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A newly recognised species of *Cryptes* Maskell 1892 (Hemiptera: Coccidae) from Western Australia

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Abstract

Cryptes utzoni Lin, Kondo & Cook **sp. n.** (Hemiptera: Coccidae) is described based on adult female morphology and DNA sequences from mitochondrial and nuclear loci. This Australian endemic species was found on the stem of *Acacia aneura* (Fabaceae) in Western Australia. All phylogenetic analyses of three independent DNA loci show that *C. utzoni* is closely related to *C. baccatus* (Maskell), the type and only species of *Cryptes* Maskell, 1892. The adult female of *C. utzoni* is described and illustrated and a table is provided of the characters that differ among adult females of the two species of *Cryptes* now recognised (*C. baccatus* and *C. utzoni*) and a morphologically similar Western Australian species, *Austrolichtensia hakearum* (Fuller). There is deep genetic divergence in *COI* among samples of *C. baccatus*, suggesting the possibility of a species complex in this taxon.

Key words: Coccomorpha, Coccoidea, wattle tick scale, Eulecaniinae, Filippiinae, *Acacia*, *COI* DNA barcode, taxonomy

Introduction

Soft scales (Hemiptera: Coccomorpha: Coccidae) are one of the most species-rich families of scale insect and are diverse on all continents except Antarctica (García Morales *et al*. 2016). Although incorporated into Hodgson's (1994) identification manual to genera, there has never been a thorough revision of the Australian soft scales and the fauna is not yet well known. However, the taxonomic database of scale insects, ScaleNet (García Morales *et al*. 2016) provides a curated checklist of species from all the published literature. Hence, by looking for available names and checking the associated literature, it is relatively easy to determine whether a new collection is an undescribed species if adult females are morphologically very distinct from described species.

During field trips of discovery funded by the Australian Biological Resources Study (Australian Government, Department of Environment and Energy) to find undescribed eriococcid scale insects (Hemiptera: Coccomorpha: Eriococcidae), numerous species of other scale insect families were also found. Some of these are currently undescribed and many have been collected at only one location. This is not out of the ordinary: in Australia, only about 25% of the insect diversity has been described (e.g., Yeates & Cassis 2017). On a trip to the Yeo Lake Nature Reserve in the Great Victoria Desert of Western Australia, an unusual and undescribed coccid (LGC02307) (Hemiptera: Coccidae) was found on a single plant of mulga (*Acacia aneura*, Fabaceae), a widespread and abundant shrubby tree of the Australian arid biome (Singh 2010). In life, adult females of the coccid (Fig. 1A) look similar to those of *Austrolichtensia hakearum* (Fuller) (Coccidae, subfamily Filippiinae) (Fig. 1B) in being partly embedded in a white wax platform but, when slide-mounted, they more closely resemble *Cryptes baccatus* (Maskell) (Coccidae, subfamily Eulecaniinae).

Cryptes baccatus (Fig. 1C), the "wattle tick scale", is the only species in the Australian endemic genus

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Cryptes. It occurs across southern Australia, including southwest Western Australia, and feeds only on *Acacia* (Farrell 1990; García Morales *et al*. 2016). All stages, including both sexes, of *C. baccatus* have been described in detail by Farrell (1990). *Austrolichtensia hakearum* (Fuller) (Fig. 1B) is also an Australian endemic species in a monotypic genus. It is restricted to southwest Western Australia (Froggatt 1915; Fuller 1897, 1899; Hodgson 1994; García Morales *et al*. 2016) where it has been reported from three genera, including *Acacia*, in different plant families. Thus, all three species (LGC02307, *C. baccatus* and *A. hakearum*) occur on *Acacia* in southwest Western Australia.

In this study, we aimed to determine whether the specimens of LGC02307 should be considered congeneric with either *A. hakearum* or *C. baccatus*. We examined morphological characters of adult females and analysed DNA sequence data from multiple loci representing both nuclear and mitochondrial genomes. Additionally, because *Austrolichtensia* and *Cryptes* are currently assigned to different subfamilies (Filippiinae and Eulecaniinae, respectively) (Hodgson 1994), our analyses also included two species of each of these in order to assess relationships among *Austrolichtensia*, *Cryptes* and LGC02307.

FIGURE 1. A. *Cryptes utzoni* **sp. n.** Adult females on twig of *Acacia aneura* (Fabaceae) from Yeo Lake Nature Reserve, Western Australia. Notice a thin layer of white wax that partially covers the insect. Photograph by L.G. Cook. **B.** An adult female of *Austrolichtensia hakearum* on the stem of *Hakea pandanicarpa* (Proteaceae) from Stirling Range National Park, Western Australia. Photograph by T.L. Semple. **C.** *Cryptes baccatus*. Young adult females (LGC03153) tended by an ant (*Leptomyrmex* sp.) on a twig of *Acacia leiocalyx* in Mt Moffatt section of Carnarvon Gorge National Park, QLD, Australia. Many of the smaller females have exit holes of parasitoids. Photograph by L.G. Cook.

Materials and methods

Species concept. Females of LGC02307 and *Cryptes baccatus* co-occur with males (Fig. 2; Farrell 1990) but, to confirm production of both male and female offspring, we stained developing embryos of LGC02307f9 with lacto-proprionic orcein, as per Cook (2001), to allow visualisation of heterochromatic bodies. These were present in most cells of some embryos and not others, indicating the presence of both male and female embryos. Because reproduction is probably obligatorily sexual, we apply the biological species concept (Mayr 1942) and consider that the existence of long-term reproductive isolation between two taxa is indicated by reciprocal monophyly across multiple nuclear genes and morphological differentiation, as per Lin *et al*. (2017b).

