National Diagnostic Protocol

Protopulvinaria pyriformis (Cockerell) Pyriform scale



NDP 33 V1

Protopulvinaria pyriformis is now present in Australia. This protocol is being kept as a resource but will not be reviewed or updated.

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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 *Protopulvinaria pyriformis* is current as at the date contained in the version control box below.

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Further information

Inquiries regarding technical matters relating to this project should be sent to: sphds@agriculture.gov.au

Contents

1	INT	RODUCTION	. 5
2	TA	XONOMIC INFORMATION	. 6
3	DE	FECTION	.7
	3.1	Signs and symptoms	. 7
	3.2	Sampling procedure	. 9
4	IDE	ENTIFICATION	10
	4.1	Methods based on morphological character states	10
	4.2	Keys	12
	4.3	Description of Protopulvinaria pyriformis – Fig. 7	17
5	COI	NTACTS FOR FURTHER INFORMATION	20
6	ACI	KNOWLEDGEMENTS	22
7	REI	FERENCES	23
8	API	PENDIX	25
	8.1	Host list for Protopulvinaria pyriformis	25
	8.2	Complete taxonomic description of <i>Protopulvinaria pyriformis</i> (Hodgson 1994)	27

1 INTRODUCTION

Protopulvinaria pyriformis (Cockerell) is a species belonging to the soft scale family or Coccidae (Hemiptera: Coccoidea) that contains many economically important pests of agriculture (Gill 1988; Williams & Watson 1990; Miller & Miller 2003). Scale insects feed on plant tissue using their sucking mouthparts to withdraw phloem sap causing wilting, distortion or stunting of shoots. The sugary honeydew they produce also encourages the growth of black sooty moulds that can cover the leaves and fruit, reducing photosynthesis (Gullan 1997).

Protopulvinaria pyriformis (pyriform scale) is a pest of fruit trees and ornamentals, particularly avocado, citrus and gardenia and has a wide range of hosts – see Appendix 1 (Ben-Dov 1993).

2 TAXONOMIC INFORMATION

Protopulvinaria pyriformis (Cockerell)

Kingdom – Animal, Phylum – Arthropoda, Class – Insecta, Order – Hemiptera, Suborder -Sternorrhyncha, Superfamily – Coccoidea, Family – Coccidae, Genus – *Protopulvinaria* Cockerell.

Synonyms:

- Pulvinaria (Protopulvinaria) pyriformis Cockerell, 1894c: 309.
- Pulvinaria newsteadi Leonardi, 1898b: 121.Synonymy by Cockerell, 1899k: 311.
- *Pulvinaria plana* Lindinger, 1911b: 34. Synonymy by Lindinger, 1912: 199.
- *Protopulvinaria piriformis*; Lindinger, 1912b: 104; Brain, 1920a: 17 Misspelling of species name.
- *Protopulvinaria piriformis*; Gomez-Menor Ortega, 1929: 4. Misspellings of species name.
- *Protopulvinaria agalmae* Takahashi, 1933: 39. Synonymy by Takahashi, 1955a:36.
- Protopulvinaria pyiformis; Tao, 1978: 82. Misspelling of species name.
- *Pulvinaria phriformis*; Pollard & Alleyne, 1986: 39. Misspelling of species name.

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

3 DETECTION

3.1 Signs and symptoms

Protopulvinaria pyriformis generally is found feeding on the underside of leaves (Merrill 1953; Swirski *et al.* 1997) (Figure 1). As each scale insect feeds it produces large amounts of honeydew that then support sooty mould growth. Heavy infestations cause leaf margins to fold, premature leaf drop and yield reduction (De Meijer *et al.* 1989; Swirski *et al.* 1997). In general, feeding damage by scales insects cause chlorosis, yellowing and wilting of the affected plant part.



Figure 1. *Protopulvinaria pyriformis* adult females on underside of leaf (Merrill, 1953)

3.1.1 Field description

Adult females 2–4 mm long, flat, pointed anteriorly and broadly rounded posteriorly (pyriform), usually asymmetrical. This pyriform shape is typical of the genus *Protopulvinaria* and this can used in field to distinguish at least the genus from other genera of Coccoidea. Nymphs and young adults are clear yellow, older adults are darker brown with broad, reddish, mottled marginal bands (Hamon & Williams 1984; Gill 1988) (Figure 2). Sometimes sclerotised areas around margin are present and there is no obvious wax covering. During egg-laying a narrow ovisac is produced, visible as a narrow white secretion around posterior margin (Figure 3) (Merrill 1953; Hamon & Williams 1984; Gill 1988). *P.pyriformis* is believed to reproduce by parthenogenesis (Ben-Dov 1993; Swirski *et al.* 1997; Miller *et al.* 2015). The entire life cycle is spent on the lower leaf surfaces (Figure 1, 2) (Gill 1988).



