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Systematic review of the Australian 'bush coconut' genus *Cystococcus* (Hemiptera: Eriococcidae) uncovers a new species from Queensland

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Abstract. Australia houses some unusual biota (insects included), much of which is undescribed. *Cystococcus* Fuller (Hemiptera : Sternorrhyncha : Coccoidea : Eriococcidae) currently comprises two species, both of which induce galls exclusively on bloodwoods (Myrtaceae: *Corymbia* Hill & Johnson). These insects display sexual dichronism, whereby females give birth first to sons and then to daughters. Wingless first-instar females cling to their winged adult brothers and are carried out of the maternal gall when the males fly to find mates – a behaviour called intersexual phoresy. Here, we use data from two gene regions, as well as morphology and host-use of the insects, to assess the status of a previously undescribed species. We describe this newly recognised species as *Cystococcus campanidorsalis*, sp. nov. Semple, Cook & Hodgson, redescribe the two existing species – *C. echiniformis* Fuller and *C. pomiformis* (Froggatt), designate a lectotype for *C. echiniformis*, and provide the first descriptions of adult males, and nymphal males and females for the genus. We have also reinterpreted a key morphological character of the adult females. This paper provides a foundation for further work on the genus, which is widespread across northern Australia and could prove to be useful for studies on biogeography and bloodwood ecosystems.

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Introduction

Gall-inducing scale insects (Hemiptera : Coccoidea) in the genus *Cystococcus* Fuller are obligatory parasites on red bloodwoods in the genus *Corymbia* Hill & Johnson (Gullan and Cockburn 1986; Gullan *et al*. 2005). The sub-spherical galls vary in size within and between species, with the largest belonging to *Cystococcus pomiformis* (Froggatt) (Froggatt 1893), at up to 90 mm in diameter (Austin *et al*. 2004). Commonly known as either 'bloodwood apples' or 'bush coconuts', they consist of a hard outer layer with a soft, white, fleshy layer lining the cavity that houses the adult female (Gullan and Cockburn 1986). The Australian Aborigines used the galls as a food source, eating the fleshy interior of the gall and the insects themselves (Froggatt 1893). Although Aboriginal tribes across central and northern Australia had several names for bush coconuts, such as 'Ballabbi' and 'Durdunga', it was thought by Fuller (1899) that they all referred to galls induced by one species – *Cystococcus echiniformis* Fuller (Fuller 1897). This is unlikely, but the Aboriginal names probably do all refer to galls induced by females of *Cystococcus*. In addition to providing food for people, the insects of *Cystococcus* species and the galls they induce are a food source for birds and other insects (e.g. moths; Turner 1942). Furthermore, the adult females are parasitised by wasps and flies (T. L. Semple and L. G. Cook, pers. obs.), and the galls provide shelter for arthropods such as tree crickets, ants and spiders (Froggatt 1893; Fuller 1899; Austin *et al*. 2004; T. L. Semple and L. G. Cook, pers. obs.).

Currently, *C. pomiformis* and *C. echiniformis* are the only described species of *Cystococcus*, and are found across large areas of northern Australia (Bowman *et al*. 2010). Froggatt (1893) described *C. pomiformis* from galls collected at Torrens' Creek in north Queensland and in the Barrier Range, near King's Sound in Western Australia, but he placed the species in the genus *Brachyscelis* Schrader (now known as *Apiomorpha* Rübsaamen). Fuller (1897) later erected the genus *Cystococcus* for the species *C. echiniformis*, which he described from material collected by R. Helms in the east Kimberley. Subsequently, Froggatt (1921) transferred *B. pomiformis* to *Cystococcus*. Gullan and Cockburn (1986) referred to a possible third species of *Cystococcus*, but provided no information on these specimens.

The life cycle and dispersal mechanism of *Cystococcus* are unusual and may be unique among insects. Females of *Cystococcus* feed, reproduce and spend the entirety of their adult lives inside their galls. They lack eyes, an anus, antennae and legs. They are soft-bodied except for a few patches of sclerotization, including a 'button' used to plug the gall entrance (Fuller 1897). Adult males have extremely elongate abdomens, which are likely an adaptation for mating through the gall entrance (Gullan *et al*. 2005), and perhaps also for intersexual phoresy (see below). Adult females control the sex allocation of their offspring, exhibited through sexual dichronism (Gullan and Cockburn 1986). A mother first gives birth to all male offspring, which develop entirely within the maternal gall. These males feed on the fleshy lining (nutritive tissue) of the gall and develop through two nymphal instars and two pupal stages, before moulting to winged adults (Gullan and Cockburn 1986). While the males are pupating, their mother produces daughters, which develop to the first instar inside the gall cavity (Gullan and Cockburn 1986) or inside their mother (T. L. Semple, pers. obs.). This co-mingling of adult males and their first-instar sisters allows for intersexual phoresy: the first-instar females grasp onto their brothers' elongate abdomens and are carried from the maternal gall as those males fly in search of mates (Grant 1965; Gullan and Cockburn 1986) (Fig. 1).

Cystococcus has been used as an exemplar of biogeographic and ecological processes in Australia (Fuller 1899; Gullan and Cockburn 1986; Bowman *et al*. 2010; Ladiges *et al*. 2010), yet the genus has never been revised taxonomically. Here, we describe a new species of *Cystococcus* from Queensland, *C. campanidorsalis*, sp. nov., and redescribe *C. echiniformis* and *C. pomiformis* based on molecular data, the morphology of adult females and adult males, and host use. We provide the

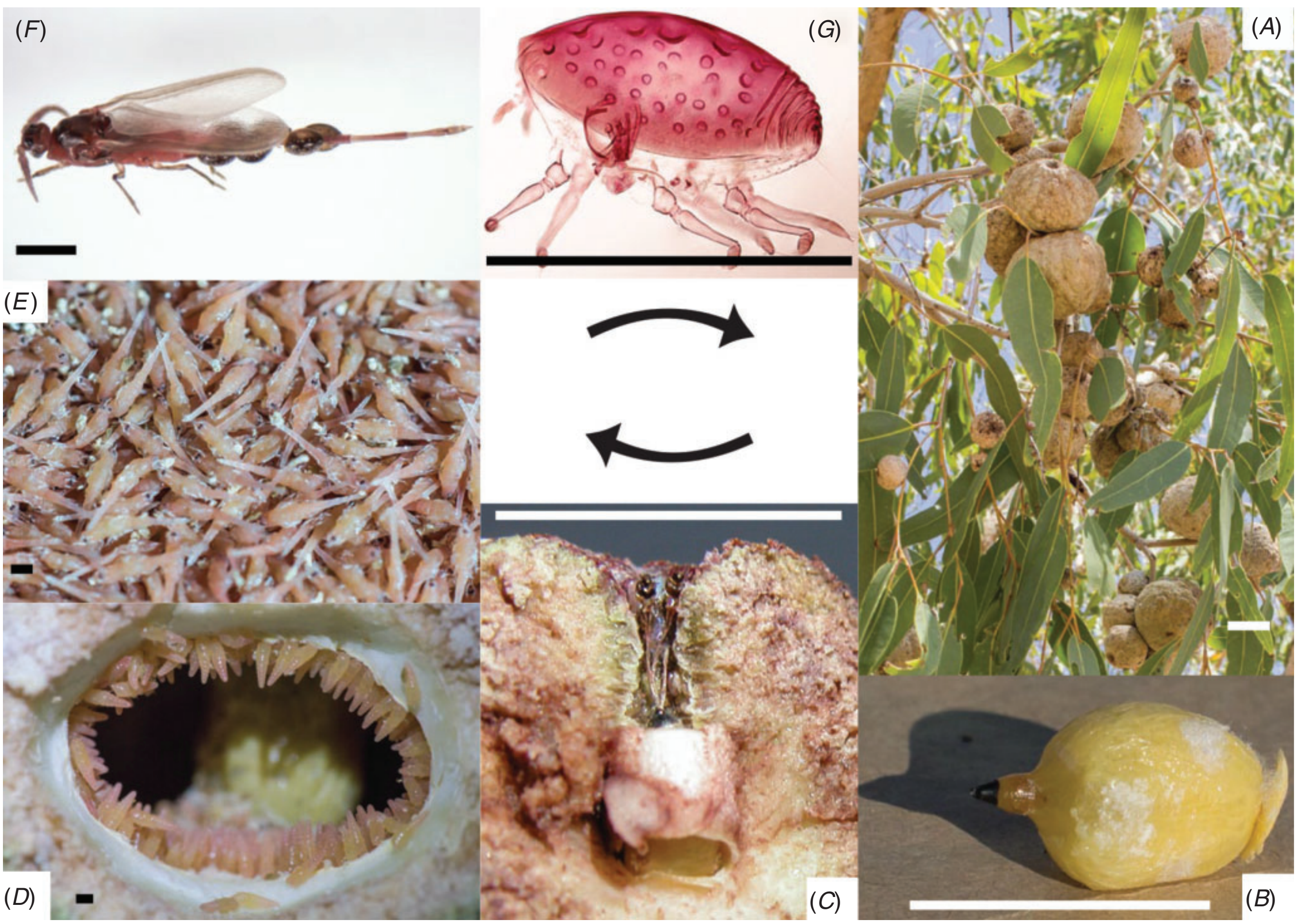

Fig. 1. Life cycle of *Cystococcus*: (*A*) galls of females on host tree (note different sizes and ages), (*B*) adult female removed from gall with plant tissue still attached on right-hand side, (*C*) multiple adult males mating with an adult female inside her gall (note long abdomens of males with their heads outside the gall), (*D*) second-instar male nymphs lining the maternal gall cavity and feeding on gall tissue, (*E*) pupal males, (*F*) adult male carrying six female crawlers on his abdomen, (*G*) slide-mounted first-instar female nymph (crawler). Black scale bars (*D*, *E*, *F* and *G*) = 0.5 mm; white scale bars (*A*, *B* and *C*) = 20 mm.

first descriptions and taxonomic illustrations of adult males, as well as nymphal stages of female and male *Cystococcus*.

Materials and methods

Species concept

Here, we recognise species as biologically distinct units, reproductively isolated from other such entities (e.g. biological species concept; Mayr 1942). We use multiple sources of data, such as DNA sequences, morphology of different life history stages, and host associations, to assess evidence of barriers to gene flow. In this way, our species concept also corresponds to that of an independently evolving gene pool ('genotypic cluster'; Mallet 1995).

Specimens and taxonomy

Specimens of *Cystococcus* were obtained from across their known range and from around south-east Queensland (Table S1, available as Supplementary material online). We also examined specimens held in Australian and overseas museums (see below). Galls were opened by cutting away a segment of the side wall with secateurs or a knife, which allowed inspection of the contents. Where present, adult females were carefully removed by pushing the sclerotized button inwards from the gall opening and cutting away a small piece of gall tissue from around the mouthparts (tissue still attached in Fig. 1*B*). The lightly sclerotized disc around the mouthparts was often stuck to the gall tissue but was removed easily, with less chance of damage, after soaking during slide mounting. The cuticle of adult females is extremely fragile and is easily damaged during removal. For molecular work, the body contents, including ovaries and eggs, were usually removed and stored separately as another source for DNA extraction and to allow penetration of ethanol into the body cavity. Males of all developmental stages and first-instar females also were collected from galls when present and preserved separately from the adult females. Almost all specimens examined for the descriptions have an associated gall, which is housed in the same institution as the adult female insect. Thus galls are not listed in the 'Material examined' sections.

All specimens collected in remote locations were removed from their galls in the field, stored in 100% ethanol and refrigerated below 4°C for transport back to the laboratory. Each collection made by TLS and LGC was assigned a unique identifier (e.g. TLS001) to allow tracking of all material derived from that tree, including insects, galls, plant material used for host identification, DNA and all other preserved forms of these (i.e. slide-mounted, ethanol-preserved and frozen specimens).

Type specimens of *C. campanidorsalis*, sp. nov. will be deposited in the Queensland Museum (QM), Brisbane, Qld, Australia, as per collection permit requirements, and some paratypes will be deposited in the Australian National Insect Collection (ANIC), CSIRO Ecosystem Sciences, Canberra, ACT, Australia. We have registered the new name published in this paper with the Official Registry of Zoological Nomenclature (ZooBank) and cite the life science identifier (LSID) after the heading for the new name. Each LSID is a globally unique identifier for the nomenclatural act of naming a new taxon. DNA and frozen specimens will be maintained at The University of Queensland for the immediate future unless storage facilities become available at state or national institutions. Insect and gall material from collections made by PJG are housed in the ANIC. PJG also examined and measured specimens from the following institutions: Agricultural Scientific Collections Unit, Orange Agricultural Institute, New South Wales, Australia (ASCU); The Natural History Museum, London, UK (BMNH); South Australian Museum, Adelaide, South Australia (SAM); the United States National Collection of Coccoidea of the National Museum of Natural History (USNM), housed at the United States Department of Agriculture (USDA), Beltsville, Maryland, USA; Western Australian Museum, Perth, Western Australia (WAM).

The International Code of Zoological Nomenclature (ICZN 1999) requires lectotypes designated after 1999 to 'contain an express statement of deliberate designation' (amended article 74.7.3). We use the statement 'we here designate' to satisfy this requirement. A lectotype has been designated for *C. echiniformis* because this name lacks a primary type specimen and an unambiguous syntype has been identified. The purpose is to provide stability of nomenclature, and designation is done in a revisionary context in accordance with the amended recommendation 74G of article 74.7.3.

Slide-mounted specimens listed for the material examined are referred to by number of individuals and slides, for example, 2/5 refers to five specimens on two slides. For *C. echiniformis* and *C. pomiformis*, the lists of specimens examined are for sequenced specimens only, but many more adult females and galls were available in museum collections; thus, the descriptions of the galls of these two species include many specimens additional to those for which collection data are listed. The data for unlisted specimens is available upon request to PJG.

Measurements were made using an ocular micrometre in the eyepiece of a compound or dissecting microscope. Body lengths and widths are maximum values, and tibiotarsal lengths of the legs of nymphs exclude the claw. In the taxonomic illustrations of nymphs and adult males, the main figure is a composite with the dorsum on the left and the venter on right. For adult males, vignettes of the more important structures are enlarged (not to scale) around the margin. For nymphs and the adult female, the draft illustrations were prepared with a drawing tube and then scanned and edited using the Adobe programs Photoshop CS and Illustrator CS.

Molecular data

DNA extraction of whole female cuticles was performed using a cetyltrimethylammonium bromide (CTAB) method in which the cuticle was incubated overnight at 55°C in CTAB buffer with 10–20 µL of proteinase K added. A chloroform wash with gentle rocking followed by centrifugation was used to separate the DNA in the aqueous layer from the organic layer and tissue debris. The DNA was precipitated from the aqueous layer using 100% isopropanol, then cleaned using two washes with 80% ethanol. Extractions of small volumes of tissue (e.g. males or parts of ovaries) were carried out with a Bioline 'Isolate 2' DNA extraction kit (cat. no. BIO-52067) or a Qiagen DNeasy blood and tissue kit (cat. no. 69506) following the manufacturer's instructions.

Extracted DNA was amplified using polymerase chain reaction (PCR) and checked using agarose gel electrophoresis. Gene regions used for analysis were the 5′ region of *18S* (small subunit nuclear rDNA, SSU rDNA) and the 'DNA barcode' region of *COI* (mitochondrial cytochrome *c* oxidase 1). Park *et al.*'s (2010) scale insect *COI* primer combination (PCO_F1 (Park *et al.* 2010) and HCO (Folmer *et al.* 1994)) was effective for many specimens of *C. pomiformis* and *C. echiniformis*, but yielded only poorly amplified or no PCR product for most specimens of *C. campanidorsalis*, sp. nov., so new primers were designed as follows. Consensus sequences for the two specimens of *C. campanidorsalis*, sp. nov. that successfully amplified during the first round of PCR were aligned with sequences from *C. pomiformis* and *C. echiniformis*. Conserved regions near the 5′ and 3′ ends were chosen for potential priming sites. Cross-binding compatibility, secondary structure and melting temperatures were considered in primer design using Geneious ver.6.1.7 (Biomatters: www.geneious.com), and compatible pairs synthesised by IDT (http://sg.idtdna.com). Details of all primers and PCR programs used are listed in Table 1. Successfully amplified DNA was purified of unincorporated primers and dNTPs using Exonuclease 1 and Antarctic Phosphatase (New England Biolabs), then sequenced by Macrogen (Republic of Korea) using Sanger sequencing.

