sometimes small for size of body14	

- 18 Dorsal surface with areolations, minute or large, only present around submargins, the areolation never joined ......*Coccus* Linnaeus Dorsal surface with areolations joined, forming a reticulated pattern .....*Neosaissetia* Tao & Wong

## 8.2 Key to *Pulvinaria* species in Australia

Key to adult females of Australian Pulvinariini species reproduced from Qin and Gullan (1992).

- ) -	
1	All marginal setae with apex blunt, truncate or rounded, never notched
2	Tubular ducts numerous dorsally, each duct with a flowery-tipped inner filament; ovisac completely or partly covering body of female
3	Spiracular setae absent; body usually wider than long <i>Pulvinaria salicorniae</i> Froggatt Spiracular setae present; body never wider than long
4	Multilocular pores oval, lateral loculi not visible under light microscope; spiracular cleft sclerotization small and triangular <i>P. flavicans</i> Maskell Multilocular pores circular, lateral loculi clearly visible under light microscope; spiracular cleft sclerotization large and horseshoe-shaped
5	Submarginal chambered ducts present; ovisac partly covering body of female <i>P. dodonaeae</i> Maskell Submarginal chambered ducts absent; ovisac completely covering body of female 
6	Body elongate, length more than twice width; dorsal setae lanceolate <i>P. elongata</i> (Newstead) Body usually oval to broadly oval, length usually less than twice width; dorsal setae never lanceolate7
7	Marginal setae truncate; anal plates with dorsal reticulations <i>P. maskelli</i> Olliff Marginal setae never truncate; anal plates without reticulations
8	Spiracular cleft sclerotization present, triangular or horseshoe-shaped <i>P. thompsoni</i> Maskell Spiracular cleft sclerotization absent
9	Submarginal tubercles always present, at least 7 present around body10 Submarginal tubercles mostly absent, occasionally 1 or 2 present
10	Dorsum with cell-like clear areas; some or all spiracles each surrounded by a sclerotic plate in mature specimens; 3 subapical setae present on each anal plate
11	Spiracular setae numbering more than 3; marginal setae deeply branched P. polygonata Cockerell Spiracular setae always numbering 3; marginal setae not deeply branched
12	Marginal setae 12–40 μm long, all finely toothed; some tubular ducts on venter each with a very long inner filament (28–42 μm) <i>P. psidii</i> Maskell Marginal setae 30–100 μm long, only some toothed; tubular ducts on venter each with an inner filament only 15–20 μm long <i>P. decorata</i> Borchsenius
13	Subdiscal seta present on each anal plate; tubular ducts numerous all over venter; marginal setae never with parallel sides <i>P. hydrangeae</i> Steinweden Subdiscal seta absent from each anal plate; tubular ducts absent from submarginal area of venter of head; some marginal setae with parallel sides <i>P. urbicola</i> Cockerell

## 8.3 Host list for *Pulvinaria iceryi*

(Ben-Dov 1993), (APG II 2003, APG III 2009, Angiosperm Phylogeny Group 2012)

Family	Genus species
* Anacardiaceae	Mangifera indica
Poaceae	Agropyron repens
	Andropogon schinzii
	Cymbopogon giganteus
	Cynodon dactylon
	Digitaria didactyla
	Digitaria scalarum
	Eleusine coracana
	Heteropogon contortus
	Panicum maximum
	Paspalidium geminatum
	Saccharum officinarum

\* Anacardiaceae, *Mangifera indica* is a doubtful host record.

## 8.4 Full taxonomic description – *Pulvinaria iceryi* (Signoret)

### See Figure 7.

Description of *Pulvinaria iceryi* reproduced from Hodgson 1994. *Saccharipulvinaria* is considered a synonym of *Pulvinaria* by some authors.