Taxon sampling and DNA extraction. Eight specimens of the Lake Yeo coccid, LGC02307, were available for DNA and morphological study. We sampled five populations of *C. baccatus*, including from Melbourne (a type locality) (Maskell 1891) and Western Australia, and two specimens of *Austrolichtensia hakearum* from different host plants and localities (Table 1). *Didesmococcus koreanus* Borchsenius and *Eulecanium kuwanai* (Kanda) (Table 1) were used to represent two additional samples of Eulecaniinae (Hodgson 1994). From the Filippiinae, we included *Ceronema banksiae* Maskell and *Metaceronema japonica* (Maskell) (Table 1), both of which are the type species of their respective genera (Hodgson 1994). Sequences from *Coccus hesperidum* L. were used to root the phylogenies because this species belongs to a different subfamily, the Coccinae (Hodgson 1994). According to Miller & Hodgson's (1997) cladistic study based on morphology, the Coccinae is distantly related to the Eulecaniinae and the Filippiinae.

Insects collected in the field were killed and preserved in absolute ethanol (> 99.5%) and then stored at 4°C. Genomic DNA was extracted from adult females using either a CTAB/chloroform protocol as per Lin *et al*. (2013) or an Isolate II Genomic DNA Kit (cat. no. BIO-52066, Bioline, Australia) following the manufacturer's instructions. After DNA extraction, cuticles were slide-mounted as vouchers following the protocol of Ben-Dov & Hodgson (1997). The genomic DNA is kept at The University of Queensland (LGC Laboratory).

FIGURE 2. The tests of male pupae of *Cryptes utzoni* **sp. n.** Photograph by L.G. Cook.

TABLE 1. Samples of scale insects used in this study. Abbreviations: ACT: Australian Capital Territory; AUS: Australia; CHN: China; JPN: Japan; NP: National Park; NR: Nature Reserve; NSW: New South Wales; QLD: Queensland; SF: State Forest; VIC: Victoria; WA: Western Australia.

Code	Host	Host family	Locality	Date	Collector
Cryptes utzoni **sp. n.**					
LGC02307	*Acacia aneura*	Fabaceae	Yeo Lake NR, WA, AUS	01.x.2013	L.G. Cook
Cryptes baccatus (Maskell)					
YPL00004	*Acacia covenyi*	Fabaceae	Canberra, ACT, AUS	20.viii.2008	M.D. Chrisp
YPL00257	*A. aulacocarpa*		Brisbane, QLD, AUS	01.v.2009	Y.-P. Lin
YPL00749	*Acacia* sp.		Wembley, WA, AUS	30.v.2009	M. Masuki & W.R. Black
YPL00798	*A. mearnsii*		Windellama, NSW, AUS	02.iii.2016	P.J. Gullan
LGC03026	*A. pycnantha*		Melbourne, VIC, AUS	28.iii.2016	L.G. Cook
Austrolichtensia hakearum (Fuller)					
YPL00757	*Hakea lissocarpha*	Proteaceae	Jarrahdale, WA, AUS	28.xi.2015	Y.-P. Lin
YPL00766	*H. pandanicarpa*		Stirling Range NP, WA, AUS	01.xii.2015	Y.-P. Lin
Ceronema banksiae Maskell					
YPL00431	*Banksia* sp.	Proteaceae	Glasshouse Mountains NP, QLD, AUS	13.vi.2010	Y.-P. Lin
Coccus hesperidum Linnaeus					
YPL00076	*Morus* sp.	Moraceae	Brisbane, QLD, AUS	17.xi.2008	Y.-P. Lin
Didesmococcus koreanus Borchsenius					
YPL00714	*Prunus persica*	Rosaceae	Taiyuan, Shanxi, CHN	11.iv.2014	X. Wang
Eulecanium kuwanai (Kanda)					
YPL00664	*Sophora japonica*	Fabaceae	Taiyuan, Shanxi, CHN	24.v.2014	Y.-P. Lin
Metaceronema japonica (Maskell)					
YPL00637	*Ilex crenata*	Aquifoliaceae	Nakamura, Okinosima, JPN	13.x.2013	H. Tanaka

The morphology of slide-mounted specimens was examined under a phase-contrast compound light microscope (Olympus BH-2 PH). Species identifications were based on Hodgson (1994) (*A. hakearum*, *C. banksiae*, *C. baccatus* and *M. japonica*) and Tang (1991) (*D. koreanus* and *E. kuwanai*). Morphological terms followed those used by Hodgson (1994). Except for six specimens (two of *Austrolichtensia hakearum* and four of LGC02307), which will be deposited in the Western Australian Museum (WAM), Perth, Australia, others are deposited in the Australian National Insect Collection (ANIC), Canberra, Australia.

PCR reactions, clean-up, gel purification and sequencing. Four gene regions from three independent loci representing a range of different rates of evolution were amplified: SSU (*18S* 5' region) and LSU (*28S* D2 and D3 regions) rRNA genes, *EF-1α* (nDNA), and *COI* (mtDNA). The primer pairs, Taq-polymerase (MangoTaq, cat. no. BIO-21083, Bioline, Australia), concentrations of PCR mixtures, thermocycles and volumes of template DNA for amplifying *18S*, *28S* and *EF-1α* followed Lin *et al*. (2013) (Table 2). All PCR runs included a negative control.