Figure 2. Nymphs and adult females of *Protopulvinaria pyriformis* (USDA Agricultural Research Service)



Figure 3. *Protopulvinaria pyriformis* adult females showing ovisac (USDA Agricultural Research Service)

3.1.2 Development

Typically for coccid species, females have four instars and males have five instars, although many species of coccids are parthenogenetic and do not produce males (Miller *et al.* 2015). Moznette (1922) reported males of *P. pyriformis* in Florida but this claim needs confirmation because elsewhere in its range *P. pyriformis* is believed to be parthenogenetic (Ben-Dov 1993; Swirski *et al.* 1997; Miller *et al.* 2015).

Protopulvinaria pyriformis overwinters as nymphs on the underside of leaves and adults can produce several hundred eggs in spring. Moznette (1922) reported that eggs were produced on avocado in early spring. First-instar nymphs emerged late spring and by early summer young adults moved onto new leaves as the trees dropped their older leaves; several overlapping generations were observed also during the year. Gill (1988) reported *P. pyriformis* as preferring gardenia, avocado and ivy and it also occurred on citrus, with several overlapping generations produced per year. Blumberg (1991) recorded that in Israel two generations of *P. pyriformis* per year occurred on avocado with eggs being produced in late spring and autumn, whereas three generations occurred on ivy with eggs being produced during early spring, late summer and autumn. The report of males by Moznette (1922) needs confirmation as *P. pyriformis* is believed to be parthenogenetic (Swirski *et al.* 1997, Ben-Dov 1993).

Large populations of *P. pyriformis* can be found along the roadside where dust and cars' exhausts disturb the biological balance. Ants and possibly repeated fungicide applications can also lead to increased infestations of *P. pyriformis* (Swirski *et al.* 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.

Nymphs of *P. pyriformis* can look like citrus whitefly (*Dialeurodes citri* Ashmead) which is found on a similar host range (Gill 1988).

Other similar *Protopulvinaria* species and *Kilifia* species can be differentiated by morphological characteristics outlined in section 4.2.2.

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% alcohol 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% alcohol and if future DNA analysis is required some specimens should also be stored in 100% alcohol.

Young adult females are best to process for slide mounting and identification (PJ Gullan pers. comm.). 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 may be visible on one individual but unclear on another (GW Watson pers. comm.).

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 4).
- 4. Identify genera:
 - use key to genera of Coccidae (4.2.1), if samples key to *Protopulvinaria*, proceed to 5
 - if specimens key to another genera samples are not *P. pyriformis* and other literature will be required.
- 5. Identify species using key to *Protopulvinaria* spp. (4.2.2), this key will determine if specimens are *P. pulvinaria* or not.

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 *Protopulvinaria pyriformis*.

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

4.1 Methods based on morphological character states

4.1.1 Procedure for mounting scale specimens onto microscope slides

Slide-mounting method for coccoidea (PJ Gullan pers. comm.) adapted from Kozarzhevskaya (1968).

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 approximately 30 mins 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 if possible.
- 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 genera of Coccidae of the tropical South Pacific region

The scale family of Coccidae (soft scales) are recognized by their characteristic anal cleft which has a pair of triangular or rounded plates (anal plates) at its base - see Figure 4.

A lucid key to the identification of soft scales can be accessed at

http://idtools.org/id/scales/key.php?key=soft

The following key is modified from Williams and Watson (1990). Footnotes are used to explain further details of characters specific to *Protopulvinaria pyriformis* (adapted from Hodgson 1994).

1. -	Marginal setae fan shaped, overlapping, forming a continuous fringe <i>Paralecanium</i> Cockerell Marginal setae, if present, flagellate or spine like ¹
2.	Second and third pairs of legs larger than first pair of legs, the second and third pairs of coxae conspicuously larger than the first coxae
-	Legs, if present, all about same size
3.	Anal plates situated at centre or at tip of sclerotized caudal process
-	Anal plates situated not at centre or at tip of sclerotized process, although sometimes the immediate area surrounding anal ring sclerotized
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
-	Stigmatic clefts, if present, without lobes containing fleshy protuberances ² . Dorsal pores, if present, various, but not flower-shaped
6.	Ventral tubular ducts numerous, present in an obvious submarginal zone
-	Ventral tubular ducts absent or, if present, a few situated in mid-region of thorax or scattered, present but not in an obvious submarginal zone
7.	Stigmatic setae opposite each spiracle single, conspicuously longDrepanococcus 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 ³
-	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 plate0
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¹ Fringed marginal setae (Fig 7a(N), 7c)