DNA sequences were checked for non-target DNA contamination (such as parasitoids and fungi) using BLAST (megablast or discontiguous megablast search: http://blast.ncbi.nlm.nih.gov), then aligned and manually edited using Geneious ver.6.1.7. PAUP* (Swofford 2003) was used to check overall base frequencies and for base composition bias among taxa for individual *COI* codon positions, as frequency differences between taxa violate the assumptions of most available tree estimation methods. Phylogenetic trees were estimated using maximum parsimony (MP) and maximum likelihood (ML), as these two methods estimate phylogenetic relationships according to different models and assumptions about the process of DNA evolution (Sleator 2011). Therefore, congruence between methods (when present) offers the strongest support from the data for relationships. Several species of *Ascelis* Schrader, which is closely related to *Cystococcus* (Cook and Gullan 2004), were used as outgroups. Diagnostic nucleotide changes were identified by using PAUP* to find synapomorphies for relevant clades (nucleotide changes with a consistency index equal to 1, i.e. no homoplasy).

Base composition bias was observed among species in the 3rd codon positions of *COI*, so MP and ML analyses were performed with and without 3rd codon positions to determine whether this bias was a confounding source of apparent divergence between species. A neighbour-joining (NJ) tree was also estimated using the LogDet transformation in PAUP*, as this method reduces the effect of grouping taxa with homoplasiously similar base frequencies (Lockhart *et al.* 1994).

Maximum parsimony

An heuristic search with 1000 random addition starting sequences was carried out in PAUP* for each gene region, with the 10 most parsimonious trees retained from each. These saved trees were then used for a second heuristic search, which was allowed to run to completion or until ~500 000 MP trees had been reached. A strict consensus tree was calculated from the resulting MP trees, and a bootstrap (BS) analysis performed using a fast-heuristic search with 1000 pseudoreplicates to calculate support values for each branch.

Maximum likelihood

Analyses were run with RAxML (Stamatakis 2006) using a generalised time reversible (GTR) model. The program uses per-site rate categories (GTR+CAT) and estimates model parameters based on the input data. We ran RAxML on the CIPRES Science Gateway (www.phylo.org) for faster processing, with a 1000 pseudoreplicate BS analysis used to calculate branch support values.

Morphology

After DNA extraction, adult females, males and immature stages were slide-mounted for morphological examination and for use as morphological vouchers for DNA sequences. Scale insect taxonomy is traditionally based on cuticular features of adult females, such as minute pores and setae, which are visible only under a compound microscope after clearing and staining. Adult males were also mounted and examined so that, as the

Table 1. Gene regions, associated primers and polymerase chain reaction (PCR) programs used in phylogenetic analyses

Gene/Primer name/PCR program	Primer sequence	Reference
18S		
2880 (F)	GTTTTCCCAGTCACGACCTGGTTGATCCTGCCAGTAG	Tautz *et al.* 1988
Br (R)	CCGCGGCTGCTGGCACCAGA	von Dohlen and Moran 1995
Program	94°C/3:00, 34x (94°C/0:30, 55°C/0:30, 72°C/1:00), 72°C/5:00	
COI		
PCO_F1	CCTTCAACTAATCATAAAAATATYAG	Park *et al.* 2010
HCO (R)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* 1994
CystCOIF	TGRTCAGGAATAATAGGAATA	This study
CystCOIR	GTATTYAAAAATCTTGTTGATATGTT	This study
Program	95°C/2:00, 5x (94°C/0:40, 72°C/1:10), 40x (94°C/0:40, 51°C/0:40, 72°C/1:10), 72°C/10:00	

dispersing adult life stage, they could be identified if ever collected outside the maternal gall.

Adult females were mounted in Canada balsam using Gullan's adaptation of the method described by Kozarzhevskaya (1968). Briefly, cuticles were cleared of contents in 10% potassium hydroxide (KOH) solution, stained in acid fuchsin, dehydrated in a series of ethanol and isopropyl alcohol baths and cleared in xylene before mounting in Canada balsam on slides. In order to prepare specimens under coverslips with a low enough profile for compound microscopy, the sclerotized button of recently mounted specimens was removed from the membranous cuticle after staining. The buttons were glued with Canada balsam to the slide, beside the coverslip. Specimens in good condition were flattened lengthways, with the mouthparts at one end and the button (removed) at the other, as this is how they flatten naturally and how existing specimens were mounted. Damaged or small females were cut along one side and 'butterflied' on the slide to allow clearer viewing of pores and setae and distinction between dorsal and ventral surfaces. Adult males and immature stages prepared by CJH and TLS were mounted using a modification of the method described in Ben-Dov and Hodgson (1997). Briefly, cuticles were cleared of contents in 10% KOH, rinsed in 2% detergent water, stained in very dilute acid fuchsin, then dehydrated in ethanol, cleared in xylene and mounted in Canada balsam. Immature stages and adult males prepared by PJG were mounted according to the method described above for adult females, except that some nymphs were mounted in Stroyan's mountant (Upton and Mantle 2010), either directly (i.e. alive) or from ethanol after thorough washing in several changes of water.

Interpretation of the anatomical position of the sclerotized 'anal button' needed revision because previous descriptions have broadly described its location as posterior abdominal or caudal (Fuller 1897), and interpretation of positioning has been difficult because abdominal segmentation is barely visible in *C. pomiformis* and *C. echiniformis*. There are few external features to help locate segments ventrally, other than the two pairs of spiracles and the vulva, and there are no clear landmarks for dorsal segmentation. Because internal structures cannot be discerned after the insect's soft tissue is macerated with KOH, some females of *C. campanidorsalis*, sp. nov., which has more defined segmentation than the other two species, were dissected before mounting in an attempt to locate the gut and ovaries, and where they attach to the cuticle. The anus is blind-ended (it does not open externally) but its location, along with that of the vulva, was expected to assist with interpretation of segmentation and the position of the sclerotized button.

Adult and first-instar females were prepared for scanning electron microscopy (SEM) after preservation and storage in 80% ethanol. Each specimen was dehydrated in a graded ethanol series, de-waxed in xylene, rehydrated through a graded ethanol series into distilled water, post-fixed in 1% aqueous osmium tetroxide, washed in distilled water and sonicated briefly to remove any black precipitate, critical point dried, glued onto a metal stub with nail varnish and coated with gold palladium under vacuum. Specimens were then examined and photographed using a Cambridge S360 scanning electron microscope.

Results and discussion

Molecular data

Both methods used for phylogeny estimation (MP and ML), and both gene regions (including *COI* without 3rd codon positions), provided strong support for the monophyly of *C. campanidorsalis*, sp. nov. (BS > 95; Fig. 2). *Cystococcus campanidorsalis*, sp. nov. was estimated to be sister to the other two species of *Cystococcus* in all analyses (BS > 70), except for the ML analysis of *COI* without 3rd codon positions, in which *C. campanidorsalis*, sp. nov. appeared nested within *C. pomiformis*. Relationships between *C. echiniformis* and *C. pomiformis* were not as clearly resolved; however, a sister relationship appears most likely, as shown by those analyses of *COI* that recovered support for reciprocal monophyly between the two species (Fig. 2). The lack of support recovered from *18S* analyses (BS < 70) was likely due to the small amount of variation between *C. echiniformis* and *C. pomiformis* in the less variable *18S* gene region.

Twenty-three collections were sequenced and analysed for both *COI* and *18S*, including four specimens of *Ascelis* spp. (outgroups) and 19 specimens of *Cystococcus* spp., sampled from across their known and newly discovered distribution (GenBank accession numbers: *18S*: KP729354–729373; *COI*: KP729331–729353). For *18S*, the final sequence alignment consisted of 611 base pairs (bp), with 51 variable sites and 38 parsimony informative sites. The alignment for *COI* consisted of 507 bp, with 183 variable sites and 129 parsimony informative sites. Across the full datasets, base frequencies were equal for *18S* but not for *COI*. In *COI*, overall nucleotide frequency means were: A = 0.43, T = 0.22, G = 0.06, C = 0.29, showing an unequal adenine to guanine ratio (i.e. a strong AT bias). The bias was greatest in 3rd codon positions, with an average AT proportion of 0.75. In addition, there was base composition bias among taxa (non-stationarity) at 3rd codon positions of *COI* (χ^2, $P < 0.001$), particularly between *C. campanidorsalis* and the other species of *Cystococcus* (Table 2). This bias between taxa could act as a confounding factor in phylogenetic analyses, exaggerating the apparent molecular separation of *C. campanidorsalis*, sp. nov. from the two other species, because most tree estimation methods assume stationarity of base composition.

Morphology

Physical characteristics of adult females of *Cystococcus* are minimal, including no eyes, antennae, legs or wings, and there is minimal sclerotization other than the button. Dissection and slide mounting of adult females of *C. campanidorsalis*, sp. nov. provided previously unknown information about their specific anatomy. Abdominal segmentation on the dorsal surface is not visible in *C. echiniformis* and *C. pomiformis*, but is defined in *C. campanidorsalis*, sp. nov. by light sclerotization between segments and a transverse row of setae and pore plates on each segment. Dissection revealed the abdominal, cuticular attachment points of the hindgut and oviduct, and confirmed the hindgut to be blind-ended with no anal opening. The oviduct appears attached to the cuticle seven or eight abdominal segments anterior to the dorsal button, on what appears to be

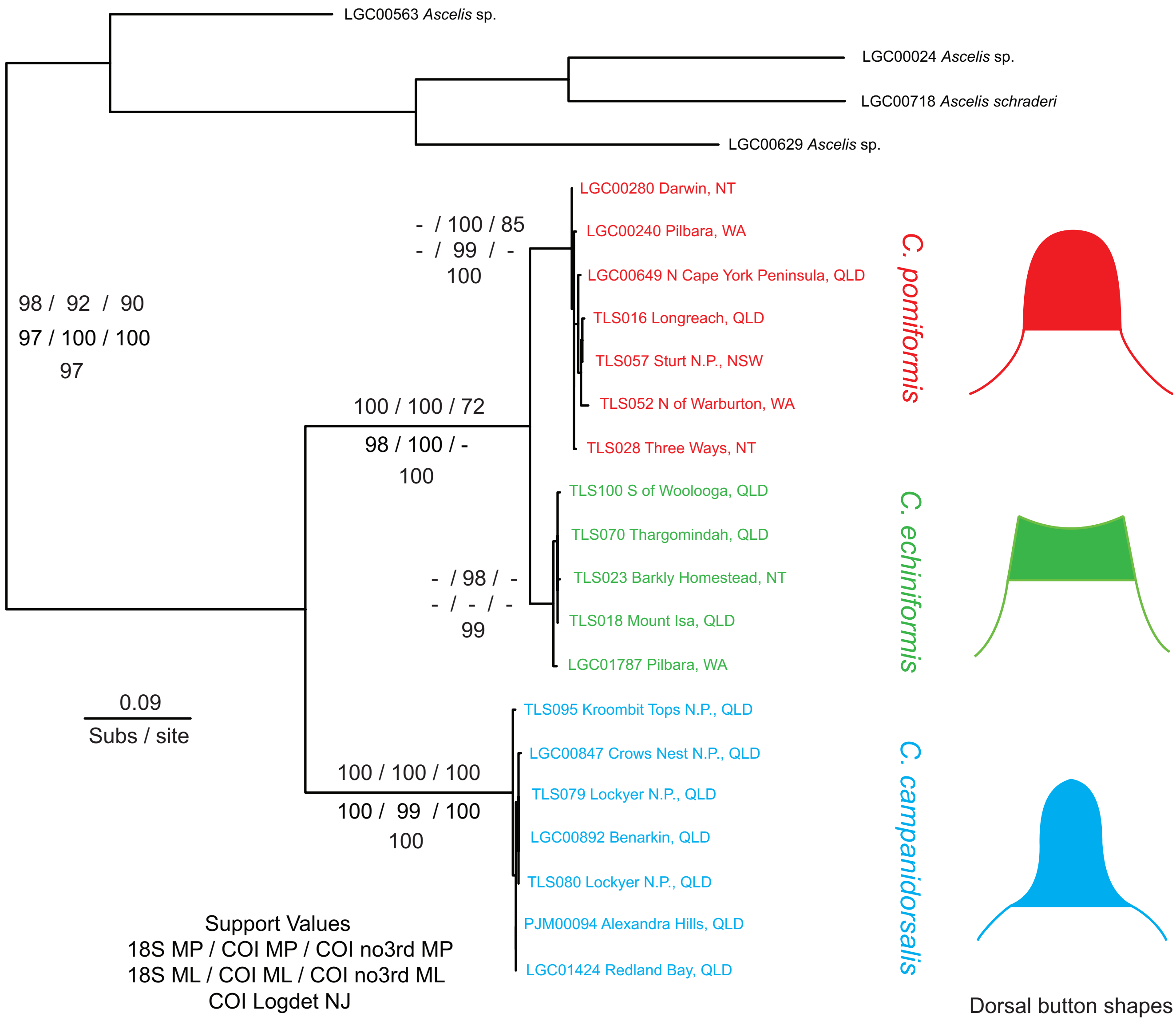

Fig. 2. Maximum likelihood phylogeny estimated from sequences of *COI* using RaxML, with bootstrap support values from all analyses displayed on branches (see key in figure). Relationships among *Ascelis* spp. were not supported in any analyses. Dorsal button shapes are shown beside their corresponding species clade. Scale bar indicates the average number of substitutions per nucleotide site.

the venter in slide-mounted specimens. That is, dorsal abdominal segments ~III through IX appear ventral and anterior to the button. This would place the button dorsally, on abdominal segment(s) II and/or III (Fig. 3).

Initial (and longstanding) misconceptions about this genus described the sclerotized button as caudal or posterior on the abdomen (see Fuller 1897; and Hardy *et al*. 2011). Froggatt (1921: 156) went so far as describing it as 'analogous with the more distinct tails of *Apiomorpha* and *Ascelis*'. In eriococcids (which include *Cystococcus* and *Ascelis*) the vulva is typically found on or between abdominal segments VII and VIII (Williams 1985; Gullan and Jones 1989). However, in *Apiomorpha* the vulva appears to have been displaced anteriorly by at least one segment (Gullan and Jones 1989). Having confirmed the location of the blind-ended hindgut and vulva of *Cystococcus*, we can confidently revise the location of the button as dorsal, on abdominal segment II and/or III (Fig. 3). The use of a sclerotized dorsum to plug gall openings is not unique to *Cystococcus*, as it appears in other eriococcid scale insects, including *Opisthoscelis* Schrader (Hardy and Gullan 2010), *Bystracoccus* Hodgson (Hodgson *et al*. 2013) and *Madarococcus* (Hardy *et al*. 2008), among others. Along with *Cystococcus*, the closely related genus *Ascelis* is thought to plug the gall opening with the sclerotized caudal area of its abdomen (Gullan *et al*. 2005). This likely requires revision because, like *Cystococcus*, the abdominal segmentation of females of *Ascelis* is not clearly defined and the genus appears very similar morphologically to *Cystococcus*.

Although little is known about nutrient uptake and waste production in *Cystococcus*, the length of their feeding stylets can help us to make inferences about feeding and to explain the lack of a functional anus. Beardsley (1984) observed that gall-inhabiting scale insects had much shorter stylets than their non-gall-inducing relatives. Indeed, the stylets of *Cystococcus* are very short (<0.6 mm long), making the gall lining (typically at least as thick as the outer wall, >1.5 mm thick) the only tissue available for feeding. Within the Coccoidea, it is the phloem-feeding groups that are best known to produce excessive volumes of sugary excrement or ‘honeydew’ (Gullan and Kosztarab 1997). Thus, if females of *Cystococcus* do not feed on phloem, as evidenced by their short stylets, then the small amount of waste produced from feeding in the special nutritive tissue might be stored and/or recycled.

Table 2. Nucleotide proportions in third codon positions of mitochondrial cytochrome *c* oxidase 1
Base composition difference between *Cystococcus campanidorsalis*, sp. nov. and the other species is highlighted in grey

Species	Adenine	Cytosine	Guanine	Thymine
C. pomiformis	0.53–0.55	0.28–0.31	0.01–0.02	0.14–0.17
C. echiniformis	0.53–0.54	0.28	0.01	0.17–0.18
C. campanidorsalis, sp. nov.	0.56–0.57	0.18	0.0–0.01	0.25
Ascelis spp.	0.60–0.66	0.18–0.23	0.01–0.03	0.15–0.18

Another unusual morphological characteristic of *Cystococcus* is the absence of loculate pores on any instar, and the presence of ‘pore plates’ on the dorsal derm of second-instar males and the ventral derm of adult females. On adult females, pore plates are numerous surrounding the spiracles and on several segments of the abdominal venter. Scanning electron microscopy (Fig. 4) shows each plate of the adult female to be composed of several rounded tubercles, the so-called ‘pores’, clustered together and surrounded by a rim of sclerotized cuticle to form a plate. There are no loculi (i.e. holes) and any exudation must be secreted across the cuticle. These pore plates appear to produce white powdery wax on live adult females. The spiracles lack this type of powdery wax but exude long (perhaps up to 400 µm), silvery filaments.