The PCR program from Park *et al*. (2010) was used for all amplifications of *COI* but using four different primer pairs to try to amplify the *COI* barcode region (Table 2). Firstly, we used the primer pair, PcoF1 and HCO. Subsequently, the reverse primer (HCO) was replaced by LepR1 (Hebert *et al*. 2004) and JerryR (the reverse compliment of CI-J-2183 from Simon *et al*. (1994)) if the gene region was not amplified previously. Finally, we used PcoF1 and CI-N-2568 (Ben) (Brady *et al*. 2000) to amplify a longer fragment (> 900 bp) for a specimen of *C. baccatus* (LGC03026) because it was not readily amplified with other primer pairs. The concentrations of the PCR mixture and used Taq-polymerase were the same as Lin *et al*. (2017b).

The successful PCR amplifications were checked on a 1% agarose gel following electrophoresis and visualised under UV illumination. The preparation of amplicons for sequencing including the reagents and protocols of clean up and gel purification for target bands followed Lin *et al*. (2017a). All PCR products were sequenced using Sanger sequencing by Macrogen Inc. (Republic of Korea).

TABLE 2. Primers and PCR protocols used.

Gene region	Primer	Direction	Primer sequence 5' to 3'	Annealing temperature	Alignment length (bp)	Reference
28S D2/D3	S3660	F	GAGAGTTMAASAGT ACGTGAAAC	55°C	634	Dowton & Austin 1998
	A335	R	TCGGARGGAACCAG CTACTA			Whiting *et al*. 1997
18S	2880	F	CTGGTTGATCCTGCC AGTAG	55°C	548	von Dohlen & Moran 1995
	B-	R	CCGCGGCTGCTGGC ACCAGA			von Dohlen & Moran 1995
COI	PcoF1	F	CCTTCAACTAATCAT AAAAATATYAG	45°C/51°C	579	Park *et al*. 2010
	HCO	R	TAAACTTCAGGGTG ACCAAAAAATCA			Folmer *et al*. 1994
	CI-J-2183 (Jerry)	R	CCAAAAAATCAAAA TAAATGTTG			Simon *et al*. 1994
	LepR1	R	TAAACTTCTGGATGT CCAAAAAATCA			Hebert *et al*. 2004
	CI-N-2568 (Ben)	R	GCWACWACRTAATA KGTATCATG			Brady *et al*. 2000
EF-1α	scutA_F	F	ATTGTCGCTGCTGGT ACCGGTGAATT	50°C	462	Hardy *et al*. 2008
	rcM52.6	R	GCYTCGTGGTGCATY TCSAC			Cho *et al*. 1995

Sequence editing and alignment. Sequences were edited using MEGA5 (Tamura *et al*. 2011) and then imported and aligned in Geneious 10.2.3 (http://www.geneious.com, Kearse *et al*. 2012). Sequences of the two rRNA genes (*18S* and *28S*) were aligned using MAFFT v7.308 (Katoh *et al*. 2002) with the default settings: algorithm = E-INS-I, scoring matrix = 200PAM / k=2 and gap open penalty = 1.53. Then Gblocks (http://molevol.cmima.csic.es/castresana/Gblocks_server.html, Castresana 2000) was used to eliminate poorly aligned positions under a less stringent selection that allows smaller final blocks with gap positions and less strict flanking positions. For the two protein-encoding regions (*COI* and *EF-1α*), unambiguous alignments were generated from amino acid translations. This was also used to check for the presence of stop codons. Intron-exon boundaries of *EF-1α* were detected using the GT-AG rule (Rogers & Wall 1980) and introns were excluded because they could not be unambiguously aligned across all species. The lengths of alignments used in the following analyses are listed in Table 2.

Phylogenetic analysis. Sequences of the different gene regions, except the two linked rRNA genes (*18S* and *28S*), were analysed separately and in concatenation. Two approaches that have different underlying assumptions, Maximum Parsimony (MP) and Bayesian Inference (BI), were used to estimate phylogenies. Before phylogenetic analyses, the same methods used by Lin *et al*. (2017b) were used for checking the presence of non-stationarity of base composition among taxa. Bootstraps (BS) or posterior probabilities (PP) were used to assess the support for particular nodes from each dataset, with BS $\geq$ 70 (Hillis & Bull 1993) and PP $\geq$ 0.95 (Huelsenbeck & Rannala 2004) considered to be good support.

Maximum parsimony (MP). MP trees were estimated using PAUP* 4.0b10 (Swofford 2003) with the heuristic searches. All settings including the weighting schemes for different gene regions, method of branch swapping, algorithm of tree starting, maximum number of kept trees, option of summarising MP trees and the number of bootstrap pseudo-replicates were the same as per Lin *et al*. (2017b).

Bayesian inference (BI). Bayesian analyses of all datasets were performed in MrBayes v.3.2.1 (Ronquist & Huelsenbeck 2003). Additional parameters (more partitions) might be a better fit to the data than using fewer parameters in Bayesian inference (Huelsenbeck & Rannala 2004), so we treated *18S* and *28S* as separate partitions

and partitioned two protein-coding gene regions by codon position. All DNA substitution models were specified by jModelTest (Darriba *et al*. 2012). The chosen models were: SYM (Zharkikh 1994) + I for *18S*, GTR (Tavaré 1986) + G for *28S*, GTR + I for *COI* (1st codon position), GTR + G for *COI* (2nd codon position), K2P (Kimura 1980) + I for *EF-1α* (1st and 2nd codon position) and HKY (Hasegawa *et al*. 1985) + G for *EF-1α* (3rd codon position). Each analysis comprised two independent runs (nruns = 2) of 40 million generations (ngen) with the default setting of four Markov chains (nchains = 4, three hot and one cold), temperature = 0.10 (temp = 0.1), starting from a random tree and sampling trees each 10000 generations (samplefreq = 10000).