² Stigmatic clefts distinct and narrow, each with 3 stigmatic spines (Fig 7a)

³ Anal plates extremely long and narrow, each with 3 small subapical setae (Fig 7a(I), 7b)

10. _	Tibia and tarsus distinct but not articulated, tibio-tarsal articulatory scleroses absent, tarsus straight. Dorsal setae cylindrical or slightly clavate apically <i>Parasaissetia</i> Takahashi Tibia and tarsus well articulated, tibio-tarsal articulatory scleroses present, tarsus usually curved. Dorsal setae tapering, sharply or bluntly pointed <i>11</i>
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 tubercules
13. _	Stigmatic furrows present, the spiracles opening some distance from margin. Anal plates triangular, the inner edges contiguous. Anal ring situated anterior to anal plates <i>Platylecanium</i> Cockerell & Robinson Stigmatic furrows barely perceptible, spiracles opening directly under bases of deep stigmatic clefts. Anal plates reniform, the inner edges not contiguous. Anal ring situated between anal plates <i>Cryptostigma</i> Ferris
14. -	Marginal setae present, conspicuous, differentiated from dorsal and ventral setae
15. _	Stigmatic setae situated on dorsal margin of each stigmatic cleft or extending on to dorsum, all spine-like and numbering 3-12; if numbering 3 only, then they are about the same size
16.	Anal plates each with antero-lateral edge 1.7-3.3 times longer than the postero-lateral edge
-	Anal plates each with outer edges about equal in length, occasionally postero-lateral edge longer than antero-lateral edge
17. -	Dorsal surface of mature specimens strongly sclerotized and divided by membranous furrows into large plates <i>Eucalymnatus</i> Cockerell Dorsal surface otherwise
18.	Dorsal surface with areolations, minute or large, only present around submargins, the aerolations never joined

- Dorsal surface with aerolations joined, forming a reticulated pattern*Neosaissetia* Tao & Wong



Figure 4 – General morphology of Coccidae (soft scales) (modified from Gill 1988)

4.2.2 Key to the species of Protopulvinaria

Compiled from Kuwana 1909; Takahashi 1955; Gill 1988; Tang 1991; Hodgson 1994; Miller *et al.* 2015; T. Kondo pers. comm.). Measurements of anal plates from drawing in Kuwana (1909) and photographs of a specimen courtesy of T. Kondo.

1. Anal plates with anterolateral margin less than 3 times as long as posterolateral margin (Figure 5).....*fukayai* Anal plates with anterolateral margin at least 4 times as long as posterolateral margin (Figure 6, Figure

7a & b)2

Adult females of *P. pyriformis* are similar to those of *Kilifia acuminata* (Signoret) (acuminate scale), *Kilifia americana* Ben-Dov and *Milviscutulus mangiferae* (Green), but differ from these three species by having extremely long sclerotised anal plates (Hamon & Williams 1984; Gill 1988). *P. pyriformis* is most similar to *P. longivalvata* Green and *P. fukayai* (Kuwana). *P. pyriformis* differs from *P. fukayai* by having anal plates with the anterolateral margin at least four times as long as posterolateral margin (*P. fukayai* has the anterolateral margin less than three times as long as posterolateral margin). *P. pyriformis* differs from *P. longivalvata* by having the marginal setae fringed, dorsal setae numerous and 8–10 μ m long (*P. longivalvata* has the marginal setae simple, dorsal setae sparse and 3–5 μ m long). Refer to the key of *Protopulvinaria* species (section 4.2.2).



Figure 5 – *Protopulvinaria fukayai* (Tang 1991)



Figure 6 – Protopulvinaria longivalvata (Miller et al. 2015)

4.3 Description of Protopulvinaria pyriformis – Fig. 7

- Marginal setae fringed,
- dorsal setae small and with rounded apex or capitate,
- anal plates located in the middle of the body anterolateral margin about 5 times longer than posterolateral margins, each anal plate with 3 or 4 apical setae and without discal setae,
- dorsal surface of older females with sclerotized pattern around body margin,
- tubular ducts abundant,
- claw without denticle,
- stigmatic setae with the middle setae longer than the lateral setae,
- antennae 7 or 8-segmented,
- multilocular pores usually scattered in medial areas of abdomen,
- preopercular pores inconspicuous and restricted to area anterior of anal plates. (Miller *et al.* 2015)

Fact sheet can accessed at <u>http://www.idtools.org/id/scales/factsheet.php?name=6902#prettyPhoto</u>)

For a complete taxonomic description see Appendix 8.2.