Species delimitation

Cystococcus campanidorsalis, sp. nov. was identified as a member of the genus *Cystococcus* by the morphology of males and females, and its induction of woody galls on stems of bloodwoods (*Corymbia* spp.). Using DNA sequence data, morphology and host use, we provide evidence for a lack of

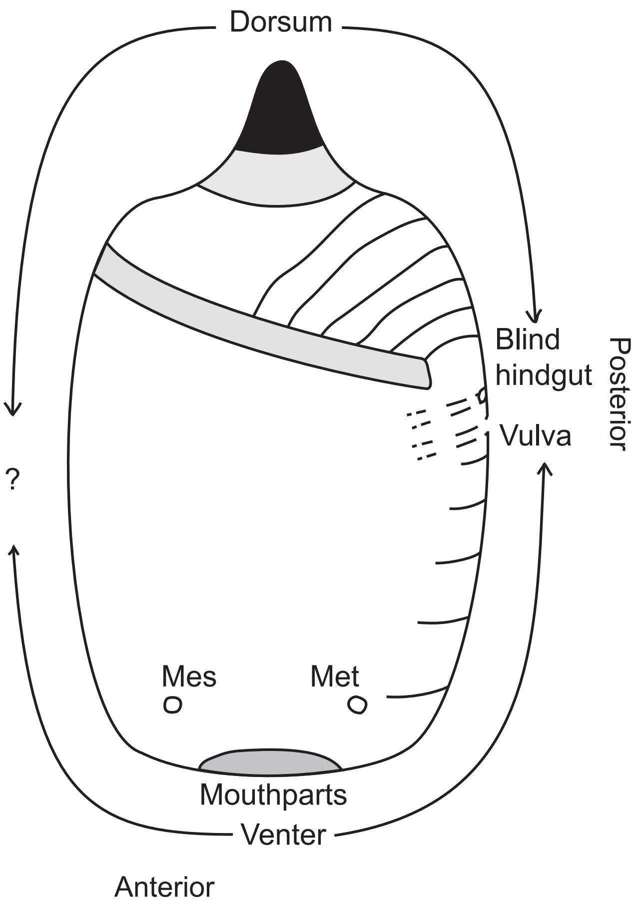

Fig. 3. Side view of an adult female of *Cystococcus* showing the revised body plan. Note the dorsal position of the sclerotized button. Mes, mesothoracic spiracle; Met, metathoracic spiracle.

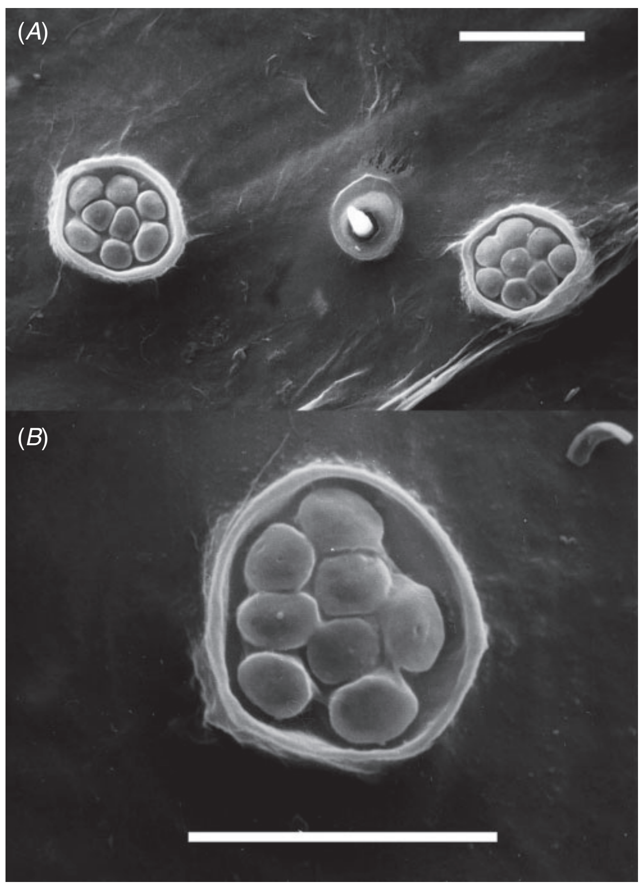

Fig. 4. Scanning electron micrograph of pore plates of adult females of *Cystococcus*: (*A*) two pore plates with a single hair-like seta between them; (*B*) a single pore plate. Scale bars = 10 µm.

gene flow between *C. campanidorsalis*, sp. nov. and other species of *Cystococcus*. We interpret this as equivalent to reproductive isolation under Mayr's (1942) biological species concept, and therefore determine *C. campanidorsalis*, sp. nov. to be a distinct species. *Cystococcus campanidorsalis*, sp. nov. is currently known to exist in near-sympatry with *C. echiniformis* and to not co-occur with *C. pomiformis*, and so reproductive isolation cannot be inferred directly from divergence in sympatry.

The reciprocal monophyly of *C. campanidorsalis*, sp. nov. and the other species of *Cystococcus* indicates that there has been no recent gene flow between these two clades (Fig. 2). This was supported by all analyses except for one – ML analysis of *COI* with 3rd codon positions removed. In the absence of 3rd codon positions, only a few synapomorphic nucleotide sites were identified for the two relevant clades. However, the same relationship was recovered in our analysis using the LogDet method from Lockhart *et al*. (1994), used to correct for base composition bias among taxa, with 3rd codon positions included.

Although the ranges of *C. campanidorsalis*, sp. nov. and *C. echiniformis* overlap, the adult females and males of these two species are easily distinguishable. In addition, *Cystococcus campanidorsalis*, sp. nov. has been collected only from *Corymbia trachyphloia*, a bloodwood species from which *C. echiniformis* has not been collected. *Corymbia trachyphloia* is considered to be a brown bloodwood (section *Apteria*) and is the sole occupant of a section of corymbias nested within the red bloodwoods (*Co.* sect. *Rufaria*) (Parra-O *et al*. 2009). *Cystococcus pomiformis* and *C. echiniformis* have been collected from numerous other species within *Co.* sect. *Rufaria*.

We also examined specimens of adult females and adult males from three collections that PJG had recognised previously as a new species (Gullan and Cockburn 1986). All specimens were from the Northern Territory from either Gunn Point (north of Darwin) or the Coburg Peninsula in Arnhem Land, and the host of one collection was recorded as *Corymbia bleeseri*. The galls are 14–20 mm in height, 17–31 mm in diameter with a wall 1–3 mm thick, and most closely resemble the galls of *C. echiniformis*. However, adult males and adult females of this putative new species most closely resemble those of *C. pomiformis*, including in the shape of the dorsal button of the female. All specimens were collected in the 1970s and 1980s and no tissue is available for DNA analysis. Fresh samples are required for molecular study to determine whether these populations represent a fourth species or a geographic or host-related variant of *C. pomiformis*.

Taxonomy

Genus *Cystococcus* Fuller

urn:lsid:zoobank.org:act:1A45C81D-B7A4-4806-A8A9-225D4D733F41

Cystococcus Fuller, 1897: 1346; 1899: 462–463 and plate XV, fig. 36.

Type species: *Cystococcus echiniformis* Fuller, by monotypy.

This genus was considered to be a junior synonym of *Ascelis* by Cockerell (1902), Fernald (1903) and Hoy (1963), but Gullan and Cockburn (1986) and Gullan *et al*. (2005) treated the two genera as distinct. *Cystococcus* is distributed broadly across northern Australia at latitudes less than 28° south (data from this study), whereas *Ascelis* has been collected mostly from south-east Australia (mainly New South Wales) (Miller *et al*. 2014). Like *Cystococcus*, most currently recognised species of *Ascelis* have been collected from *Corymbia*, especially *C. gummifera* (formerly *Eucalyptus corymbosa*, as listed in Miller *et al*. (2014)). Galls of *Cystococcus* are always on the stems, whereas those of *Ascelis* are on leaves (Froggatt 1921).

Generic diagnosis

Adult female

Body up to 25 mm long and 13 mm wide, elliptical to subspherical and roughly circular in transverse cross-section, with a prominent dorsal, heavily sclerotized button 1.0–2.4 mm in diameter and 0.3–2.0 mm long, ranging from convexly dome- or bell-shaped, to squat and concave-ended (dependent on species; Fig. 5), used to plug gall orifice. Integument mostly membranous, except for sclerotized dorsal button and light derm sclerotization surrounding button and mouthparts and sometimes marking intersegmental lines on ventral abdomen. Eyes, antennae and legs absent. Mouthparts with prominently enlarged apodemes (aliform expansions) of clypeolabral shield. Stylets 275–600 µm long. Spiracles subequal in size, with dense bunches of trachea radiating into body; mesothoracic spiracles often appearing dorsal, due to incorrect perception of body plan (actually ventral, anterior to mouthparts, near margin); metathoracic spiracles posterior to mouthparts, near margin. Unclear where venter meets dorsum around head, due to absence of head structures (only guide is mesothoracic spiracles). Sparse, short hair-like setae on dorsum and venter. Loculate pores absent but venter with clusters of pore plates around spiracles and grouped on ventral abdomen (Fig. 5). Anus sometimes visible posterior to vulva, but blind-ended and non-functional.

First-instar female

Based on all three species, but only *C. echiniformis* is illustrated as morphology is almost constant among species: body up to 440 µm long and 360 µm wide. Dorsum sclerotized and convex, with 30–35 pits on each side of thorax, each pit up to 35 µm in diameter, distributed submedially to submarginally. Antennae three-segmented; apical segment longest and with robust fleshy setae. Legs subequal; tibia and tarsus fused; tarsal digitules capitate, one longer and thinner than other. Claw with distinct subapical denticle; one claw digitule capitate, other with lance-shaped apex. Body setae hair-like, mostly minute, except for a few longer setae on ventral head and a pair of longer apical setae on posterior abdomen. Tubular ducts and loculate pores absent. A small pore plate adjacent to each thoracic spiracle.

First-instar male

Based on *C. campanidorsalis*, sp. nov. and *C. pomiformis*, but only *C. pomiformis* is illustrated: body turbinate, up to 540 µm long and 340 µm wide. Derm membranous, both surfaces covered by microtrichia. Antennae three-segmented; apical segment longest, with hair-like and fleshy setae but none bifid. Legs subequal in size with tibia and tarsus fused; tarsal digitules capitate, one longer and thinner than other. Claw with a small

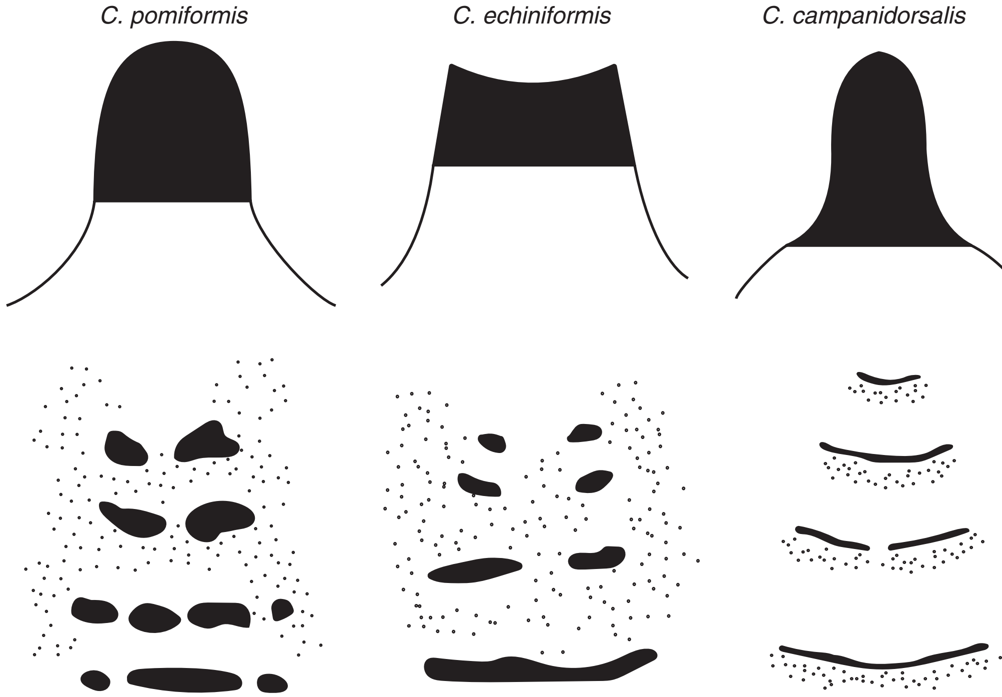

Fig. 5. Differences in button shape (top) and ventral pore-plate patterns (bottom) in adult females of *Cystococcus* species.

subapical denticle; claw digitules capitate and subequal. Body setae all hair-like, mostly minute, except for a few longer setae on ventral head and thorax and a pair of longer apical setae on posterior abdomen. Tubular ducts, loculate pores and pore plates absent.

Second-instar male

Based on all three species, but only *C. pomiformis* is illustrated: body turbinate, 0.7–2.5 mm long, 0.4–0.8 µm wide. Derm membranous, both surfaces covered by microtrichia. Antennae three-segmented; apical segment longest, with hair-like and fleshy setae and a few robust bifid fleshy setae. Legs subequal in size with tibia and tarsus fused; tarsal digitules capitate, one longer than other. Claw with a small subapical denticle; claw digitules capitate and subequal. Body setae all hair-like, mostly minute, except for a few longer setae on ventral head and thorax and two pairs of longer apical setae on posterior abdomen. Tubular ducts absent. Loculate pores absent, but most segments of dorsal abdomen with a few to several pore plates, also sometimes on dorsum of thoracic segment III.

Adult male

Body up to 9.5 mm long with abdominal segments III–VII extremely long and narrow, making up between 2/3 and 3/4 of total body length. Antennae short, about twice length of head, with most flagellar segments fused, with spinose and/or broad fleshy setae, and several, sometimes digitate, antennal bristles. Mesothoracic wings of typical form, but alar setae absent; hamulohalteres absent. Body with very few setae, mostly hair-like; pores absent. Glandular pouches absent. Penial sheath elongate and bluntly pointed. Males collected solely from within the galls induced by females, often as nymphs, prepupae and/or pupae, occasionally as adults.

Gall

Diameter up to 90 mm; subspherical, but sometimes squat, dumpy or pear-shaped. Surface texture ranging from smooth to very lumpy and knobbled. With a small orifice at apex, plugged by female dorsal button, but allowing mating with adult males and egress of male and female offspring. Females induce gall growth on small, young branches of numerous species of *Corymbia*.

Remarks

The most commonly found form of *Cystococcus* (adult female in gall) usually can be identified to species level without opening the gall, or even removing it from the tree. The shape of the sclerotized dorsal button (which can be seen from outside the gall) is usually sufficient to identify individuals in the field. Adult males can be difficult to distinguish, due to minimal differences among species and variation within species, especially in the absence of good quality slide-mounted specimens. Molecular data or adult female morphology are much more reliable for identification (when available).

Key to species of *Cystococcus* based on adult females (Fig. 5)

1. Sclerotized dorsal button concave-ended .... *C. echiniformis*
   Sclerotized dorsal button convex-ended 2
2. Dorsal button bell-shaped (flaring outwards at base); pore plates on venter in clearly separated transverse bands ....
   ....*Cystococcus campanidorsalis*, sp. nov.

Dorsal button ranging from dome-shaped to bluntly conical; pore plates in cluster around vulva, not in clearly separated transverse bands .... *C. pomiformis*

Key to species of *Cystococcus* based on adult males

1. Antennal pedicel with numerous broad fleshy setae; antennae without digitate antennal bristles; scutum with scutal setae in two broad bands of ~20 setae each; posterior abdominal segments and penial sheath with many fleshy setae ....*C. campanidorsalis*, sp. nov. (Fig. 6)
   Antennal pedicel without fleshy setae; antennae with some digitate antennal bristles; scutum with scutal setae in two narrow bands; posterior abdominal segments (except sometimes segment VIII) and penial sheath with few fleshy setae 2
2. Antennal flagellum without (or with very few) broad, fleshy setae .... *C. echiniformis* (Fig. 7)
   Antennal flagellum with numerous broad, fleshy setae........................... .... *C. pomiformis* (Fig. 8)

Species descriptions

Cystococcus campanidorsalis, sp. nov. Semple, Cook & Hodgson

urn:lsid:zoobank.org:act:A78C6002-72A0-4141-A01B-F0E78DC C38F8

Material examined

Holotype. Adult ♀. Australia: Queensland, Lockyer National Park (–27.452, 152.23), on *Corymbia trachyphloia* (Myrtaceae), 19.xii.2013, T. L. Semple (ID: TLS080) (QM: 1/1 ♀). GenBank accession numbers: *18S*: KP729371; *COI*: KP729351.

Paratypes. Fourteen slides with: 10 ♀ (ID: LGC00892, LGC01227, LGC01424, PJM00187, PJM00193, TLS079, TLS081, TLS082, TLS083, TLS084; see Table S1) and 10 adult ♂ (ID: LGC00886, PJM00094) (QM: 5/5♀, 2/5♂; ANIC: 5/5♀, 2/5♂) and two slides with: 10 first-instar ♀ (ID: PJM00394) (QM: 1/5; ANIC: 1/5).