In addition to the criteria and methods listed in Lin *et al*. (2017b), we also used the AICM comparisons (Raftery *et al*. 2007) of likelihoods to check the performance of each Bayesian analysis. The calculations of AICM and *P* values, which indicate whether any two runs were not converged, were as per Lin *et al*. (2017a). The settings for the numbers of trees discarded from the burn-in period (burnin) varied with each analysis, depending on when stationarity was reached. A maximum clade credibility topology with posterior probability values from the two runs of each analysis was found by TreeAnnotator v.1.8.3 (Drummond & Rambaut 2007) using the trees sampled post-burnin.

Results and discussion

Adult female morphology. In life, the specimens collected from Yeo Lake Nature Reserve differ from both *Austrolichtensia hakearum* and *C. baccatus* in body shape, colour and appearance of wax secreted around body margin (Fig. 1). Adult females of LGC02307 differ from those of *C. baccatus* also in where the eggs develop. In *C. baccatus*, eggs develop under the female, which remains attached to the stem: the female's body gradually shrinks centrally to form a brown hollow sphaerical shell (the "berry"). In the Yeo Lake specimens (LGC0307), the female's body is raised posteriorly from the stem as the ovisac extends and the eggs are housed within the ovisac between female and the stem. In *A. hakearum*, eggs are also housed within an ovisac between the female and the stem.

The morphology of the eight slide-mounted adult females of LGC02307 (f1 to f8) fit the generic description of *Cryptes* by Hodgson (1994) but differ from *C. baccatus* in having dorsal tubular ducts, sharply setose marginal setae, and two discal setae on each anal plate (Table 3). LGC02307 also differs from *C. baccatus marmoreus* Fuller, also off *Acacia* from Western Australia. Although the type material of this subspecies is probably lost (P. Gullan, 1990, personal communication to Yair Ben-Dov; in García Morales *et al*. 2016), and there have been no records since its original description, Fuller's (1897) description accords closely with that of *Cryptes baccatus*. Fuller (1899) stated that the females of this subspecies are white, polished and globular but differ from the typical *C. baccatus* in having smaller body size and differing in "several anatomical details". The Lake Yeo specimens also differ from *A. hakearum* in that they have no sclerotised areas on the derm near the stigmatic spines, semi-circular anal plates, reduced anal cleft, 8-segmented antennae, a claw denticle and no tibio-tarsal articulatory sclerosis (Table 3).

Molecular phylogenetics. All our sequence data are available in GenBank (Table 4). No premature stop codons were found in protein coding gene datasets. Non-stationarity among taxa was detected in the third codon position of the *COI* dataset ($P < 0.001$) and, therefore, only first and second codon positions of this gene region (386 bp) were used in phylogenetic analyses. No significant non-stationarity in base frequencies was detected in the other two datasets (*18S* + *28S* and *EF-1α*), with *P* values ranging from 0.23 to 1.00.

The sequences of all amplified gene regions from the three specimens of LGC02307 are identical. Uncorrected p-distances in *COI* between specimens of LGC02307 and *C. baccatus* ranged from 14.16%–17.1%, and to *A. hakearum* was 21.9%. There was deep divergence in *COI* (c. 6.2%) between populations of *C. baccatus* in Queensland and those from New South Wales/ACT, which warrants investigation using multi-locus nuclear data and additional populations to determine whether the current concept of *C. baccatus* represents a species complex.

MP analyses resulted in one tree of length 416 (CI = 0.82, RI = 0.79) for the *18S* + *28S*, three trees of length 438 (CI = 0.75, RI = 0.75) from the *COI*, one tree of length 351 (CI = 0.68, RI = 0.69) from the *EF-1α* and one tree of length 1225 (CI = 0.74, RI = 0.73) from the concatenated dataset. The two independent runs of all Bayesian analyses were converged after burn-ins of 10% (*18S* + *28S*) and 50% (*COI*, *EF-1α* and concatenated datasets) of generations. LGC02307 and *C. baccatus* formed a well-supported clade in phylogenies estimated from all datasets

in all analyses but the relationships between this clade and other species were uncertain (Fig. 3). Neither Eulecaniinae nor Filippiinae were supported as monophyletic in any analyses.