Protopulvinaria pyriformis (Cockerell)

Figure 7a - Protopulvinaria pyriformis (Hodgson 1994)

Legend to letters: A=dorsal setae, B=dorsal tubercules, D=dorsal microducts, G=preopercular pores, H=pocket-like sclerotisations, I=anal plates, K=ano-gential fold, L=pregenital disc-pores, M=spiracluar disc-pores, N=marginal setae, O=stigmatic spines, R=ventral microducts, S=legs, T=antennae, U₁ U₂ U₃=ventral tubular ducts, W=pre-antennal pores.



Figure 7b – *Protopulvinaria pyriformis* (image by DJ Tree)



Figure 7c – *Protopulvinaria pyriformis* marginal fringed setae (image by DJ Tree)

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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 Host list for *Protopulvinaria pyriformis*

The following host list is compiled from Ben-Dov 1993, APG II 2003, APG III 2009 and Angiosperm Phylogeny Group 2012.

<u>Family</u>	<u>Species</u>
Acanthaceae	Adhatoda vasica
Anacardiaceae	Mangifera indica
Apocynaceae	Araujia sericofera
	Carissa grandiflora
	Nerium
	Plumeria
	Plumeria tricolor
	Trachelospermum jasminoides
Aquifoliaceae	Ilex canariensis
	Ilex perado
Araceae	Dizygotheca
Araliaceae	Agalma lutchuense
	Aralia
	Brassaia actinophylla
	Fatsia japonica
	Hedera
	Hedera canariensis
	Hedera helix
	Schefflera
	Schefflera octophylla
	Tetrapanax papyriferum
Asparagaceae	Dracaena duranti
Cannaceae	Canna indica
Caprifoliaceae	Caprifolium
	Lonicera
	Lonicera etrusca
	Viburnum tinus
Caricaceae	Carica papaya
Convolvulaceae	Іротоеа
Ebenaceae	Diospyros erianthi
Elaeocarpaceae	Elaeocarpus elliptica
	Elaeocarpus serratus
Euphorbiaceae	Antidesma
	Antidesma bunius
Fabaceae	Bauhinia chamioni
	Bauhinia vahlii

<u>Family</u>	<u>Species</u>
Lauraceae	Apollonias barbujana
	Cinnamomum
	Cinnamomum camphora
	Cinnamomum cassia
	Cinnamomum zeylanicum
	Laurus
	Laurus azorica
	Laurus canariensis
	Laurus nobilis
	Ocotea foetens
	Persea
	Persea americana
	Persea borbonia
	Persea gratissima
Lythraceae	Lagerstroemia indica
	Punica
Malpighiaceae	Malpighia glabra
Malvaceae	Hibiscus sinensis
Moraceae	Ficus
Musaceae	Musa cavendishi
Myricaceae	Myrica
Myrtaceae	Amomis
	Eucalyptus
	Eugenia
	Eugenia jambolana
	Myricaria
	Myrtus communis
	Psidium guajava
Orchidaceae	Cymbidium
	Epidendrum
Passifloraceae	Passiflora
Phyllanthaceae	Richeria grandis
Pittosporaceae	Pittosporum tobira
Rubiaceae	Gardenia
	Gardenia fortunei
	Gardenia jasminoides
Rutaceae	Choisya ternata
	Citrus
	Citrus aurantium
Saxifragaceae	Peltophyllum peltarum
Scrophulariaceae	Veronica
Verbenaceae	Clerodendrum

8.2 Complete taxonomic description of *Protopulvinaria pyriformis* (Hodgson 1994)

Refer to Fig 7a,b,c.

Mounted material. Body pyriform, broadest in abdomen, but often slightly asymmetrical, with slight indentations at each stigmatic cleft; anal cleft deep, about 1/3rd body length. Length 1.6-3.0 mm, width 1.0-3.0 mm.