DNA sequence data (synapomorphic nucleotide sites mapped to the GenBank reference sequence listed)

18S: Reference sequence: TLS080: GenBank KP729371. Site# 16(A), 18(A), 103(C), 125–126(TT), 149(A), 155(A), 241(T), 248(G), 257(T), 268(T), 313(T), 586(G).

COI: Reference sequence: TLS080: GenBank KP729351. Site# 44(C), 56(T), 101(C), 169(G), 203(T), 263(T), 275(T), 303(T), 326(C), 339(G), 377(T), 392(T), 404(C), 480(T), 521(C).

Description

Adult female (Fig. 9) (11/11: three poor, three fair, two good, three excellent)

Mounted material. Body up to 16 mm long and 12 mm wide. Sclerotized button 1.6–1.8 mm diameter at base, 1.3–1.5 mm long, bell-shaped with slightly raised point at apex; located dorsally on abdominal segments II and/or III. Spiracles 160–220 µm in diameter. Mouthparts of older individuals surrounded by sclerotized derm disc, 1.3–2.0 mm diameter; stylets 380–530 µm long, but often lost along with supporting aliform expansions when female removed from gall tissue.

Dorsum. Majority of cuticle with sparsely scattered, short hair-like setae (hs), each 12.5–20.0 µm long. Long hs, 37.5–55.0 µm long, present on abdomen in clear bands posterior to dorsal button, separated by very light bands of sclerotization.

Venter. Majority of cuticle with sparsely scattered, short hs, each 12.5–17.5 µm long. Median, posterior half of venter with transverse rows of alternating hs, 12.5–17.5 µm long, and pore plates, each 10–15 µm diameter with 4–12 pores (each 2–3 µm diameter). Some very faint sclerotization separating rows of setae and pore plates. Pore plates and hs also clustered densely around spiracles, these pore plates each 10–17 µm in diameter with 4–14 pores (each 2–3 µm diameter).

Adult male (Fig. 6) (3/3, one fair–good, two fair)

Material examined (Three of three from this locality used for description)

Australia: Queensland. Scribbly Gums Conservation Area, Alexandra Hills (–27.535, 153.232), on *Corymbia trachyphloia* (Myrtaceae), 25.ii.2010, A. Mather and P. J. Mills, ID: PJM00094. Measurements for body length, antennal length, and wing length and width are supplemented with data from other paratype specimens.

Mounted material. Body of moderate size but with an exceptionally long abdomen (length of head, thorax + abdominal segments I–III 2.0–3.0 mm; total body length 5.3–9.2 mm). Ocular sclerite without reticulations, but extending more or less around head, with two pairs of large simple eyes. Body with very few setae, almost all hair-like (hs), each 10–16 µm long; setae on legs and antennae mainly rather longer and stronger, many becoming spur-like at distal end of legs, but with an occasional fleshy seta (fs) on dorsal margin of tibia. Claws with a denticle near apex and another near base of claw; claw and tarsal digitules capitate; one claw digitule arising from basal denticle. Wings normal, without alar setae or pores. Hamulohalteres absent. Glandular pouches absent.

Head. Appearing rather broad in dorsoventral view, but probably with a distinct posteroventral bulge for ventral simple eyes; width across ocular sclerites ~355–375 µm. Median crest broad and parallel-sided, sclerotized, not reticulated, with ~14–16 hs dorsal head setae on either side plus one to three above each dorsal simple eye. Postoccipital ridge present, represented by a bowtie-shaped sclerotized area posterior to median crest. *Mid-cranial ridge*: dorsal ridge obscure or short; ventral ridge with poorly developed lateral arms extending to each scape, and with an indistinct medial ridge extending a short distance posteriorly; area laterad to ventral mid-cranial ridge not apparently sclerotized or reticulated, with a group of ~25 hs ventral mid-cranial ridge setae on each side, plus a few head setae extending between ventral simple eyes and with a pair on posterior margin of ocular sclerite. Genae mildly sclerotized but not reticulated, each with a group of 8–14 hs genal setae. Eyes: two pairs of round simple eyes, subequal in size, each 62–69 µm wide. Ocelli distinct, not touching postocular ridge, each ~28–30 µm wide. Ocular sclerite well sclerotized but not polygonally reticulated, sclerites almost meeting ventrally. Preocular ridge absent, represented by anterior margin of ocular sclerite, with a small articulation with antennae. Postocular ridge represented by posterior margin of ocular sclerites. Dorsal ocular setae absent. Preoral ridge well developed; mouth opening distinct. Cranial apophysis not detected.

Antennae. Length 620–780 µm. Segments between pedicel and apical segment apparently fused. Scape: 37–53 µm long, 74–106 µm wide, with five or six fs. Pedicel: length 75–80 µm, width 63–90 µm, with a few ridges distally, with 22–29 broad fs and five or six hs, mainly ventral. Flagellar segments fused, broadest near pedicel (~55–70 µm wide) narrowing gradually to apical segment (28–30 µm wide), with numerous rather large spinose setae (35–50 µm long), broad fs (11–23 µm long), and shorter, more hair-like setae (25–35 µm long); each seta mainly on a small convexity in an area of sclerotization and arranged more or less in rings; also with three to five antennal bristles (ab), mostly quite long (36–65 µm long), on distal half

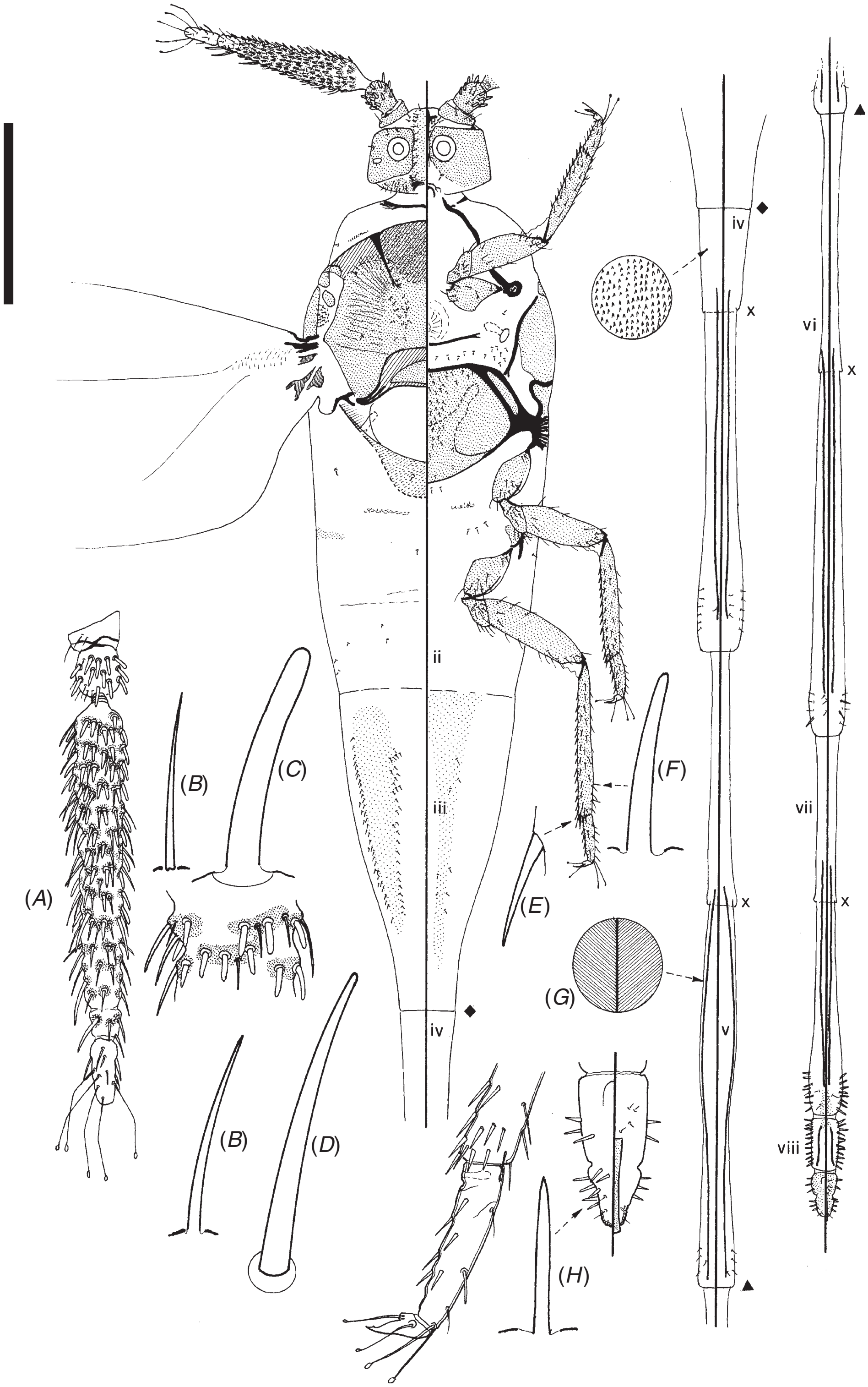

Fig. 6. *Cystococcus campanidorsalis*, sp. nov. Semple, Cook & Hodgson. Adult male. Abdomen drawn in three sections due to length, with segments indicated in Roman numerals: (*A*) detail of antenna; (*B*) spinose seta on antenna; (*C*) fleshy seta on antenna; (*D*) antennal bristle; (*E*) spur-like seta on tibia; (*F*) fleshy seta on tibia; (*G*) detail of rod-like structures inside abdominal segments IV–VIII; (*H*) stout fleshy seta on penial sheath. Scale bar = 0.5 mm.

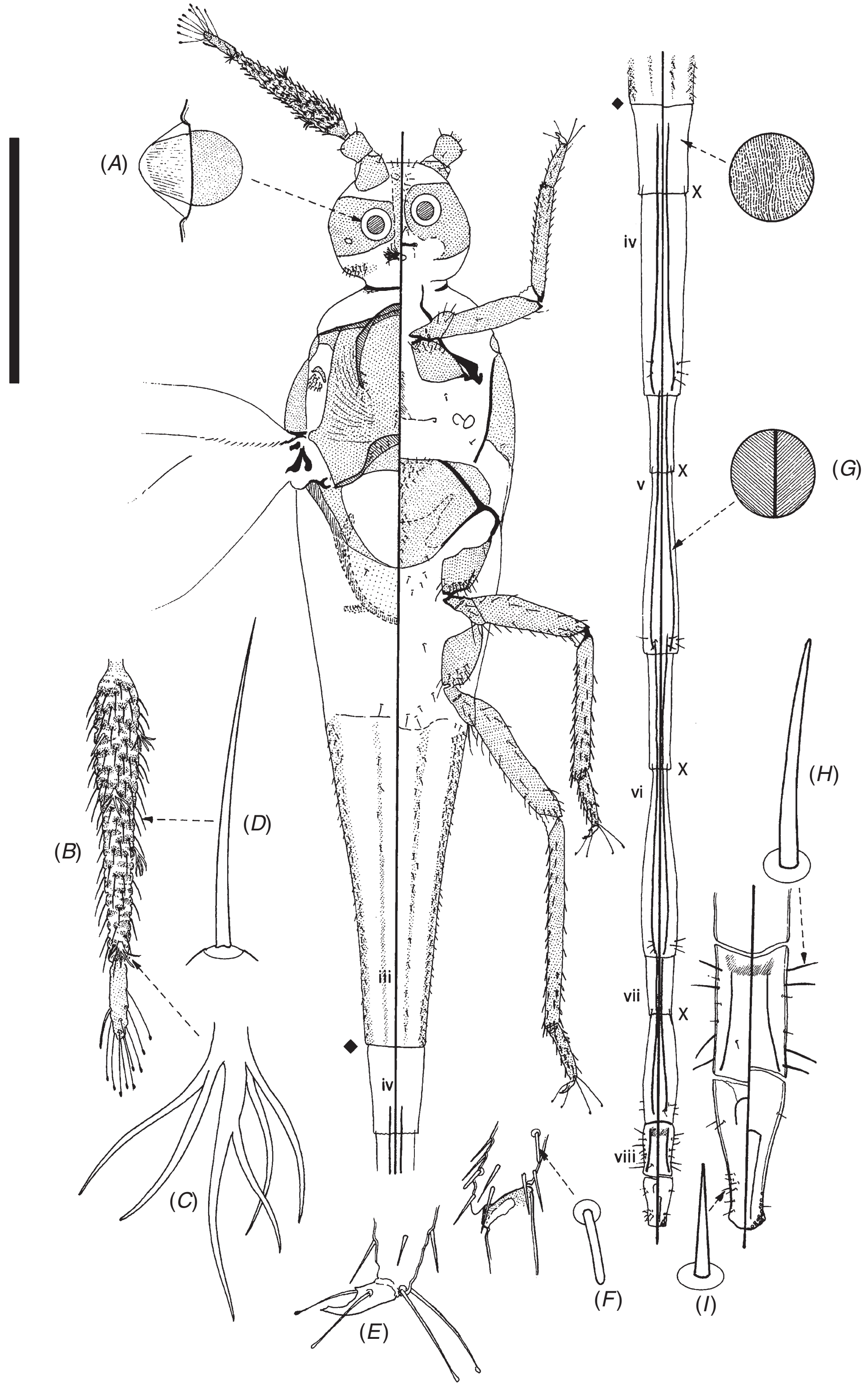

Fig. 7. *Cystococcus echiniformis* Fuller. Adult male. Abdomen drawn in two sections due to length, with segments indicated in Roman numerals: (*A*) detail of eye; (*B*) detail of antenna; (*C*) digitate antennal bristle; (*D*) spinose fleshy seta on antenna; (*E*) detail of tarsus and claw, showing tarsal digitules (right) and denticles on claw (left); (*F*) peg-like seta on tibia; (*G*) detail of rod-like structures inside abdominal segments IV–VIII; (*H*) fleshy seta on abdominal segment VIII; (*I*) stout fleshy seta on penial sheath. Scale bar = 0.5 mm.

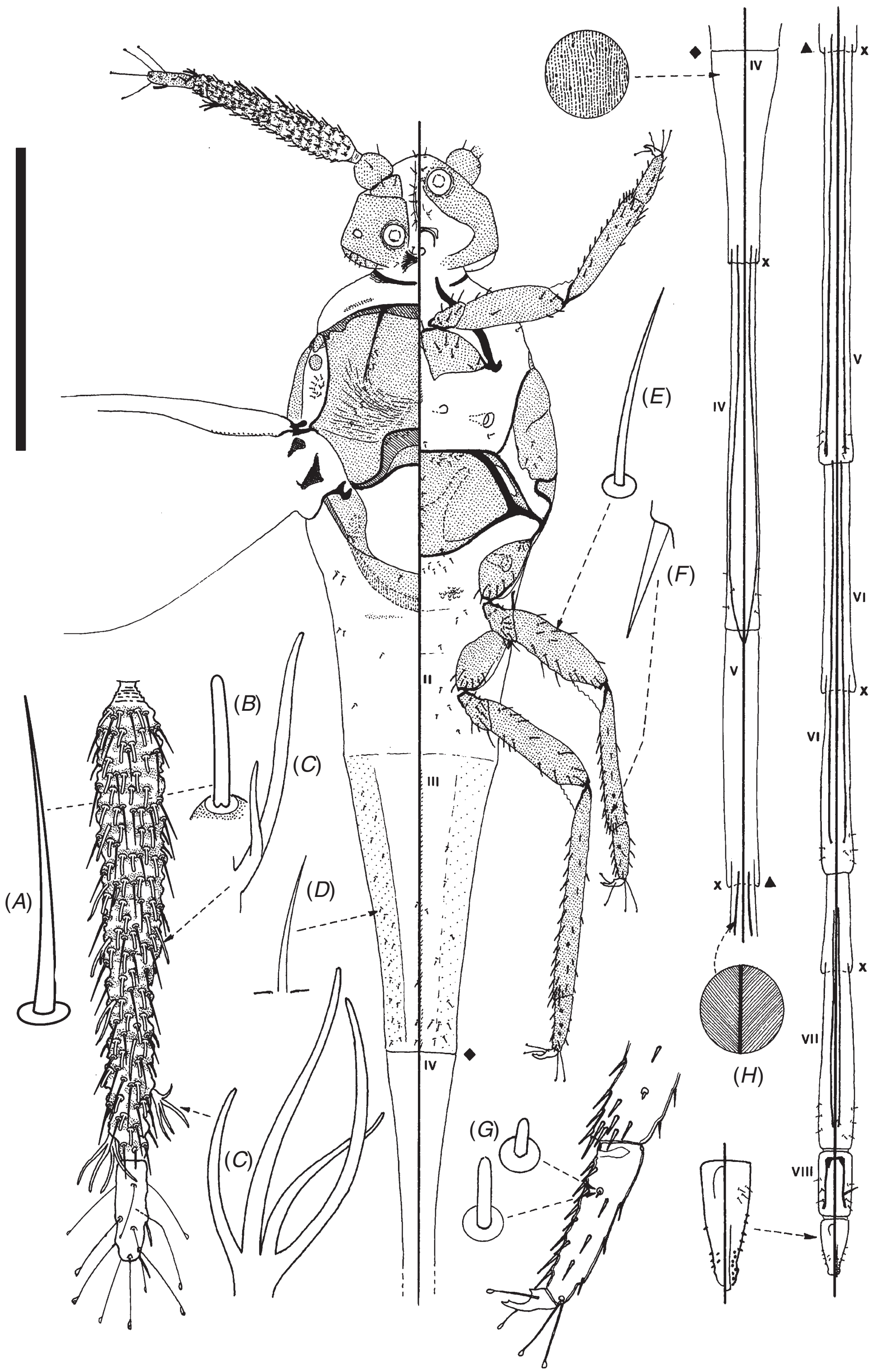

Fig. 8. *Cystococcus pomiformis* (Froggatt). Adult male. Abdomen drawn in three sections due to length, with segments indicated in Roman numerals: (*A*) spinose seta on antenna; (*B*) broad fleshy seta on antenna; (*C*) digitate antennal bristles; (*D*) hair-like seta on abdomen; (*E*) hair-like seta on femur; (*F*) apical spurs on tibia; (*G*) peg-like seta on tarsus; (*H*) detail of rod-like structure inside abdominal segments IV–VIII. Scale bar = 0.5 mm.