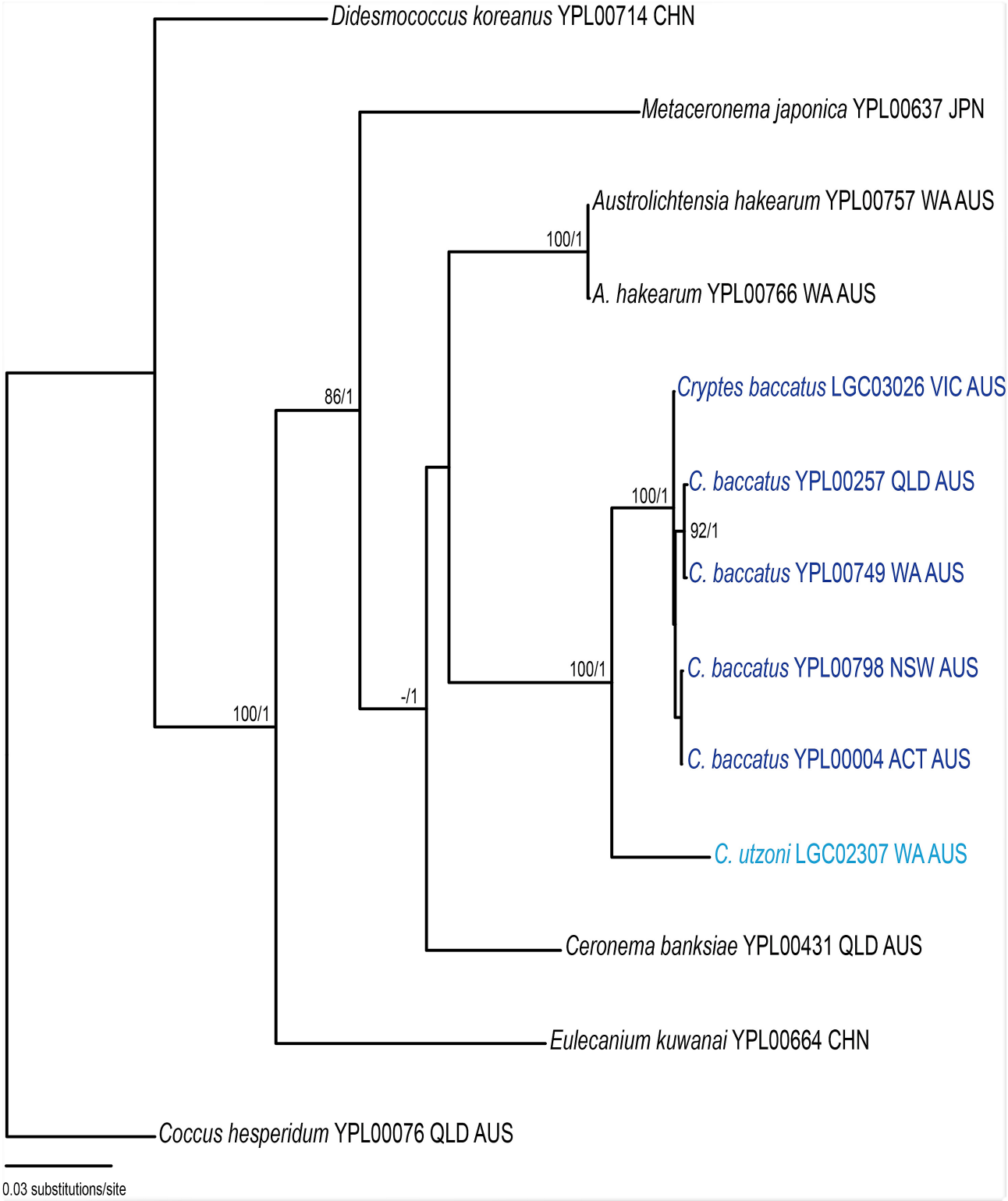

FIGURE 3. The Maximum Clade Credibility (MCC) tree from analysis of the concatenated dataset (2030 bp). The tree was rooted using sequences from *Coccus hesperidum*. Branch support is indicated on internal branches (MP bootstrap/Bayesian posterior probability). Only bootstrap values ≥ 70% and posterior probabilities ≥ 0.95 are shown. Abbreviations as per Table 1.

Considering (i) a series of fixed morphological differences (Table 3), (ii) the reciprocal monophyly of all three ingroup species in all analyses of multiple gene regions (Fig. 3), and (iii) the level of DNA differentiation between

LGC02307 and other species, we conclude that the specimens of LGC02307 represent a distinct biological species, which we describe below.

The Yeo Lake species is strongly supported as sister to *C. baccatus* rather than *A. hakearum* and so, given the similarity of adult female morphology, we place it in *Cryptes* to avoid erecting a new monotypic genus at this stage.

TABLE 3. Comparison of morphological features of adult females that differentiate *Cryptes utzoni* **sp. n.** from *C. baccatus* and *Austrolichtensia hakearum*. The descriptions and measurements of *C. baccatus* and *A. hakearum* are based on Hodgson (1994) and the specimens used in this study.

	C. utzoni **sp. n.**	*C. baccatus*	*A. hakearum*
Dorsal setae	2 sizes: (i) 8–10 μm long, scattered throughout dorsum and (ii) 20–40 μm long, restricted to submedian areas of dorsum	1 size, 10–18 μm long	1 size, 12–18 μm long
Dorsal tubular ducts	Present	Absent	Present
Marginal setae	Sharply setose, 30–55 μm long	Bluntly setose, 18–54 μm long	Sharply setose, 12–41 μm long
Sclerotised areas on derm near stigmatic spines	Absent	Absent	Present
Number of stigmatic spines	2 anteriorly and 1 posteriorly	2 anteriorly and 1 posteriorly	1 in each stigmatic area
Anal plate shape	Half-circular	Half-circular	Triangular
Number of anal plate setae	Each anal plate with 3 apical and 2 discal setae	Each anal plate with 3 apical setae	Each anal plate with 5 setae along posterior margin
Anal cleft	Presents but shallow	Presents but shallow	Reaches anal ring
Antenna	With 8 segments	With 7–9 segments	With 6 segments
Cup-shaped invaginations of ventral tubular ducts	2 sizes: (i) 5–7 μm wide, mostly present in a broad ventral marginal band and (ii) 4–5 μm wide, mostly present in near mouthparts	1 size	1 size
Tibio-tarsal articulatory sclerosis	Absent	Absent	Present
Claw denticle	Present	Present	Absent
Claw digitules	Both slender	Both slender	Alike and with broad apices

Taxonomy

Cryptes utzoni Lin, Kondo & Cook sp. n.

(Fig. 4)

urn:lsid:zoobank.org:act:8509AE5F-35CB-4546-9E78-2217ED214152

Material examined. *Holotype*. Adult female (ID: LGC02307f6). Yeo Lake Nature Reserve, WA,/ Australia. -28.08° S, 124.32° E./ ex *Acacia aneura*, 1.x.2013,/ L. G. Cook (WAM: 1 female on 1 slide). GenBank accession numbers: *18S*: MH844470; *28S*: MH886632; *COI*: MH886618; *EF-1α*: MH886635.