Dorsum. Derm membranous on young specimens, but with a thickened area around the anal plates and with numerous small areolations which appear to form a tessellated pattern medially but this is less clear submarginally; on older specimens derm becomes sclerotised submarginally, with a clear area around each eyespot and with clear radial rays at each stigmatic cleft and a further 3 pairs on broadest part of abdomen and another medially on head. Dorsal setae each rod-shaped, often with slightly clavate apex and with a well-developed basal-socket; each seta 8-10µm long; common throughout dorsum. Dorsal pores all minute, sunken and simple, with a long inner filament, present in most areolations. Preopercular pores each small and flat, with a granulate surface, present in a group of 11-33 pores around anterior end of anal plates and extending slightly anteriorly. Dorsal tubular ducts absent. Dorsal tubercles rather small, in a sparse submarginal ring, with 2 or 3 pairs present on abdomen, 1 pair between stigmatic clefts and 0 or 1 pair on prothorax and head. Pocket-like sclerotisations present between dorsal tubercles and margin; very sparse (total 0-3 on each side), much less frequent than dorsal tubercles and possibly absent on some specimens. Anal plates extremely long and narrow (about 3-3.5x longer than their combined width), each with 3 small subapical setae; length of plates 440-578µm, width of single plate 74-84µm; each plate with a Yshaped supporting bar. Ano-genital fold with 2 pairs of long setae present on anterior margin and none laterally. Anal ring with probably 6 setae present.

Margin. Marginal setae spinose, each with a broadly fimbriate apex (though they can appear pointed when seen sideways) and a well-developed, broad basal-socket; each seta 12-28µm long; with 20-30 setae on each side between stigmatic clefts. Stigmatic clefts distinct and narrow, each with 3 stigmatic spines, median spine much the longest, rather longer than marginal setae, and generally bent posteriorly, with a well-developed, broad basal-socket; length of each median spine 24-26µm; lateral spines each rather sharply conical with a narrow basal-socket; length of each lateral spine 5-8µm. Eyespots present submarginally on dorsum, in a clear area of derm; width of each lens 12-18µm.

Venter. Derm membranous. Pregenital disc-pores each mainly with 7 loculi; abundant around genital opening, becoming less frequent mediolaterally on more anterior abdominal segments; a few also generally present lateral to each metacoxa. Spiracular disc-pores each mainly with 5 loculi; present in a line one pore wide between margin and each spiracle, with few disc-pores present more medially; with 19-28 disc-pores in each anterior band and 31-41 in each posterior band. Ventral microducts minute, most abundant in a submarginal ring, although also occasionally present more medially. With a single preantennal pore present near base of each antenna. Ventral tubular ducts of 3 types present: (i) a duct with a short, wide, outer ductule and a filamentous inner ductule; present in a complete submarginal ring, extending medially to about level with spiracles; (ii) a duct with a long outer ductule, a well-developed cup-shaped invagination and a wide inner ductule with a large terminal gland; abundant medially on head, thorax and first 2 abdominal segments; and (iii) a duct rather similar to (ii), but outer ductule with a shallow cup-shaped invagination and a rather thin inner ductule; present medially and mediolaterally on more posterior abdominal segments. Ventral setae: 3 pairs of long

setae present between antenna; also with a single pair of long pregenital setae; other setae sparse. Spiracles small; width of each peritreme: anterior $25-32\mu$ m, posterior $37-40\mu$ m. Legs well developed; each with a tibio-tarsal articulation and articulatory sclerosis; claws without a denticle; claw digitules both broad and slightly shorter than tarsal digitules; dimensions (iii): trochanter + femur 200-208 μ m, tibia 124-150 μ m and tarsus 72-82 μ m. Antennae each with 7 or 8 segments, 4th segment often with a pseudo-articulation; apical segment rather long; total length 264-318 μ m. Mouthparts often displaced slightly to one side; labium with 4 pairs of setae; width 70-90 μ m.

Material examined. **COSTA RICA**, Turrialba, ex *Altidesma bunius* (Stilaginaceae), 1.iii.1983, *J.H. Martin* (BMNH:1 /1).

DOMINICAN REPUBLIC, ex Persea Americana (Lauraceae), 28.i.1972, M.J. Sommeijer (BMNH: 1/2).

GRENADA, no data, ex collection J.D.A Cockerell (BMNH: 1/1).

St LUCIA, ex umbrella plant (? *Peltophyllum peltarum* (Saxifragaceae)), -.ii.1988, no collector (BMNH: 1/3).

Discussion

The above description agrees well with those given by Hamon & Williams (1984) and Gill (1988) and with the generic diagnosis of Williams & Watson (1990), except that here there are considered to be three types of ventral tubular duct present and pocket-like sclerotisations were occasionally noted near the dorsal tubercles. *Protopulvinaria*, along with *Milviscutulus*, belongs to the *Protopulvinaria* group within the Pulvinariini. For further comment see under *Milviscutulus*, from which *Protopulvinaria* differs in having ventral tubular ducts abundant on the head.

Protopulvinaria contains 3 species, one restricted to Japan and China, the other 2 with an almost cosmopolitan distribution (Ben-Dov, 1993).