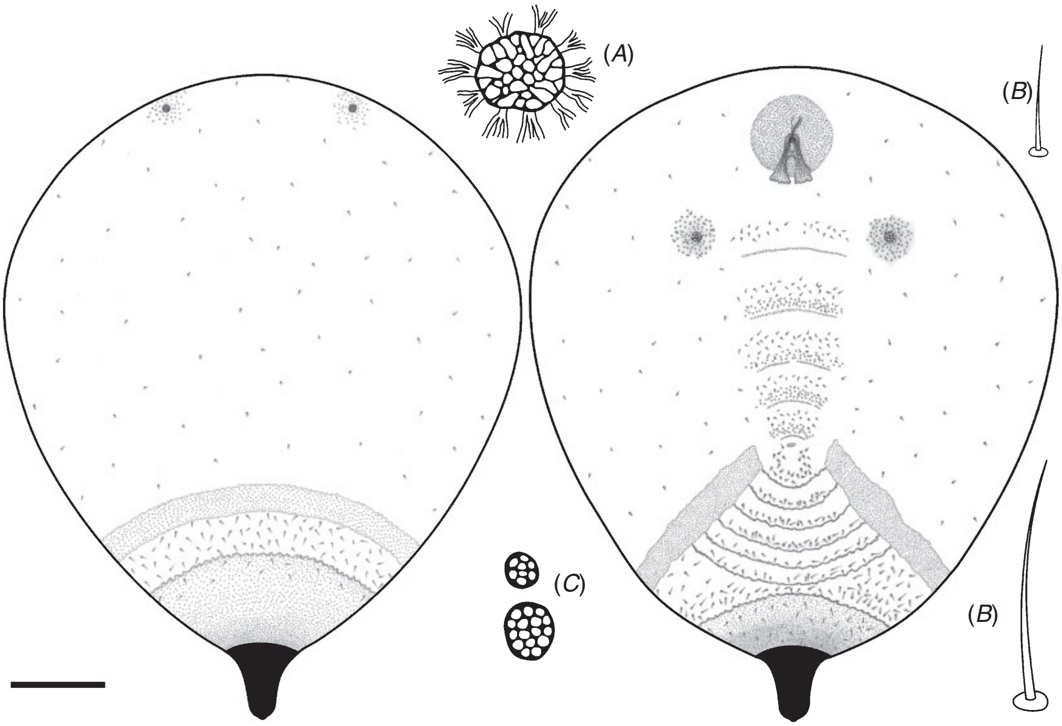

Fig. 9. *Cystococcus campanidorsalis*, sp. nov. Semple, Cook & Hodgson. Adult female. Illustrated as seen on slide-mounted specimens. Left side includes: mesothoracic spiracles, some venter, and dorsum to dorsal button and abdominal segment II and/or III. Right side includes: (ventral) mouthparts, metathoracic spiracles and vulva, plus dorsal abdominal segments II–VIII, including the dorsal button: (*A*) detail of spiracles; (*B*) hair-like setae (length relative to location on body); (*C*) detail of pore plates. Scale bar = 2 mm.

of flagellum; all non-digitate. Preapical segment sometimes fused to flagellum, ~35–42 µm long, 28–36 µm wide, with five or six rather spinose setae and a large ab. Apical segment parallel-sided, not constricted apically, 75–110 µm long, 28–30 µm wide, with probably seven or eight capitate setae, about four or five fs, two or three large ab and one small ab; apparently without sensilla basiconica.

Thorax. Prothorax: pronotal ridge well developed, possibly fused dorsally, broadening laterally into a small, ridged lateral pronotal sclerite; pronotal ridge extending ventrally, articulating with cervical sclerite. Almost all prothoracic setae absent, except no or one lateral pronotal seta. Post-tergites thought to be present. Proepisternum and cervical sclerite well developed; propleural apophysis particularly large. Sternum lightly sclerotized; transverse ridge present with distinct sternal apophyses; median ridge absent, but with radial ridges; prosternal and anteprosternal setae absent; antemesospiracular setae generally absent, one occasionally present. Mesothorax: prescutum ~292–304 µm wide anteriorly, narrowing to ~95 µm wide posteriorly; nodulated; prescutal ridges present but prescutal suture absent, with two to seven prescutal setae along lateral margins. Scutum: median area not membranous, strongly sclerotized, with light transverse microridges, particularly laterad to prescutum, with two bands of 12–20 small setae extending medioposteriorly from margin of prescutum; marginal areas of scutum laterad to scutellum sclerotized but not reticulated; prealare and triangular plate present; scutal apodeme probably present on anterior margin. Scutellum 265–290 µm wide, 100–105 µm long, with an inverted U-shaped scutellar ridge; scutellar setae absent; posterior notal wing process strong. Basisternum 475 µm wide, 305–350 µm long, without a median ridge but bounded anteriorly by a strong marginal ridge and posteriorly by strong precoxal ridges; basisternal setae in a medial line and in a broad band along marginal ridge, with a total of 55–60 hs; lateropleurite fairly narrow but with an elongate membranous area medially, each with a sclerotized extension from marginal ridge along entire margin; furca well developed, broadly waisted, arms very divergent and extending at least 4/5ths to marginal ridge. Mesepimeron large, sclerotized and appearing digitate and nodulated. Mesopostnotum and postnotal apophysis well developed, the latter quite deep. Area bounded anteriorly by scutellum and laterally and posteriorly by mesopostnotum not sclerotized. Mesepisternum not reticulated; subepisternal ridge well developed, arising from anterior margin of lateropleurite. Postalare not reticulated anteriorly, without postalare setae. Mesothoracic spiracle: peritreme 40–46 µm wide. Postmesospiracular setae: ~30 extending across entire width. Tegula present, with 13–15 tegular hs on each side. Metathorax: with one metatergal hs on each side. Metapostnotum small, narrow, slightly nodulated. Dorsospiracular setae: ~0–3 hs. Dorsal part of metapleural ridge present but without a suspensorial sclerite. Ventral part of metapleural ridge well developed; episternum mildly sclerotized, with three postmetaspiracular hs on either side. Metepimeron sclerotized and elongate, without setae. Antemetaspiracular setae absent. Metathoracic spiracle: width of peritreme 43–50 µm. Metasternum probably membranous, with 3–5 anterior metasternal hs and no posterior metasternal setae.

Wings. Hyaline, 1545–2100 µm long, 600–900 µm wide (ratio of length to width 1 : 0.47); alar lobe present, setae absent. Hamulohalteres absent.

Legs. Metathoracic legs clearly longest. Coxae: I 158–170, II 175–190, III 185–190 µm long; coxa III with ~14 setae, probably hs. Trochanter + femur: I 330–335, II 330, III 375–380 µm long; trochanter III with ~13 setae, probably hs; long trochanter seta not differentiated; femur III with ~17 setae, probably hs. Tibia: I 280–285, II 305–320, III 435–440 µm; tibia III with many setae, mainly spur-like setae but with probably two peg-like blunt fs, with a group of stout apical spurs, length 25–27 µm, one occasionally bifid. Tarsi one-segmented (although a pseudo-articulation present on several legs): I 108–115, II 130–135, III 142–145 µm long (ratio of length of tibia III to length of tarsus III 1 : 0.33); tarsus III with ~9 setae, mainly rather spur-like; short peg-like setae absent; tarsal spurs ~20–25 µm long; tarsal campaniform pore, if present, very small; tarsal digitules capitate, slightly longer than claw. Claws rather small but clearly longer than width of tarsi, with a small denticle near apex and another near base of claw; length: III 40–43 µm; claw digitules capitate, longer than claw, one arising from distal margin of basal denticle.

Abdomen. Segments I–VII: segments I and II reasonably normal but segments III–VII extremely long and narrow, representing ~2/3rds total body length; posterior margins of these segments recognisable by presence of a small group of setae, mainly along margins, as follows (on each side): IV 10 or 11 hs, V six hs, VI five or six hs and one to three fs; VII two to six hs and 11–15 fs; each segment occasionally with a fold about halfway along, where posterior part of segment telescopes into anterior part (marked 'x' on figure). Tergites and sternites of I–VII considered absent. Caudal extensions of segment VII absent. Setae few on segments I and II, but segment III with two longitudinal lines of short hs on both sides of dorsum, plus a sparse band medially on venter. Segments IV–VII each also with a pair of internal rod-like structures, those of IV and V shorter than segment but those of VI–VII about same length as segments; each rod with very fine lines running diagonally (function unknown). Segment VIII quite short (145–150 µm long), parallel-sided, with a pair of internal rod-like structures about same length as segment, and 2–4 hs and 16–20 fs on each side. Caudal extensions, glandular pouches and glandular pouch setae absent. Genital segment: penial sheath elongate and bluntly pointed, 120–128 µm long, 70 µm wide at base, only lightly sclerotized, with a shallow constriction about halfway along margins. Anus visible dorsally (~15 µm wide), but functionality not confirmed (as in adult females). Ventrally, with aedeagus 80–85 µm long, 10 µm wide at apex, parallel-sided but widening slightly at apex and extending slightly past apex of penial sheath; basal rod apparently absent. Setae mainly marginal, with ~13–16 rather short, stout fs (mostly ~14–17 µm long), but with two to five very short hs ventrally, each ~8 µm long. Apex of penial sheath with a group of penial sheath sensilla.

Galls (based on nine specimens)

Sub-spherical in shape (mean height : diameter ratio = 1 : 1.09); height 18–28 mm (mean = 21 mm), diameter 18–28 mm (mean = 23 mm) and side wall thickness 3–7 mm (mean = 4.5 mm). Gall surface usually with a loose, flaky outer layer, similar to bark that flakes off juveniles of host *Corymbia trachyphloia*, and light to dark mottled brown in colour; paler coloured, slightly flattened or recessed ring around opening in some individuals.

Remarks

Females of *C. campanidorsalis* have dorsal buttons most closely resembling those of *C. pomiformis*, but flaring out at the base in a bell shape (Fig. 5). These two species also can be distinguished by the pattern of pore plates on the venter of adult females, anterior to the vulva. *Cystococcus campanidorsalis* has clear, transverse bands of pore plates, in contrast to the unpatterned clustering on *C. pomiformis* and *C. echiniformis* (Fig. 5). Due to the small number of discernible differences between adult females of *Cystococcus* species, only one whole female illustration is included in this paper. Adult males of *C. campanidorsalis* can be distinguished from those of *C. pomiformis* and *C. echiniformis* by the presence of numerous broad, fleshy setae on the antennal pedicel and the absence of digitate bristles on the flagellum (Fig. 6).

Distribution and host plants

Known from south-east Queensland, north to 24°S and west to 151°E. Only known host tree is *Corymbia trachyphloia*.

Etymology

The name *campanidorsalis* comes from the bell-shaped (campana = bell in Latin), sclerotized dorsal button, and also describes the location of this button as being dorsal rather than caudal.

Cystococcus echiniformis Fuller

Cystococcus echiniformis Fuller, 1897: 1346; 1899: 462–463. plate XV, fig. 36.

Ascelis echiniformis (Fuller); Cockerell, 1902: 114. Change of combination, not accepted by subsequent authors.

Fuller's (1897) original description of this species is very brief, but later he (Fuller 1899) provided a more detailed description accompanied by line drawings of the adult female and its gall. The only insect specimen with label data that clearly match collection information in Fuller (1897, 1899) is in the Brain collection (#438) in the USNM (examined by PJG). The specimen is incomplete and split between two slides: one has just a piece of cuticle with two spiracles and the other only the apex of the abdomen. The basal width of the abdominal button is 1.1 mm and it is concave-ended. The slide label data are: 'Cystococcus/echiniformis/cuticle' and 'Cystococcus/echiniformis/apex of abdomen', and both slides have '[On Eucalyptus tesselaris/E. Kimberly [sic]. Australia/R. Helms Coll.]/438'. We here designate the remains of this adult female as the lectotype.

There is also a gall of *C. echiniformis* in the USNM (also examined by PJG), but it has a 6 mm diameter hole in the side wall, no gall contents and no locality or collector data. The box label is 'Ascelis echiniformis (Full.)/TYPE/Ckll. Coll.' and thus there is no evidence that this gall is associated with the remains of the adult female in the Brain collection.

There also are two galls (one complete and one half) in the SAM that clearly are part of Fuller's original material as they have locality data of East Kimberley, Western Australia. The galls were received at the SAM in July 1897, which is before Fuller's formal naming of the species as *C. echiniformis* in August 1897, and the names on the labels with the galls are 'Cystococcus Fuller (n.g.)/Eucalypti, Fuller, nov. sp.', and 'Cystococcus n.g./Eucalypti n.sp. Fuller', and 'Cystococcus nov. gen./Eucalypti n.sp. Fuller m.s.' (there are three labels with the two galls). Thus, Fuller must have been planning to call his species '*Cystococcus eucalypti*', but changed the species name before publication. There are no insects associated with these SAM galls.

Material examined

DNA sequence data (synapomorphic nucleotide sites mapped to the GenBank reference sequence listed)

18S: No synapomorphic sites (gene region too conserved).

COI: Reference sequence: LGC01787: GenBank KP729341. Site# 47(T), 113(G), 320(T), 413(C), 434(T).

Redescription

Adult female (11/11: one poor, four fair, four good, two very good condition)

Material examined

Australia: Queensland, Northern Territory and Western Australia, on *Corymbia terminalis* (Myrtaceae), (ID: LGC01787, TLS002, TLS004, TLS005, TLS006, TLS008, TLS018, TLS023, TLS025, TLS043, TLS070) (ANIC: 11/11 ♀).

Mounted material. Body up to 13 mm long and 13 mm wide. Sclerotized button 1.1–1.6 mm diameter at base, 0.3–0.7 mm long, shaped like a volcanic caldera rim, concave at the end (Fig. 5); located dorsally, assumed to be on anterior abdominal segments (similar to *C. campanidorsalis*), but exact location unknown due to lack of visible dorsal abdominal segmentation. Spiracles 100–200 µm diameter. Mouthparts of older individuals surrounded by sclerotized derm disc 1.35–2.25 mm diameter. Stylets 275–400 µm long, but often lost along with supporting aliform extensions when female removed from gall tissue.

Dorsum. Majority of cuticle with sparsely scattered, short hs, each 10.0–17.5 µm long. Long hs present on abdomen, each 12.5–27.5 µm long, posterior to dorsal button.

Venter. Majority of cuticle with sparsely scattered, short hs (each 7.5–15 µm long), and pore plates (each 5.0–27.5 µm diameter) with 4–46 pores (each 2–3 µm diameter), in median, posterior half of venter. Some very faint, transverse bands of sclerotization medially, in between mouthparts and vulva, apparently separating abdominal segments. Pore plates and hs also clustered densely around spiracles, these pore plates each 7.5–22.5 µm in diameter with 4–40 pores (each 2–3 µm diameter).

Descriptions

First-instar female (Figs 10, 11) (3/10: all in good to very good condition)

Material examined

Australia: Queensland, Carnarvon Gorge lodge, on *Corymbia* sp., 9.xii.1993, L. G. Cook (ANIC: 2/60+ first-instar ♀); Northern Territory, ~50 km N of Tennant Creek, near Stuart Hwy, on *Corymbia* sp., early vi.1977, S. L. Wentworth (ANIC: 1/50+ first-instar ♀).