Paratype. Adult female (ID: LGC02307f4). Same data as for holotype (WAM: 1 female on 1 slide). GenBank accession numbers: *18S*: MH844468; *28S*: MH886630; *COI*: MH886616; *EF-1α*: MH886633.

Paratype. Adult female (ID: LGC02307f5). Same data as for holotype (ANIC: 1 female on 1 slide). GenBank accession numbers: *18S*: MH844469; *28S*: MH886631; *COI*: MH886617; *EF-1α*: MH886634.

Paratypes. Adult females (ID: LGC02307f1, LGC02307f2). Same data as for holotype (WAM: 2 females on 2 slides).

Paratypes. Adult females (ID: LGC02307f3, LGC02307f7, LGC02307f8). Same data as for holotype (ANIC: 3 females on 3 slides).

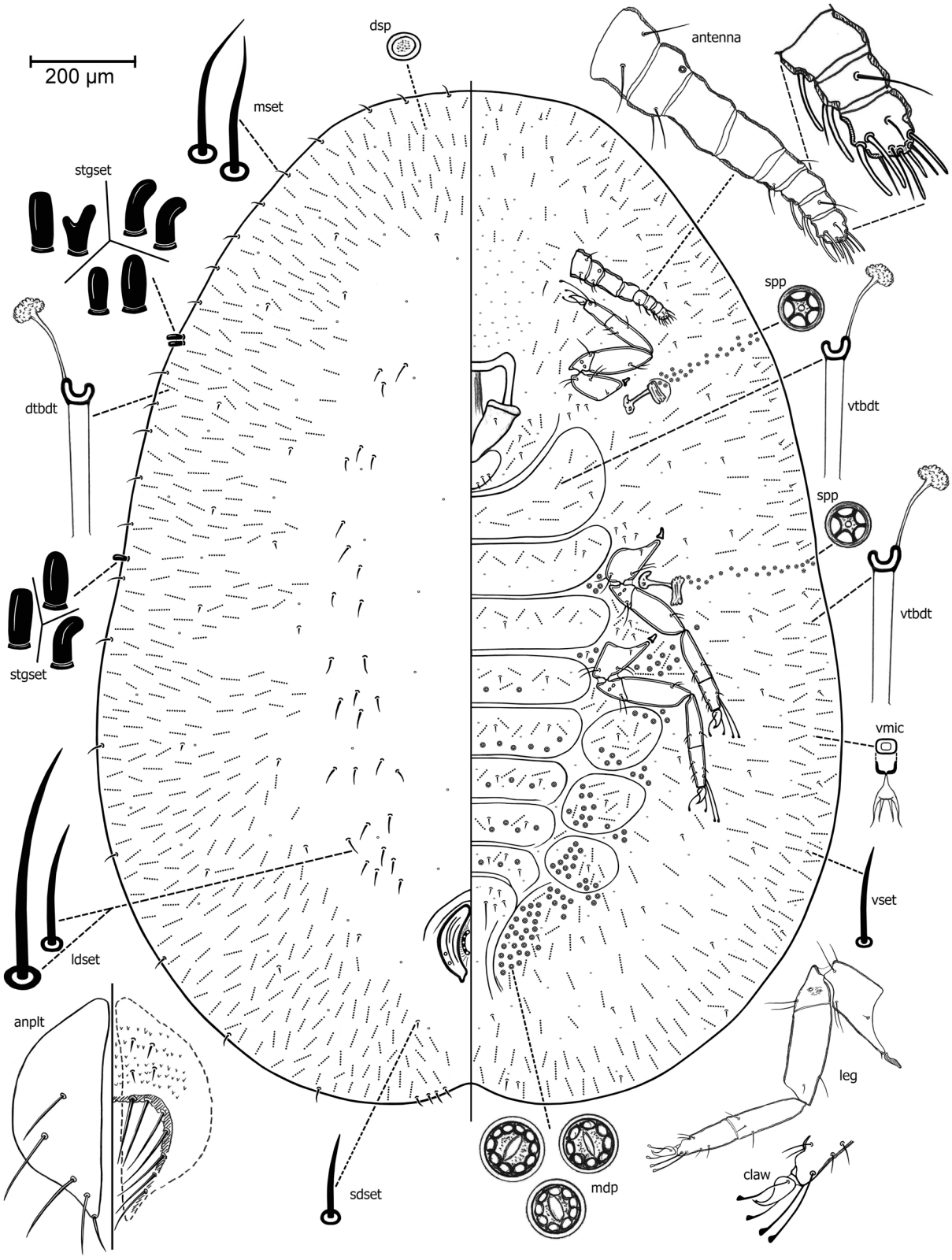

FIGURE 4. Adult female of *Cryptes utzoni* Lin, Kondo & Cook **sp. n.** dsp: dorsal simple pore; mset: marginal seta; stgset: stigmatic seta; dtbdt: dorsal tubular duct; ldset: long dorsal seta; sdset: short dorsal seta; anplt: anal plate; mdp: multilocular disc-pores; vset: ventral seta; vmic: ventral microduct; vtbdt: ventral tubular duct; spp: spiracular disc-pore.