### Saccharipulvinaria iceryi (Signoret)

### Lecanium iceryi Signoret, 1869b: 95

**Unmounted material**. "Adult female extremely elongate or elliptical, extremities more or less attenuated, dorsum convex and shiny; cephalic region flattened in mature females; eyes black, fringed by white glassy filaments. Before production of ovisac, female more or less membranous and its colouration varies from pale straw-colour to pale brownish-yellow; submarginal and submedian areas speckled with greyish, blackish or deep bordeaux-red or violet spots which sometimes occur in patches especially in the submedian zone; these spots absent from the broad median area which is straw-coloured. Beginning of formation of ovisac marked by appearance all round the female of pure white secretory matter on which the female rests. At oviposition time, female becomes darker throughout and is more sclerotized; posterior extremity transversely ridged, tilted upwards and rests on waxy secretions forming the ovisac. Ovisac well developed, sometimes longer than the insect, pure white in colour, with very feebly marked carinae; extending anteriorly to about the region of the eyes. Venter of female before oviposition membranous but afterwards becoming somewhat sclerotized" (Mamet, 1958).

**Mounted material**. Body elongate oval, narrowing almost equally at both ends, with slight indentations at each stigmatic cleft and sometimes near each eyespot; anal cleft about 1/7<sup>th</sup> body length; dorsum probably not very convex in life. Length 2.8–4.5 mm, width 1.0–2.0 mm.

**Dorsum**. Derm membranous throughout on available material (but becoming slightly sclerotized at maturity). Dorsal spines each rather small and lanceolate, with a marked constriction at base, rather bluntly pointed and in a well-developed basal-socket; each seta 4.7  $\mu$ m long; frequent throughout dorsum but usually arranged segmentally medially on abdomen and thorax. Dorsal pores of 2 types present, both frequent throughout: (i) a minute microdcut; and (ii) a rather larger, round to oval, flat pore with a granulate surface. Preopercular pores rather similar to type (ii) but much larger and more oval, in an elongate group of 14–20 pores in front of anal plates. Dorsal tubular ducts of one type present, each with a squat outer ductile, and a fine inner ductile with a minute terminal gland. Dorsal tubercles present. Anal plates together quadrate, each plate with outer margins subequal and with 3 small subapical setae and a small apical seta; length 135–158  $\mu$ m, width of single plate 70–82  $\mu$ m. Anogenital fold with 2 pairs of robust setae present along anterior margin and 3 pairs laterally. Anal ring with 3 or 4 pairs of setae present.

**Margin**. Marginal setae each spinose and often leaning posteriorly, with rather parallel sides and a sharp apex, which is occasionally bifid; in a broad basal-socket; length of each seta 17–58 μm (the shorter setae sometimes appearing to be set slightly onto dorsum); with 19–25 setae on each side between stigmatic clefts; those on either side of anal cleft not longer than elsewhere. With 3 stigmatic spines in each cleft; median spine about 3x as long as each lateral spine, usually curved posteriorly, rather broad and bluntly pointed, in a narrow basal-socket; length of each median spine 35–53 μm; lateral spines stout at base, abruptly narrowing about half-way along their length on some specimens

but narrowing gradually on others, each in a narrow basal-socket; length of each lateral spine 14– $18 \mu$ m. Eyespot present on margin; width of each lens 18–20  $\mu$ m.