Mounted material. Body tortoiseshell-like, 400–440 µm long, 310–360 µm wide; lightly sclerotized dorsally, membranous ventrally. Eyespot on dorsal submargin, with lens 7.5–11.0 µm in diameter set in ring 12–16 µm in diameter. Antennae three-segmented, 40–60 µm long, with hs 7–25 µm long on all segments; apical segment 25–30 µm long, with four robust fs, 27–40 µm long, plus three to four slender fs 10–15 µm long. Clypeolabral shield 80–90 µm long. Labium without segmentation, 25–33 µm long, 31–37 µm wide. Spiracles very small, <20 µm long including peritreme, each with a small pore plate, 5.0–7.5 µm in diameter with three to four pores, adjacent to atrium. All legs subequal in size; trochanter + femur 70–75 µm, with femur widest (22–34 µm) in basal half; tibia and tarsus fused, 40–48 µm long; claw 13–16 µm long, with distinct subapical denticle; tarsal digitules capitate 25–37 µm long, one longer and thinner than other; claw digitules 19–25 µm long, one capitate, other with lance-shaped apex. Anus visible, but possibly blind-ended and non-functional (as in adult females).

Dorsum. Sclerotized throughout, with 33–35 pits on each side of thorax, each pit 12–25 µm in diameter, distributed submedially to submarginally. Margin without a fringe of setae; all minute hs 2–5 µm long, in a sparse submarginal line on thorax, a marginal line on abdomen with one seta on each side of each segment, and a few submedially on thorax and head. Tubular ducts and pores absent.

Venter. Hair-like setae mostly 2–16 µm long, few in number, present submedially on head and thorax and on abdominal segments in two longitudinal lines of setae submarginally and one longitudinal line medially to submedially (three pairs of lines in total), posterior segments also with longer setae 20–28 µm, and a pair of very long apical setae, 100–135 µm long. Tubular ducts and loculate pores absent.

Adult male (Fig. 7) (13/24: poor to very good condition, but all structures clear on at least one specimen; drawing based on Cyst E1 males)

Material examined

Australia: Northern Territory, ~50 km N of Tennant Creek, near Stuart Hwy, on *Corymbia* sp., early vi.1977, S. L. Wentworth (ANIC: 3/3 ♂); Queensland, S of Cooktown, Mt Elephant, Desailly Creek, early x.1977, P. Fell (ID: cp14/77) (ANIC: 5/16 ♂); Queensland, Paluma Road (–18.98, 146.30), on 'bloodwood', 2.vii.1993, P. J. Gullan and L. G. Cook (ID: CystE1) (5/5 ♂).

Mounted material. Body of moderate size but with an exceptionally long abdomen (length of head, thorax + abdominal segments I–III 1.75–2.35 mm; total body length 4.1–5.6 mm). Ocular sclerite without reticulations, but extending more or less around head, with two pairs of large simple eyes. Body with very few setae, almost all hs, each 8–16 µm long; hs and fs hard to differentiate on legs and antennae, where setae mainly rather longer and stronger, although many becoming spur-like at distal end of legs; some setae on legs peg-like, short and parallel-sided. Claws with a large denticle near apex and another near base of claw; claw and tarsal digitules capitate. Wings normal, without alar setae or pores. Hamulohalteres absent. Glandular pouches absent.

Head. Appearing rather broad in dorsoventral view but probably with a distinct posteroventral bulge for ventral simple eyes; width across ocular sclerites ~330 µm. Median crest lightly sclerotized, not reticulated, with ~4–6 hs dorsal head setae on either side. Postoccipital ridge present, represented by a bowtie-shaped sclerotized area posterior to median crest. Mid-cranial ridge: dorsal ridge obscure or absent; ventral ridge probably absent; area laterad to ventral mid-cranial ridge (vmcr) not apparently sclerotized or reticulated, with 8–10 hs vmcr setae on either side of vmcr, plus with a few ventral head setae in a narrow band extending posteriorly between ventral simple eyes. Genae mildly sclerotized but not reticulated, with a group of 8–13 genal hs on each side. Eyes: two pairs of round simple eyes, subequal in size, each 60–90 µm wide. Ocelli distinct, not touching postocular ridge, each ~23–29 µm wide. Ocular sclerite

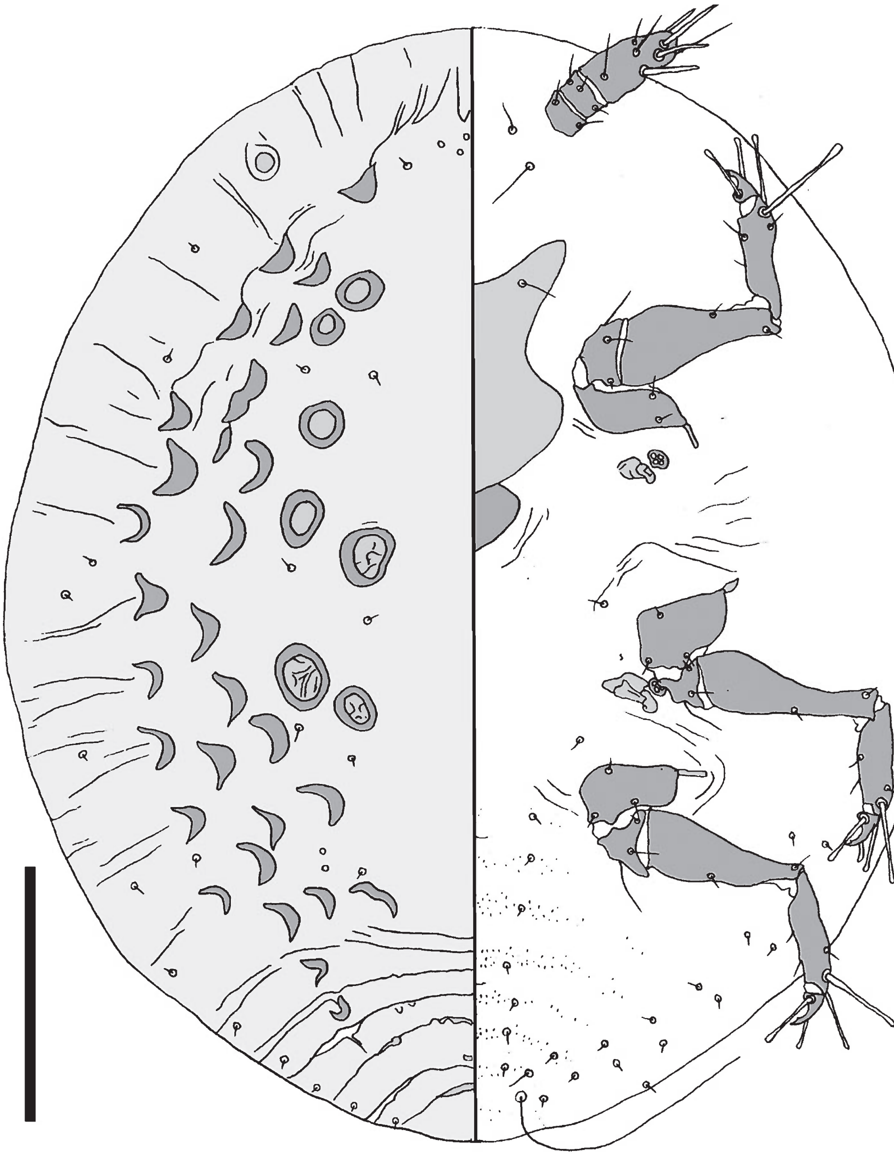

Fig. 10. *Cystococcus echiniformis* Fuller. First-instar female. Scale bar = 0.1 mm.

well sclerotized but not polygonally reticulated, sclerites almost meeting ventrally, without setae. Preocular ridge represented by anterior margin of ocular sclerite; not articulating with antennae. Postocular ridge represented by posterior margin of ocular sclerite. Dorsal ocular setae absent. Preoral ridge well developed; mouth opening distinct. Cranial apophysis not detected.

Antennae. Length 520–700 µm, with segments between pedicel and apical segment apparently fused. Scape 60–65 µm long, 70–75 µm wide, with two fs. Pedicel length 60–70 µm, width 65 µm, with a few ridges distally and six or seven hs. Flagellar segments fused, broadest near pedicel (~50 µm wide) narrowing gradually to apical segment (25 µm wide), with numerous, slender spinose fs, each 33–40 µm long, on a small convexity in small areas of sclerotization, latter more or less forming rings; also with up to 10 antennal bristles, mostly large and digitate (up to 60 µm long, with four to six fingers), rarely one shorter and parallel-sided. Preapical segment sometimes fused to apical segment, parallel-sided, ~30–35 µm long, 25 µm wide, with no to two setose setae. Apical segment parallel-sided, not constricted apically, 80–85 µm long, 25–27 µm wide, with at least nine capitate setae, one or two large ab but no other setae.

Thorax. Prothorax: pronotal ridge well developed, possibly fused dorsally, broadening laterally into a small lateral pronotal sclerite; pronotal ridge extending ventrally, articulating with cervical sclerite. Post-tergites possibly absent. Sternum lightly sclerotized; transverse ridge moderately well developed with distinct sternal apophyses; median ridge absent, but indicated by an area of slightly denser sclerotization; all prothoracic setae absent apart from one pair of antemesospiracular setae present. Mesothorax: prescutum ~215 µm wide, 230 µm long; sclerotized but not nodulated; prescutal ridges present but

Fig. 11. *Cystococcus echiniformis* Fuller. Scanning electron micrograph of first-instar female. Scale bar = 0.1 mm.

prescutal suture absent, with two to four prescutal setae along lateral margins. Scutum: median area not membranous, strongly sclerotized, with light transverse microridges, with two bands of four or five small setae extending medioposteriorly from margin of prescutum; marginal areas of scutum laterad to scutellum sclerotized but not reticulated; prealare and triangular plate present; scutal apodeme present on anterior margin. Scutellum 195–200 µm wide, 75–90 µm long, with an inverted U-shaped scutellar ridge; scutellar setae: four or five hs on each side; posterior notal wing process strong. Basisternum 445–465 µm wide, 240–260 µm long, without a median ridge; bounded anteriorly by a fairly weak marginal ridge and posteriorly by strong precoxal ridges; basisternal setae in a medial line and in a broad band along marginal ridge, with a total of ~50 hs; lateropleurite fairly narrow, without a median membranous area, each without a sclerotized extension from marginal ridge; furca well developed, broadly waisted, arms very divergent and extending ~3/4 to 4/5ths to marginal ridge. Mesopostnotum and postnotal apophysis well developed. Area bounded anteriorly by scutellum and laterally and posteriorly by mesopostnotum not sclerotized. Mesepisternum not reticulated, but without setae; subepisternal ridge well developed, arising from anterior margin of lateropleurite. Postalare not reticulated anteriorly, without postalare setae. Mesothoracic spiracle: peritreme 30–34 µm wide. Postmesospiracular setae: none or one hs just posterior to each spiracle, but none medially. Tegula present, with 10–15 tegular hs on each side. Metathora: with one metatergal hs on each side. Metapostnotum small, narrow. Dorsospiracular setae possibly absent. Dorsal part of metapleural ridge present but without a suspensorial sclerite. Ventral part of metapleural ridge well developed; episternum mildly sclerotized, with one pair of hs postmetaspiracular setae on either side. Metepimeron well sclerotized, without setae. Antemetaspiracular setae absent. Metathoracic spiracle: width of peritreme 34 µm. Metasternum probably membranous, with eight or nine short anterior metasternal hs and ~7 posterior metasternal hs.

Wings. Hyaline 1860–2200 µm long, 710–850 µm wide (ratio of length to width 1 : 0.36–0.39); alar lobe present, setae absent. Hamulohalteres absent.

Legs. Metathoracic legs clearly longest. Coxae: I 165–170, II 170–180, III 175–190 µm long; coxa III with ~11 strong hs. Trochanter + femur: I 345–405, II 360–370, III 405–425 µm long; trochanter III with ~5 hs; long trochanter seta not differentiated; femur III with ~22 hs. Tibia: I 290–300, II 335–345, III 500 µm; tibia III with many spur-like setae plus 6–10 peg-like setae distally, each 7–12 µm long; apical spurs not differentiated from other spur-like setae, longest 25–35 µm long. Tarsi one-segmented (although a pseudo-articulation present on several legs): I 105–120, II 125–135, III 135–145 µm long (ratio of length of tibia III to length of tarsus III 1 : 0.28); tarsus III with several spur-like setae plus 5–10 short peg-like setae; tarsal spurs not differentiated; tarsal campaniform pore, if present, very small; tarsal digitules capitate, slightly longer than claw. Claws rather small, clearly longer than width of tarsi, with a conspicuous denticle near apex and another near base of claw; length III 33–35 µm; claw digitules capitate, longer than claw.

Abdomen. Segments I–VII: segments I and II reasonably normal but segments III–VII extremely long and narrow, so that these segments represent ~3/4 total body length; posterior margins of these segments recognisable by presence of a small group (~8–13) of hs, mainly along margins. Most segments with a fold about halfway along, where posterior part of segment telescopes into anterior part (marked by 'x' on figure). Tergites and sternites of I–VII considered absent. Caudal extensions of segment VII absent. Setae few on segments I and II, but with a fairly dense marginal band of short hs on dorsum of III, plus a sparse band ventrally; as indicated above, segments IV–VII with small groups of hs near posterior margins, rather variable in length, each 13–45 µm long. Segments IV–VII each also with a pair of internal rod-like structures, those of IV and V shorter than segment but those of VI and VII about same length as segments; each rod with very fine lines running diagonally (function unknown). Segment VIII quite short (125–130 µm long), parallel-sided, with a few pairs of inner rods, similar to those more anteriorly, with four or five hs plus three or four fs on each side. Caudal extensions, glandular pouches and glandular pouch setae absent. Genital segment: penial sheath elongate and bluntly pointed, 115–125 µm long, 70–75 µm wide at base, only slightly sclerotized. Anus visible dorsally (~20 µm wide), but functionality not confirmed (as in adult females). Ventrally, with aedeagus 70–75 µm long, 18 µm wide at apex, parallel-sided, extending as far as apex of penial sheath; basal rod apparently absent. Setae mainly marginal, with ~4–7 rather short, stout fs (mostly ~8–16 µm long), but with one to three similar fs on ventral surface anteriorly. Apex of penial sheath with a group of penial sheath sensilla.

Galls (based on 60 specimens)

Sub-spherical (mean height : diameter ratio = 1 : 1.13), but variable in shape; height 11–39 mm (mean = 23 mm), diameter

16–49 mm (mean = 26 mm) and side wall thickness 1.3–6.5 mm (mean = 2.5 mm). Gall surface smooth to roughly textured; pale cream in colour, but changing to grey or black with age.

Remarks

Females of *C. echiniformis* are most easily distinguished by the short, concave-ended dorsal button, which contrasts with the convex buttons of *C. pomiformis* and *C. campanidorsalis*. Found in sympatry with *C. pomiformis*, the galls of *C. echiniformis* lack the depression around the apical orifice typical of *C. pomiformis*. However, the shape of the dorsal button is a more reliable characteristic (when the adult female is present).

Distribution and host plants

Known from north Western Australia to 23°S, the Northern Territory as far south as 22°S, and Queensland. Host trees include *Corymbia cliftoniana*, *Co. collina*, *Co. deserticola*, *Co. dichromophloia*, *Co. drysdalensis*, *Co. erythrophloia*, *Co. hamersleyana*, *Co. intermedia* and *Co. terminalis* (only records with positive identifications included).

Cystococcus pomiformis (Froggatt)

Brachyscelis pomiformis Froggatt, 1893: 367.
Apiomorpha pomiformis (Froggatt); Cockerell, 1896: 328. Change of combination.
Cystococcus pomiformis (Froggatt); Froggatt, 1921: 156–157. Change of combination.
Ascelis pomiformis (Froggatt); Lindinger, 1957: 545. Change of combination, not accepted by subsequent authors.

In the original description of this species, Froggatt (1893: 367) listed two localities: 'Torrens Creek, N.Q., on *E*. sp. (– Chisholm); Barrier Range, King's Sound, N.W.A., on *E*. sp. (W. W. Froggatt)'. He also said that there was only a single very large gall specimen from the north Queensland locality (Torrens Creek, near Charters Towers), and that 'Only one gall contained the remains of a female; the anal segments appear to be robust and dark coloured'. Froggatt must have been referring to the sclerotized abdominal button of the adult female and it is most likely that this was not retained as it has not been found in Froggatt's collection, which is split between ANIC and ASCU. Froggatt (1893) did not designate a type. However, Froggatt (1921: 157) clearly made a subsequent type designation: 'The type specimen came from North Queensland, and was described on the gall and the remains of a female coccid as a *Brachyscelis*; ...'. Froggatt's collection has two large cut-open galls in each of ANIC and ASCU (four large galls in total; examined by PJG), but the label associated with each lot of galls refers to both northern Western Australia and northern Queensland and it seems that the galls may have been used for display purposes with the associated data probably referring to the distribution known at the time of display and not to the collection site of the galls. Thus we cannot identify the one large gall from Torrens Creek in north Queensland that Froggatt (1921) designated as the type. However, based on Froggatt's (1893, 1921) descriptions of the galls, there is no doubt as to species identity.