Diagnosis. Adult females of *C. utzoni* can be identified and distinguished from *C. baccatus* and *A. hakearum* by the following combination of morphological character states (the contrasting states for the other two species are given in Table 3): (i) live adult female with a pale linear stripe with irregular black borders running longitudinally from head to area anterior to anal plates on mid dorsum; slide-mounted female with (ii) dorsal setae of two sizes; (iii) tubular ducts abundant on body margin and submargin on dorsum; on venter, present throughout more abundant near margin, but absent from anterior to mouthparts (iv) each anal plate almost half-circular in shape, appearing crescentic when open, with three apical setae and two discal setae; (v) marginal setae setose, often with slightly curved apices; (vi) sclerotised areas on derm near stigmatic spines absent; (vii) anterior stigmatic areas each with two stigmatic spines, posterior stigmatic areas each with a single stigmatic spine; (viii) anal cleft shallow; (ix) each antenna 8-segmented; (x) cup-shaped invaginations of ventral tubular ducts of two sizes; (xi) tibio-tarsal articulatory sclerosis absent; (xii) claw denticle present; and (xiii) both claw digitules slender.

Cryptes utzoni differs from *C. baccatus* at the following DNA sequence positions (mapped to the GenBank reference sequence listed for each gene). Dashes (-) represent deletions.

18S: Reference sequence: *Cryptes baccatus* (ID: LGC03026): GenBank accession number: MH844467. Site# 156 (A), 171 (T), 252 (T), 314 (T).

28S: Reference sequence: *Cryptes baccatus* (ID: LGC03026): GenBank accession number: MH886629. Site# 11 (T), 29 (G), 31–53 (TGGTCGTCGCGCTCGCGCGACGG), 55–56 (TT), 146 (C), 155 (A), 162–163 (GT), 176–193 (ACGTTTAGGCGTGCGTGG), 195 (T), 209 (A), 249 (A), 275 (G), 277–288 (TG), 280–281 (GT), 283 (A), 285 (G), 289–296 (AAAT----), 298–300 (TTA), 303–305 (CGC), 337 (C), 420 (A), 429–430 (--), 437–445 (---------), 447–450 (----), 453 (G), 456–459 (---A), 461 (A), 467 (A), 469–470 (AA), 477 (C), 485 (G), 554 (C), 572 (G), 576 (C), 598 (T), 600 (A), 604 (A), 607–608 (CT), 617 (G), 649–650 (AT), 661 (C), 677–688 (TGCTTTTCGGAG), 694–695 (CG), 701 (A).

COI: Reference sequence: *Cryptes baccatus* (ID: LGC03026): GenBank accession number: MH886615. Site# 6 (T), 9 (G), 27 (T), 32 (T), 64 (T), 66 (A), 69 (C), 90 (C), 96 (G), 108 (C), 120 (A), 126 (A), 133 (A), 135 (T), 142 (A), 159 (C), 177 (T), 207 (T), 221–223 (CAA), 227 (T), 234 (G), 241–242 (AC), 245–246 (GA), 248–249 (GA), 252 (G), 257 (A), 264 (G), 273 (A), 279 (T), 282–283 (AT), 288 (C), 300 (T), 303 (T), 306 (T), 342–343 (TA), 351 (A), 355 (T), 363 (T), 366 (G), 380–381 (GG), 387–388 (AT), 390 (G), 397 (A), 399 (T), 404 (G), 408 (G), 410 (A), 412 (A), 415–417 (TAT), 420 (T), 423–426 (AAGA), 435 (C), 438 (G), 447 (T), 453 (T), 456 (A), 459 (A), 466 (G), 477 (C), 489 (A), 492 (G), 534 (G), 543 (C), 546–547 (CT), 558 (A), 573 (C), 579 (T).

EF-1α: Reference sequence: *Cryptes baccatus* (ID: LGC03026): GenBank accession number: MH886640. Site# 15 (G), 66 (C), 87 (C), 102–103 (AC), 107 (G), 111 (C), 113 (C), 117–119 (TGG), 124 (T), 128 (A), 130–132 (TGT), 136–137 (AG), 143–144 (GT), 151 (C), 153 (T), 157 (A), 161 (C), 195 (C), 207 (C), 219 (T), 222 (A), 258 (C), 267 (C), 279 (C), 300 (T), 321 (G), 339 (C), 369 (G), 372 (T), 378–379 (TT), 399 (T), 408 (C), 423 (G), 426 (T), 451 (T), 455 (A), 458 (C), 463 (T), 467–470 (TTGT), 474–475 (GC), 483 (G), 486 (A), 490–491 (GT), 495 (C), 500 (A), 508–510 (TCT), 516 (C), 522 (C), 561 (T), 573 (T), 597 (T), 600 (T), 609 (T).

Description. Adult female (Figs 4, 1B) (drawing and measurements based on eight specimens: LGC02307f1 to LGC02307f8, all in good condition).

Unmounted specimens. Live adult female (Fig. 1B) body highly convex, truncated dorsally, yellowish to light brown in colour; with a white longitudinal stripe with irregular black border that is composed of pigments (which disappear during slide-mounting processes) running longitudinally from head to area anterior to anal plates on mid dorsum (Fig. 1); with some small, raised, irregularly-rounded black spots on the dorsum of unmounted specimens. Part of body margin covered by a thin layer of white wax, with ventral and upper part of body devoid of wax, at least on young females. All specimens were found on the stems of the host plant.

Slide-mounted specimens. Body of young adult female (Fig. 4) circular, 2.0–3.6 mm long, 1.5–3.4 mm wide.