**Venter**. Derm membranous throughout (but becoming slightly sclerotized at maturity). Pregenital disc-pores each mainly with 7 or 8 loculi; abundant around genital opening, becoming progressively less frequent across preceding abdominal segments; occasionally with 1 or 2 present just mesad to each metathoracic spiracle. Spiracular disc-pores each mainly with 5 loculi present; present in bands 2-4 pores wide between margin and each spiracle, with 0-4 pores present more medially; with 22-28 disc-pores in each anterior band and 26–42 in each posterior band. With 2 or 3 preantennal pores present near base of each antenna. Ventral microducts and a small simple pore, similar to type (ii) on dorsum, present scarcely throughout. Ventral tubular ducts of 3 types present: (i) a small duct, rather similar to that on dorsum, with a squat outer ductile and fine inner ductile with a minute terminal gland; almost restricted to submarginal tubular duct band, most frequent on either side of anal cleft, least frequent anterior to antennae; with a few also present medially on pregenital segment; (ii) a duct with a long, moderately thin outer ductile, a shallow cup-shaped invagination and a thin inner ductile with a well-developed terminal gland; most abundant medially on more posterior abdominal segments but also frequent in submarginal band; and (iii) a stouter duct, with a broad outer ductile, welldeveloped cup-shaped invagination and a wide inner ductile (which is as broad as the outer ductile in some specimens); particularly frequent towards posterior end but frequent throughout submarginal band and least frequent on head; only duct present medially on anterior abdominal segments and on meso- and metathorax, but inner ductile becomes rather thinner on more anterior ducts and can appear similar to type (ii) ducts; no ducts detected medially on prothorax. Ventral setae: pairs of long setae present medially on 3 pregenital segments; also with 2 pairs of long and 2–4 pairs of shorter setae between antennae; setae of moderate length present medially on abdomen and thorax; other setae small. Spiracles normal; width of each peritreme: anterior 38–53 μm; posterior 53–70 μm. Legs well developed; each with a tibio-tarsal articulation and articulatory sclerosis; each claw rather narrow, without a denticle; claw digitules both rather narrow but broader than slightly longer tarsal digitules; dimensions (iii): trochanter + femur 226–209 µm, tibia 188–248 µm and tarsus 116–126 µm. Antennae each with 8 segments; total length  $250-418 \mu m$ . Labium: width 79–94  $\mu m$ .

### Material examined.

**KENYA**, Endebess, ex *Agropyron repens* (Gramineae), 22.viii.1951, collector unnamed (BMNH: 2/2). **MAURITIUS**, Reduit, ex sugar cane (*Saccharum officianarum*) (Gramineae), 21.vii.1976, J.R. Williams (BMNH: 1/2).

**SOUTH AFRICA**, Natal, Durban, sugar cane, no date, no collector (BMNH: 1/3).

#### Discussion

Whilst the designation Tao and Wong (in Tao *et al.* 1983) of *Pulvinaria iceryi* as the type species of their new genus *Saccharipulvinaria* is quite clear, they illustrated a type species which is clearly not *S. iceryi* as it has only 6-segmented antennae and lacks ventral tubular ducts medially. The illustration was considered to represent *Pulvinaria elongata* Newstead by Williams and Watson (1990). As implied by Williams and Watson, the exact status of this genus will have to await a complete revision of the grass-inhabiting group of Pulvinariini, as the present facies of *Saccharipulvinaria* does not cover all such species. However, the lanceolate form of the dorsal setae and the elongate shape of the body separates *S. iceryi* and closely related genera from other genera in the Pulvinariini. The authorship of this genus is explained by Kosztarab *et al.* (1986).

Six species of Pulvinaria have been recorded from Poaceae, mainly from the Afrotropcial region, but how many of these are congeneric with S. iceryi remains to be seen. *Saccharipulvinaria* is considered a synonym of *Pulvinaria* by some authors.

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### Comments (PJ Gullan pers. comm. June 2012)

Hodgson's drawing of *P. iceryi* includes numerous dorsal ducts. However after examination of dissected females collected from Mauritius in 2012, very few dorsal ducts were observed, possibly fewer than 20. It is possible that Hodgson had difficulty seeing the dorsal ducts and thus illustrated a greater number than really existed on the specimens. The types and distribution of ventral ducts that Hodgson describes match the Mauritius females except that there are no ducts on the head between and immediately anterior to the antennae in the Mauritius females.

**Comment** – Hodgson (1994) mentions in the last paragraph above that there are six species of *Pulvinaria* known from grasses. There are only five species included in the key to species known from grasses in this report (4.2.2). The sixth species, *P. bambusicola*, was described by Tang 1991 from bamboo, in China. This is the only known description of *P. bambusicola* and it is written in Mandarin Chinese. This description is very brief and, once translated to English, it was clear that the description has very few of the character states that are included in the descriptions of other *Pulvinaria* species from grasses. Moreover, included in Tang (1991) is a description of *P. iceryi* that states this species has six antennal segments, which of course is incorrect (see *P. iceryi* description above). Thus, *P. bambusicola* is of doubtful identity and is not included in the key to *Pulvinaria* species from grasses.