A slide of an adult female in the ANIC with a printed label saying 'HOLOTYPE' and two handwritten labels ('1921/ Cystococcus pomiformis Frg/Hardly full grown/Loc. Broome WA/Coll. L.J. Newman' and 'Type. Drawn') has no type status and the type label is clearly an erroneous subsequent addition.

Material examined

DNA sequence data (synapomorphic nucleotide sites mapped to the GenBank reference sequence listed)
18S: No synapomorphic sites (gene region too conserved).
COI: Reference sequence: TLS016: GenBank KP729343. Site# 56(A), 89(C), 111(T), 127(A), 177(C), 317(T), 371(C), 374(G), 392(G).

Redescription

Adult female (12/12: six fair, six good condition)

Material examined

Australia: Queensland, Northern Territory and Western Australia, on *Corymbia* spp. (Myrtaceae), (ID: TLS007, TLS016, TLS024, TLS026, TLS028, TLS031, TLS034, TLS035, TLS037, TLS041, TLS045, TLS052) (ANIC: 12/12 ♀).

Mounted material. Body up to 25 mm long and 12 mm wide. Sclerotized button 1.0–2.4 mm diameter at base, 0.9–1.9 mm long, roughly dome-shaped, ranging from broad and round-ended (Fig. 5) to angular and pointed at end, located dorsally, probably on anterior abdominal segments (similar to *C. campanidorsalis*), but exact location unknown due to lack of visible dorsal abdominal segmentation. Spiracles 170–260 µm diameter. Mouthparts of older individuals surrounded by sclerotized derm disc 0.9–3.4 mm diameter. Stylets 325–600 µm long, but often lost along with supporting aliform expansions when female removed from gall tissue.

Dorsum. Majority of cuticle with sparsely scattered, short hs, each 12.5–20 µm long. Long hs, each 37.5–55 µm long, present on abdomen posterior to dorsal button.

Venter. Majority of cuticle with sparsely scattered, short hs, each 10–15 µm long. Slightly longer hs, 12.5–17.5 µm long, and pore plates each 7.5–27.5 µm diameter with 3–36 pores (each 2–3 µm diameter), densely clumped in median, posterior half of venter. Some faint, transverse bands of sclerotization medially, between mouthparts and vulva, presumably separating abdominal segments. Pore plates and setae also clustered densely around spiracles, these pore plates each 7.5–22.5 µm in diameter with 3–26 pores (each 2–3 µm diameter).

Descriptions

First-instar male (Fig. 12) (7/13: five poor, eight good condition)

Material examined

Australia: Northern Territory, Mt Bundey (–13.20, 131.18), on *Corymbia* sp., early iv.1991, M. Horak and M. Upton (ANIC: 6/many first-instar ♂); South Australia, Amata Aboriginal Reserve, near NT border, on *Corymbia polycarpa* var. *oligocarpa*, 10.ii.1970, F. D. Morgan, specimen index number 20/72 (ANIC: 1/4 first-instar ♂).

Mounted material. Body turbinate, 420–540 µm long, 230–340 µm wide; completely membranous. Eyespot on dorsal submargin, 10–15 µm in diameter. Antennae three-segmented, 80–115 µm long, with hs 7–23 µm long on all segments, longest at apex; apical segment 40–70 µm long, with three robust fleshy setae, 15–28 µm long, plus five to six slender fleshy setae 5–13 µm long. Clypeolabral shield 90–108 µm long. Labium without segmentation, 35–48 µm long, 45–65 µm wide. Spiracles including peritreme 30–35 µm long, without pores. All legs subequal in size; trochanter + femur 74–82 µm, with femur widest (30–45 µm) in basal half; tibia and tarsus fused, 53–65 µm long; claw 18–23 µm long, with small subapical denticle; tarsal digitules capitate, 27–40 µm long, one longer and thinner than other; claw digitules capitate and subequal, 20–27 µm long. Anus visible but indistinct; functionality not confirmed (as in adult females).

Dorsum. Derm covered with microtrichia, 2–5 µm long. Margin without a fringe of setae; all hs minute 2–4 µm long, sparsely distributed on thorax and head. Tubular ducts and pores absent.

Venter. Derm covered with microtrichia 1–3 µm long. Hair-like setae mostly 2–8 µm long, a few on head and thorax 15–20 µm long, three setae marginally to submarginally on each side of each abdominal segment, and a pair of very long apical setae, probably up to 75 µm long (often broken). Tubular ducts and pores absent.

Second-instar male (Fig. 13) (4/9: all good condition)

Material examined

Australia: Northern Territory, Mt Bundey (–13.20, 131.18), on *Corymbia* sp., early iv.1991, M. Horak and M. Upton (ANIC: 3/39 s-instar ♂, six first-instar ♂ and some first-instar exuviae); South Australia, Piltardi Waterhole, no host data, viii.1962, F. D. Morgan (this appears to be an error; collector should be D. A. Maelzer), specimen index number 110/62 (ANIC: 1/10 s-instar ♂).

Mounted material. Body turbinate, 770–1380 µm long, 400–680 µm wide; completely membranous. Eyespot on dorsal submargin, 18–22 µm in diameter. Antennae three-segmented, 100–125 µm long, with hs 15–30 µm long on all segments, longest at apex; apical segment 60–75 µm long, with three to four robust fleshy setae, 30–42 µm long and mostly bifid, plus five to six slender fleshy setae 10–30 µm long. Clypeolabral shield 170–185 µm long. Labium probably two-segmented, 52–75 µm long, 70–75 µm wide. Spiracles including peritreme 40–50 µm long, without pores. All legs subequal in size; trochanter + femur 107–120 µm, with maximum width of femur 48–60 µm; tibia and tarsus fused, 80–100 µm long; claw 25–28 µm long, with small subapical denticle; tarsal digitules capitate, 35–44 µm long, one slightly longer than other; claw digitules capitate and subequal, 30–35 µm long. Anus visible but indistinct; functionality not confirmed (as in adult females).

Dorsum. Derm covered with microtrichia, 3–10 µm long. Margin without a fringe of setae; all hs short, 4–8 µm long, sparsely distributed submarginally to submedially on head, thorax and abdomen. Pore plates, each irregularly circular to oval with 3–24 'pores' and 7–20 µm in maximum width, usually present on abdominal segments I–VI and sometimes on thoracic segment III, with one to seven plates per segment and 21–34 in total. Tubular ducts and loculate pores absent.

Venter. Derm covered with microtrichia 3–10 µm long. Hair-like setae mostly 5–8 µm long on abdomen, 18–25 µm long on head and thorax, with several pairs on head between antennae and each side of venter with submedial seta per thoracic and anterior abdominal segment and one to two pairs marginally on each side of each abdominal segment; two adjacent pairs of longer apical setae, one pair 35–45 µm long and other 15–28 µm long. Tubular ducts, loculate pores and pore plates absent.

Comment. The second-instar nymphs from northern South Australia (specimen index number 110/62) have slightly more pore plates (29–34) than the nymphs from the Northern Territory locality (21–27) and most plates on the former are of a more uniform, smaller size.

Adult male (Fig. 8) (15/45: poor to very good condition, but all structures clear on at least one specimen; drawing based mainly on LGC01266 males)

Material examined

Australia: Northern Territory, Durack memorial, opposite Bullita access Rd (–15.739, 130.506), on *Corymbia greeniana* (Myrtaceae), 1.x.2009, L. G. Cook (ID: LGC01266) (ANIC: 2/2 ♂); Northern Territory, Barkly Hwy (–19.409, 134.466), on *Co. terminalis*, 23.ix.2013, M. Cosgrove (ID: TLS029) (ANIC: 5/12 ♂); Queensland, near Willum Swamp, near Weipa, on either *Corymbia nesophila* or *Co. polycarpa*, 27.viii.1980, A. G. Morton. (ANIC: 8/31 ♂).

Mounted material. Body of moderate size but with an exceptionally long abdomen (length of head, thorax + abdominal segments I–III 1.5–2.4 mm; total body length 5.4–7.0 mm). Ocular sclerite without reticulations, but extending more or less around head, with two pairs of large simple eyes. Body with very few setae, almost all hs, each 8–16 µm long; hs and fs hard to differentiate on legs and antennae, where setae mainly rather longer and stronger, although many becoming spinose and even spur-like at distal end of legs; some setae on legs peg-like, short and parallel-sided. Claws with a large denticle near apex and another near base of claw; claw and tarsal digitules capitate. Wings normal, without alar setae or pores. Hamulohalteres absent. Glandular pouches absent.

Head. Appearing rather broad in dorsoventral view but probably with a distinct posteroventral bulge for ventral simple eyes; width across ocular sclerites ~325 µm. Median crest lightly sclerotized, quite broad, not reticulated, with ~8 dorsal head hs on either side. Postoccipital ridge present, represented by a bowtie-shaped sclerotized area posterior to median crest. Mid-cranial ridge: dorsal ridge obscure or absent; ventral ridge with poorly developed lateral arms extending to each scape, and possibly with an indistinct medial ridge extending a short distance posteriorly; area laterad to vmcr not apparently sclerotized or reticulated, with 8–10 vmcr hs on either side of ridge and with a few ventral head setae in a narrow band extending posteriorly between ventral simple eyes. Genae mildly sclerotized but not reticulated, with a group of 7–10 hs genal setae on each side. Eyes: two pairs of round, rather bulging, simple eyes, subequal in size, varying from 45–60 µm wide. Ocelli distinct, not touching postocular ridge, each ~20–23 µm wide. Ocular sclerite well sclerotized but

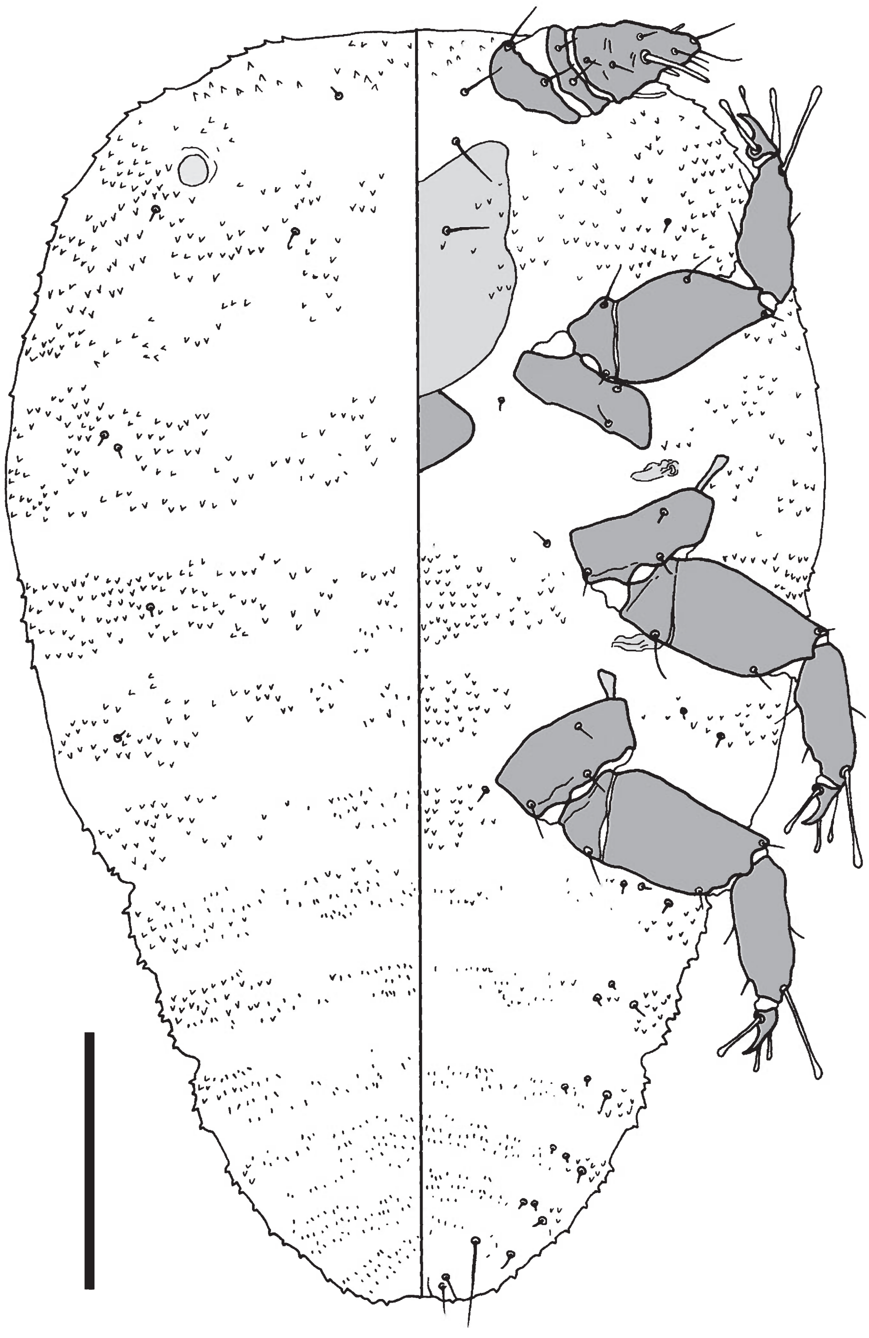

Fig. 12. *Cystococcus pomiformis* (Froggatt). First-instar male. Scale bar = 0.1 mm.

not polygonally reticulated, sclerites almost meeting ventrally, without setae. Preocular ridge absent, represented by anterior margin of ocular sclerite, without an articulation with antennae. Postocular ridge represented by posterior margin of ocular sclerite. Dorsal ocular setae absent. Preoral ridge well developed; mouth opening distinct. Cranial apophysis not detected.

Antennae. Length 495–700 µm, with segments between pedicel and apical segment apparently fused. Scape: 40–45 µm long, 65–70 µm wide, with three fs. Pedicel: length 55 µm, width 48–53 µm, with a few ridges distally, with two or three fs. Flagellar segments fused, broadest near pedicel (45–80 µm wide) narrowing gradually to apical segment (25 µm wide), with numerous broad fs (each 8–18 µm long), interspersed with longer spinose fs (each 30–40 µm long), in small areas of sclerotization, often more or less forming rings; also with up to 10 antennal bristles, some rather short, others much longer (up to 50 µm long); these becoming digitate from about halfway along segment, those nearest apical segment largest, divided into about five or six fingers. Preapical segment partially fused to previous segments, ~25–27 µm long, 27–30 µm wide, with three setae and

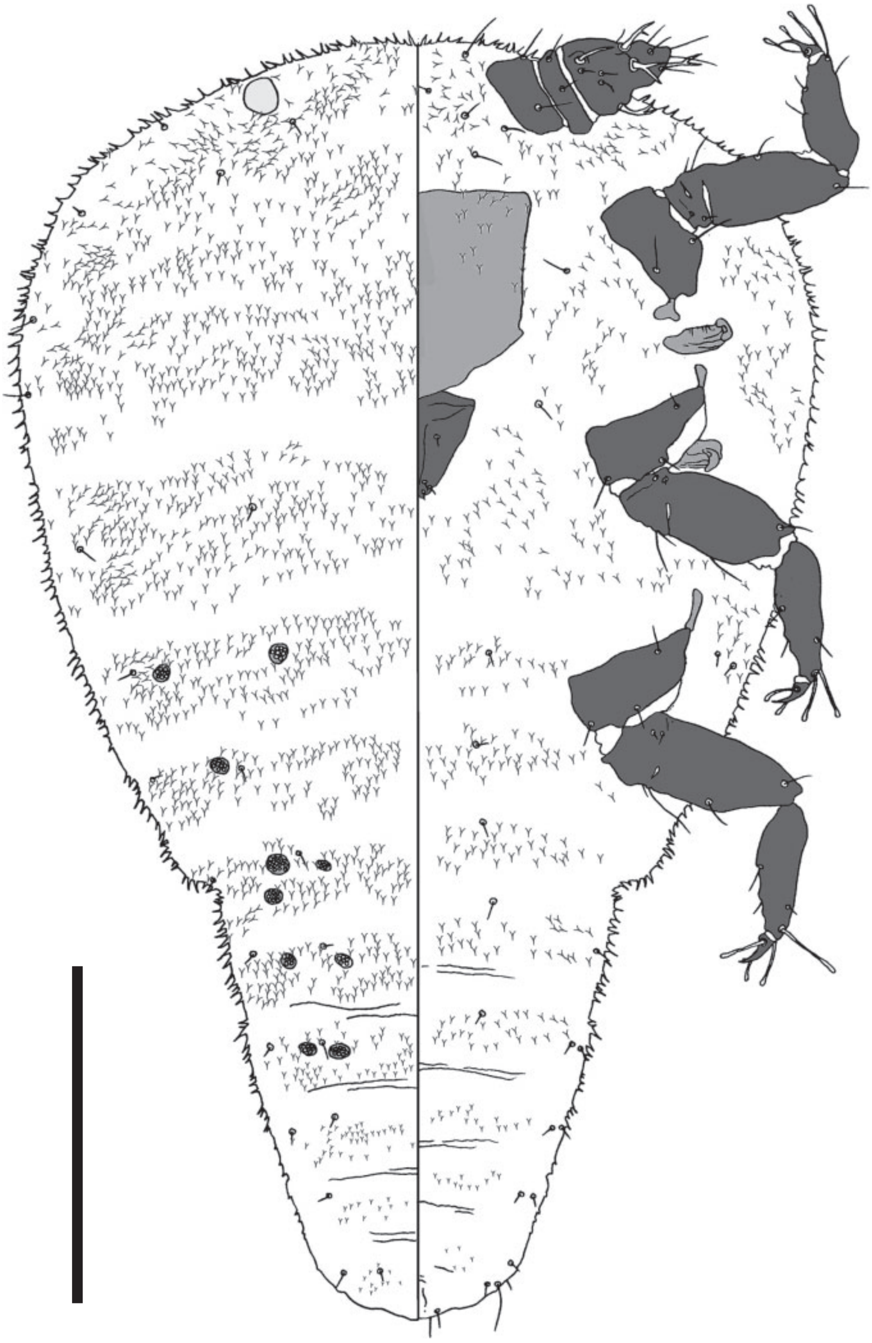

Fig. 13. *Cystococcus pomiformis* (Froggatt). Second-instar male. Scale bar = 0.3 mm.

a large digitate ab. Apical segment parallel-sided, not constricted apically, 65–70 µm long, 25–27 µm wide, with at least six capitate setae, ~4 fs, apparently no sensilla basiconica, and with four short bristles.