Dorsum. Dorsum mostly membranous but sclerotised around anal plates on older specimens (not illustrated on Figure 4 as drawing was based on a young adult female). Dorsal setae setose and of 2 sizes: (i) shorter setae each 8–10 μm long, sparsely scattered throughout dorsum except absent medially from end of head to anal plates; and (ii) longer setae each 20–40 μm long, restricted to a broad band submedially on each side. Dorsal tubular ducts of 1 type, each with a cup-shaped invagination 5–7 μm wide, a broad outer ductule 25–40 μm long, a narrow inner ductule 13–20 μm long, with a well-developed terminal gland; abundant in a broad marginal to submedial band around dorsum. Dorsal pores flat, simple and ovoid, each about 3–4 μm in maximum dimension, scattered throughout dorsum. Dorsal microducts, dorsal tubercles and preopercular pores absent. Anal plates each half-

circular, 145–165 μm long, 50–65 μm wide; with 3 setae apically on each plate plus 2 discal setae, each seta 45–55 μm long. Ano-genital fold probably with 2 pairs of setae on anterior margin and 5 pairs laterally. Anal ring well sclerotised, 45–50 μm in diameter, probably bearing 5 pairs of setae, each about 125 μm long (but only 1 female, LGC02307f5, could be measured).

Margin. Marginal setae setose and often with apex slightly bent, 30–55 μm long, arranged in a single marginal row; with 11–18 setae on head between stigmatic areas, 2–5 on each side between anterior and posterior stigmatic areas, and 10–14 on each side of abdomen; marginal setae at apex of abdomen not differentiated from others. Anal cleft present, shallow. Stigmatic cleft absent; stigmatic spines each 21–30 μm long with a rounded apex, some with apex slightly bent; sometimes bifurcated in anterior stigmatic area (e.g., on specimen LGC02307f7); with 2 spines in each anterior stigmatic area and with 1 in each posterior stigmatic area. No eyespots detected.

TABLE 4. Sequences used in this study.

Species and Code	GenBank accession no. (*18S*)	GenBank accession no. (*28S*)	GenBank accession no. (*COI*)	GenBank accession no. (*EF-1α*)
Cryptes utzoni **sp. n.**				
LGC02307f4	MH844468	MH886630	MH886616	MH886633
LGC02307f5	MH844469	MH886631	MH886617	MH886634
LGC02307f6	MH844470	MH886632	MH886618	MH886635
Cryptes baccatus (Maskell)				
YPL 00004	MH844463	MH886625	MH886611	MH886636
YPL 00257	MH844464	MH886626	MH886612	MH886637
YPL 00749	MH844465	MH886627	MH886613	MH886638
YPL 00798	MH844466	MH886628	MH886614	MH886639
LGC03026	MH844467	MH886629	MH886615	MH886640
Austrolichtensia hakearum (Fuller)				
YPL00757	MH844461	MH886623	MH886609	MH886641
YPL00766	MH844462	MH886624	MH886610	MH886642
Ceronema banksiae Maskell				
YPL00431	MH844460	MH886619	MH886605	MH886643
Coccus hesperidum Linnaeus				
YPL00076	JX566902	JX627324	JX843722	JX945995
Didesmococcus koreanus Borchsenius				
YPL00714	MH844459	MH886622	MH886608	MH886646
Eulecanium kuwanai (Kanda)				
YPL00664	MH844458	MH886621	MH886607	MH886645
Metaceronema japonica (Maskell)				
YPL00637	MH844457	MH886620	MH886606	MH886644

Venter. Derm entirely membranous; segmentation visible on mid-areas of thorax and abdomen. Ventral setae setose, each 10–15 μm long, sparsely scattered across venter. Pregenital segment (VII) with a single pair of pregenital setae, each seta 23–33 μm long. Multilocular disc-pores each about 8 μm in diameter and with 8–10 loculi; abundant around genital opening, becoming progressively less frequent across preceding abdominal segments where present in irregular transverse rows, plus in submedial clusters on each abdominal and meta- and mesothoracic segments. Each stigmatic furrow with a band of spiracular disc-pores, each pore mostly with 5 loculi and about 6 μm in diameter, with 22–25 pores present between each spiracle and body margin. Ventral microducts each with an outer ductule 3 μm wide and an inner ductule that divides into 2–4 long filaments; sparsely scattered throughout venter but abundant on head between antennae and posterior to labium. Ventral tubular ducts each with a broad outer ductule 25–33 μm long, and a narrow inner ductule 13–15 μm long with a well-developed terminal

gland; ducts of two types: one with a cup-shaped invagination 5–7 μm wide, mostly present in a broad marginal to submarginal band and sparsely present in median areas of abdomen and thorax but absent from the area immediately anterior to mouthparts; and another with a cup-shaped invagination 4–5 μm wide present medially on thorax, especially near mouthparts. Spiracles well developed: anterior spiracle + peritreme 102–114 μm long, peritreme 48–66 μm wide; posterior spiracle + peritreme 108–120 μm long, peritreme 60–66 μm wide. Legs well developed; each with tibio-tarsal articulation but no articulatory sclerosis; each claw 33–36 μm long, with a denticle; both claw digitules fine and slightly shorter than thin tarsal digitules; trochanter + femur 150–180 μm and tibia + tarsus 150–180 μm. Antennae each with 8 segments, total length 210–252 μm; scape and pedicel each with about 2 setae, segments III and IV without setae, segment V with 1 short seta and 2 longer setae, segment VI with 1 fleshy seta, segment VII with 1 fleshy and 2 setose setae, and segment VIII with a pair of fleshy setae, about 4 stiff setae and 3 setose setae. Clypeolabral shield 192–210 μm long, 186–210 μm wide. Labium 66–72 μm long, 90–120 μm wide, with 3 pairs of setae.

Etymology. The species epithet honours Danish architect Jørn Utzon, who designed the UNESCO World Heritage-Listed Sydney Opera House (Sydney, Australia) and frequently used sculptural curves in his designs. In life, the adult females of *C. utzoni* (Utzon's scale) and their tests invoke the curves of the arching white shells of the Sydney Opera House.

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