Thorax. Prothorax: pronotal ridge well developed, possibly fused dorsally, broadening laterally into a small, ridged lateral pronotal sclerite; pronotal ridge extending ventrally, articulating with cervical sclerite; almost all prothoracic setae absent, except no or one lateral pronotal seta. Post-tergites apparently well developed. Sternum lightly sclerotized; transverse ridge absent but with distinct sternal apophyses; median ridge absent, without either radial ridges or prosternal setae; anteprosternal and antemesospiracular setae absent. Mesothorax: prescutum ~260 µm wide, sclerotized but not nodulated; prescutal ridges present but prescutal suture absent, with four or five prescutal setae along lateral margins. Scutum: median area not membranous, strongly sclerotized, with light transverse ridging, with two bands of five or six small setae extending medioposteriorly from margin of prescutum; marginal areas of scutum laterad to scutellum sclerotized but not reticulated;

prealare and triangular plate present; scutal apodeme present on anterior margin. Scutellum 161 µm wide, 65 µm long, with an inverted U-shaped scutellar ridge; scutellar setae: four or five hs on each side; posterior notal wing process strong. Basisternum 350–375 µm wide, 195–220 µm long, without a median ridge, but bounded anteriorly by a strong marginal ridge and posteriorly by strong precoxal ridges; basisternal setae in a medial line and in a broad band along marginal ridge, with a total of ~30 hs; lateropleurite fairly narrow but with an elongate membranous area medially, each lateropleurite with a sclerotized extension from marginal ridge along entire margin; furca well developed, broadly waisted, arms very divergent and extending ~4/5ths to marginal ridge. Mesopostnotum and postnotal apophysis well developed, the latter quite deep. Area bounded anteriorly by scutellum and laterally and posteriorly by mesopostnotum not sclerotized. Mesepisternum not reticulated, but with three to five small setae; subepisternal ridge well developed, arising from anterior margin of lateropleurite. Postalare not reticulated anteriorly, without postalare setae. Mesothoracic spiracle: peritreme 30–34 µm wide. Postmesospiracular setae: none to two just posterior to each spiracle plus none or one medially. Tegula present, with 10–12 tegular hs on each side. Metathorax: with one metatergal hs on each side. Metapostnotum small, narrow. Dorsospiracular setae: ~1–4 hs on each side. Dorsal part of metapleural ridge present but without a suspensorial sclerite. Ventral part of metapleural ridge well developed; episternum mildly sclerotized, each with two postmetaspiracular hs. Metepimeron well sclerotized, without setae. Antemetaspiracular setae absent. Metathoracic spiracle: width of peritreme 34 µm. Metasternum probably membranous, with eight or nine short anterior metasternal hs and two or three posterior metasternal hs.

Wings. Rather distorted, hyaline, 1250–1900 µm long, 410–800 µm wide (ratio of length to width 1 : 0.38–0.44); alar lobe present, setae absent. Hamulohalteres absent.

Legs. Metathoracic legs clearly longest. Coxae: I 132–145, II 145, III 150–158 µm long; coxa III with ~13–17 strong setae, probably hs. Trochanter + femur: I 260, II 275–305, III 295–345 µm long; trochanter III with ~8 or 9 setae, probably hs; long trochanter seta not differentiated; femur III with ~19 or 20 hs. Tibia: I 245–253, II 273–290, III 345–410 µm; tibia III with a total of ~45 setae, mainly spur-like but a few short parallel-sided and peg-like, with a group of stout apical spurs, length 16–18 µm (poor specimen with one spur clearly bifurcated on both metathoracic legs, ~21 µm long). Tarsi one-segmented (although a pseudo-articulation present on several legs), lengths (µm): I 152–175, II 152, III 159 µm long (ratio of length of tibia III to length of tarsus III 1 : 0.41); tarsus III with ~9 setae, mainly spur-like; also with up to six short peg-like setae; tarsal spurs ~27 µm long; tarsal campaniform pore, if present, very small; tarsal digitules capitate, slightly longer than claw. Claws rather small, clearly longer than width of tarsi, with a conspicuous denticle near apex and another near base of claw; length: III 35–42 µm; claw digitules capitate, longer than claw.

Abdomen. Segments I–VII: segments I and II reasonably normal but segments III–VII extremely long and narrow, so that these segments represent ~3/4 total body length; posterior margins of these segments recognisable by presence of a small group (~8–13) of hs, mainly along margins. Most segments with a fold about halfway along, where posterior part of segment telescopes into anterior part (marked by 'x' on figure). Tergites and sternites of I–VII considered absent. Caudal extensions of segment VII absent. Setae few on segments I and II, but with a marginal band of short hs on dorsum of III, plus a sparse band ventrally; as indicated above, segments IV–VII with small groups of hs near posterior margins. Segments IV–VII each also with a pair of internal rod-like structures, those of IV and V shorter than segment but those of VI–VII about same length as segments; each rod with very fine lines running diagonally (function unknown). Segment VIII quite short (120–130 µm long), parallel-sided, and with two short inner rod-like structures, with 8–12 hs and variable numbers of fs (ranging from 2–10 to more than 30). Caudal extensions, glandular pouches and glandular pouch setae absent. Genital segment: penial sheath elongate and bluntly pointed, 114–125 µm long, 50–55 µm wide at base, only slightly sclerotized. Anus visible dorsally (~23 µm wide), but functionality not confirmed (as in adult females). Ventrally, with aedeagus 68–80 µm long, 8 µm wide at apex, parallel-sided, extending slightly past apex of penial sheath; basal rod apparently absent. Setae mainly marginal, with ~13–15 rather short, stout fs (mostly ~3–7 µm long) but with a few short hs on ventral surface anteriorly, each ~8 µm long. Apex of penial sheath with a group of penial sheath sensilla.

Comment. Adult males of this species show morphological variation between geographically separated populations, within populations, and even within individual galls. Note the variation in eye diameter, width of the antennal flagellum, density of broad fs on the flagellum, and number of fs on abdominal segment VIII.

Galls (based on 95 specimens)

Sub-spherical (mean height : diameter ratio = 1 : 1.14), but shape variable and often deformed, usually with an uneven or lumpy surface; height 10–80 mm (mean = 36.5 mm), diameter 13–90 mm (mean = 41.5 mm) and side wall thickness 4–18 mm (mean = 7 mm), usually widest at mid-point, but sometimes pear-shaped. Opening typically recessed into centre of a raised, circular lip at terminal apex. Gall surface pale and creamy in colour when insect is alive, but darkens and can become very knobbled on surface once inhabitant dies.

Remarks

Adult females of *C. pomiformis* have a convex button, varying from broad and dome-shaped to pointy and conical, but easily distinguishable from those of *C. echiniformis* (concave ended) and *C. campanidorsalis* (bell-shaped).

Distribution and host plants

Known from north Western Australia to 26°S, the Northern Territory, west Queensland to 148°E (all latitudes) and Sturt National Park in far-north-west New South Wales. Host trees include *Corymbia chippendalei*, *Co. clarksoniana*, *Co. foelscheana*, *Co. greeniana*, *Co. lenziana*, *Co. polycarpa*, *Co. ptychocarpa* and *Co. terminalis* (only records with positive identifications included).

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Table S1. Collection information for specimens of *Cystococcus* for which at least some molecular data are available

Co., *Corymbia*; N.P., National Park; NT, Northern Territory; QLD, Queensland; WA, Western Australia; (?) indicates uncertainty in *Corymbia* spp. identification; latitude and longitude stated in degrees

ID	*Cystococcus* species	Slide-mounted specimens	Date collected	Collector	Host plant	Location	Latitude	Longitude
LGC00847	*C. campanidorsalis*		26.iv.2008	LGC	*Co. trachyphloia*	Crows Nest N.P., QLD	–27.26	152.117
LGC00865	*C. campanidorsalis*		26.iv.2008	LGC	*Co. trachyphloia*	Crows Nest N.P., QLD	–27.26	152.117
LGC00886	*C. campanidorsalis*	Adult males	18.v.2008	LGC	*Co. trachyphloia*	Toohey Forest, QLD	–27.542	153.053
LGC00892	*C. campanidorsalis*	Adult female	4.v.2008	LGC	*Co. trachyphloia*	Benarkin, QLD	–26.858	152.15
LGC01227	*C. campanidorsalis*	Adult female	12.ix.2009	LGC	*Co. trachyphloia*	Burbank, QLD	–27.542	153.166
LGC01363	*C. campanidorsalis*	Nymphal stages	14.xii.2009	P. Mills	*Co. trachyphloia*	Redland Bay, QLD	–27.614	153.282
LGC01424	*C. campanidorsalis*	Adult female, pupal males	17.vi.2010	P. Mills	*Co. trachyphloia*	Redland Bay, QLD	–27.644	153.275
LGC01430	*C. campanidorsalis*	Adult and pupal males	12.vi.2010	LGC	*Co. trachyphloia*	Mt Tibrogargan, QLD	–26.93	152.936
PJM00094	*C. campanidorsalis*	Adult males	25.ii.2010	P. Mills + A. Mather	*Co. trachyphloia*	Alexandra Hills, QLD	–27.535	153.232
PJM00187	*C. campanidorsalis*	Adult female	25.xi.2010	P. Mills	*Co. trachyphloia*	Capalaba, QLD	–27.552	153.207
PJM00193	*C. campanidorsalis*	Adult female	28.xi.2010	P. Mills	*Co. trachyphloia*	Mount Cotton, QLD	–27.647	153.24
PJM00394	*C. campanidorsalis*	Nymphal stages	8.x.2011	P. Mills	*Co. trachyphloia*	Brisbane Koala Bushland, QLD	–27.571	153.164
TLS079	*C. campanidorsalis*	Adult female	19.xii.2013	TLS	*Co. trachyphloia*	Lockyer N.P., QLD	–27.452	152.23
TLS080	*C. campanidorsalis*	Adult female	19.xii.2013	TLS	*Co. trachyphloia*	Lockyer N.P., QLD	–27.452	152.23
TLS081	*C. campanidorsalis*	Adult female	19.xii.2013	TLS	*Co. trachyphloia*	Lockyer N.P., QLD	–27.452	152.23
TLS082	*C. campanidorsalis*	Adult female	19.xii.2013	TLS	*Co. trachyphloia*	Lockyer N.P., QLD	–27.452	152.23
TLS083	*C. campanidorsalis*	Adult female	19.xii.2013	TLS	*Co. trachyphloia*	Lockyer N.P., QLD	–27.454	152.251
TLS084	*C. campanidorsalis*	Adult female	19.xii.2013	TLS	*Co. trachyphloia*	Lockyer N.P., QLD	–27.475	152.29
TLS091	*C. campanidorsalis*		25.ii.2014	TLS	*Co. trachyphloia*	Kroombit Tops N.P., QLD	–24.395	151.045
TLS093	*C. campanidorsalis*		25.ii.2014	TLS	*Co. trachyphloia*	Kroombit Tops N.P., QLD	–24.439	150.993
TLS095	*C. campanidorsalis*		25.ii.2014	TLS	*Co. trachyphloia*	Kroombit Tops N.P., QLD	–24.45	150.944
LGC01787	*C. echiniformis*	Adult female	18.ix.2011	A. Thornhill	*Co. hamersleyana*	Mt Nameless, WA	–22.721	117.757
TLS002 f1	*C. echiniformis*	Adult female	19.ix.2013	TLS	*Co. terminalis*	Diamantina Development Rd, QLD	–26.641	144.807
TLS004 f1	*C. echiniformis*	Adult female	20.ix.2013	TLS	*Co. terminalis*	Diamantina Development Rd, QLD	–26.079	143.486
TLS005	*C. echiniformis*	Adult female	20.ix.2013	TLS	*Co. terminalis*	Diamantina Development Rd, QLD	–25.993	143.431
TLS006	*C. echiniformis*	Adult female	20.ix.2013	TLS	*Co. terminalis*	Thomson Development Rd, QLD	–25.325	142.656
TLS008	*C. echiniformis*	Adult and nymphal females	21.ix.2013	TLS	*Co. terminalis*	Near Cloncurry, QLD	–21.043	140.971
TLS023 f1	*C. echiniformis*	Adult female	23.ix.2013	TLS	*Co. terminalis*	Barkly Hwy, NT	–20.079	136.783
TLS025 f1	*C. echiniformis*	Adult female	23.ix.2013	TLS	*Co. terminalis*	Barkly Hwy, NT	–19.382	135.263
TLS043 f1	*C. echiniformis*	Adult female	22.ix.2013	TLS	*Co. terminalis*	Near Camooweal, QLD	–19.980	138.505
TLS070	*C. echiniformis*	Adult female	19.x.2013	TLS	*Co. terminalis*	Near Thargomindah, QLD	–27.957	143.804
LGC01266	*C. pomiformis*	Adult males	1.x.2009	LGC	*Co. greeniana*	East of Timber Creek, NT	–15.739	130.506
TLS007 f1	*C. pomiformis*	Adult female	21.ix.2013	TLS	*Co. terminalis*	Landsborough Hwy, QLD	–23.262	144.114
TLS016 f1	*C. pomiformis*	Adult female	21.ix.2013	TLS	*Co. terminalis*	Landsborough Hwy, QLD	–23.262	144.114
TLS024 f1	*C. pomiformis*	Adult female	23.ix.2013	TLS	*Co. terminalis*	Barkly Hwy, NT	–20.079	136.783
TLS026 f1	*C. pomiformis*	Adult female	23.ix.2013	TLS	*Co. terminalis*	Barkly Hwy, NT	–19.382	135.263
TLS028 f1	*C. pomiformis*	Adult female	23.ix.2013	TLS	*Co. terminalis*	Barkly Hwy, NT	–19.409	134.466
TLS029	*C. pomiformis*	Adult males	23.ix.2013	M. Cosgrove	*Co. terminalis*	Barkly Hwy, NT	–19.409	134.466
TLS031 f1	*C. pomiformis*	Adult female	24.ix.2013	TLS	*Co. terminalis*	Stuart Hwy, NT	–20.004	134.218
TLS034 f1	*C. pomiformis*	Adult female	24.ix.2013	TLS	*Co. chippendalei* (?)	Stuart Hwy, NT	–21.936	133.529
TLS035 f1	*C. pomiformis*	Adult female	24.ix.2013	TLS	*Co. chippendalei*	Stuart Hwy, NT	–21.936	133.529
TLS037 f1	*C. pomiformis*	Adult female	24.ix.2013	TLS	*Co. terminalis*	Stuart Hwy, NT	–22.804	133.419
TLS041 f1	*C. pomiformis*	Adult female	24.ix.2013	TLS	*Co. terminalis*	Stuart Hwy, NT	–23.383	133.814
TLS045 f1	*C. pomiformis*	Adult female	25.ix.2013	TLS	*Co. terminalis*	Ellery Creek Big Hole, NT	–23.779	133.073
TLS052 f1	*C. pomiformis*	Adult female	30.ix.2013	TLS	*Co. lenziana* (?)	Old Gunbarrel Hwy, WA	–25.552	126.565