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A newly recognised Australian endemic species of *Austrolecanium* Gullan & Hodgson 1998 (Hemiptera: Coccidae) from Queensland

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Abstract

Austrolecanium cryptocaryae Lin & Cook **sp. n.** is described based on adult female morphology and DNA sequences from mitochondrial and nuclear loci. This Australian endemic species was found on the underside of leaves of *Cryptocarya microneura* (Lauraceae) in Queensland. All phylogenetic analyses of four independent DNA loci and a concatenated dataset show that *A. cryptocaryae* is monophyletic and closely related to *A. sassafras* Gullan & Hodgson, the type species of *Austrolecanium* Gullan & Hodgson. The adult female of *A. cryptocaryae* is described and illustrated and a table is provided of the characters that differ among adult females of the three species of *Austrolecanium* currently recognised (*A. cappari* (Froggatt), *A. cryptocaryae* **sp. n.** and *A. sassafras*).

Key words: Coccomorpha, Coccoidea, Paralecaniini, *Cryptocarya*, *COI* DNA barcode, taxonomy

Introduction

Austrolecanium (Hemiptera: Coccidae) was erected by Gullan & Hodgson (1998) for two Australian endemic species of soft scales, *A. cappari* (Froggatt) and *A. sassafras* Gullan & Hodgson, with *A. sassafras* designated as the type species of the genus. In the published records (Froggatt 1915; Gullan & Hodgson 1998), both species were regarded as monophagous under the definition of Lin *et al*. (2015) (feeding on a single host plant family) and as restricted to New South Wales (NSW), but isolated from each other geographically and differing in their patterns of host use. *Austrolecanium cappari* was described by Froggatt (1915) (as *Lecanium cappari*) and has been collected only from *Capparis mitchellii* (Capparaceae) in inland areas of north-western NSW. Live adult females were described as dark chocolate brown with the body margins lighter in colour (Froggatt 1915). In contrast, adult females of *A. sassafras* are bright green in life and are now known to occur in wet forests of NSW and south-eastern Queensland (Gullan & Hodgson 1998; this study) on hosts belonging to two plant families: *Doryphora sassafras* (Atherospermataceae) and *Endiandra sieberi* (Lauraceae) (a new host record added in this study). The adult females and first-instar nymphs of *A. cappari* and *A. sassafras* were described and illustrated in detail by Gullan & Hodgson (1998), who placed *Austrolecanium* in the tribe Paralecaniini of the subfamily Coccinae in the Coccidae. This tribe contains at least 12 genera (Hodgson 1994), including three (*Melanesicoccus* Williams & Watson, *Neosaissetia* Tao, Wong & Chang and *Platylecanium* Cockerell) that share some morphological similarities with *Austrolecanium* (Gullan & Hodgson 1998).

Several soft scales (Fig. 1A), collected by LGC from Mt. Glorious in D'Aguilar National Park (Queensland, Australia) in 2007 and 2008, appeared very similar to *A. sassafras* but were on the leaves of *Cryptocarya microneura* (Lauraceae), which belongs to the same order (Laurales) as the hosts of *A. sassafras* (*Doryphora* and *Endiandra*). In this study, we sought to determine whether these specimens were conspecific with *A. sassafras* or represented an undescribed species of *Austrolecanium.* We examined morphological characters of adult females

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and analysed DNA sequence data from multiple loci representing both nuclear and mitochondrial genomes. Our analyses included four populations of *A. sassafras* from across its range: three from New South Wales, including one from the type locality and one (Fig. 1B) from *Endiandra sieberi* (Lauraceae), and one population from south-eastern Queensland (representing an extension of the known geographic range), to test the current concept of *A. sassafras* and assess the relationships of the population from *Cryptocarya*.

Materials and methods

Species concept. Here, we apply the biological species concept (Mayr 1942) because our study organisms probably reproduce sexually: males have been reported for *A. cappari* (Froggatt 1915) and *A. sassafras* (Gullan & Hodgson 1998). We consider evidence of lack of recent gene flow (reciprocal monophyly across multiple nuclear genes, and morphological differentiation) to indicate that there has been long-term reproductive isolation.

Taxon sampling, morphological assessment and DNA extraction. Four specimens of the putative new species of *Austrolecanium* from *Cryptocarya* were available for DNA extraction and morphological study. Ideally, both the previously described species of *Austrolecanium* should be sampled to assess the relationships of the newly discovered population, but no material of *A. cappari* was available for molecular studies. We obtained specimens for DNA extraction from four populations of the type species of the genus, *A. sassafras* (Table 1). Among them, one specimen (coccid4) was from the type locality, but not the type collection (Table 1). Other members of Paralecaniini were represented as outgroups in the molecular analyses by specimens of three genera (*Neosaissetia*, *Paralecanium* and *Platylecanium*), including the type species of the tribe, *Paralecanium frenchii* (Maskell) (Hodgson 1994) (Table 1). Sequences from *Coccus hesperidum* Linnaeus were used to root phylogenies because that species represents a different tribe, Coccini (Hodgson 1994). Coccini, together with Ceroplastinae, Pulvinariini and Saissetiini, formed a clade sister to Paralecaniini in Miller & Hodgson's (1997) cladistic study based on morphology.

Although no material was available for DNA analysis, we examined three slide-mounted females of *A. cappari*, each on a separate slide, collected from Nyngan (NSW, Australia) on *Capparis*, 11.xi.1921 (collection number: WWF 1061) deposited in the ANIC (Australian National Insect Collection, Canberra, Australia). These three specimens had been included in the description of the adult female by Gullan & Hodgson (1998).

TABLE 1. Samples of Coccidae used in this study. Abbreviations: AUS (Australia); MYS (Malaysia); NP (National Park); NSW (New South Wales); QLD (Queensland); SF (State Forest); TWN (Taiwan).

Code	Host	Host family	Locality	Date	Collector
Austrolecanium cryptocaryae **sp. n.**					
LGC00727	*Cryptocarya microneura*	Lauraceae	D'Aguilar NP, QLD, AUS	28.iv.2007	L.G. Cook
LGC00831	*C. microneura*		D'Aguilar NP, QLD, AUS	15.iii.2008	L.G. Cook
Austrolecanium sassafras Gullan & Hodgson					
coccid4	*Doryphora sassafras*	Atherospermataceae	Yadboro SF, NSW, AUS	15.iii.1998	P.J. Gullan & C.J. Hodgson
LGC00551	*D. sassafras*		Dorrigo NP, NSW, AUS	19.ii.2006	L.G. Cook
LGC02279	*D. sassafras*		Lamington NP, QLD, AUS	7.viii.2013	L.G. Cook
YPL00691	*Endiandra sieberi*	Lauraceae	Bombah Point, NSW, AUS	5.xi.2014	Y.-P. Lin
Paralecanium frenchii (Maskell)					
YPL00278	*Banksia integrifolia*	Proteaceae	Brisbane, OLD, AUS	7.viii.2009	Y.-P. Lin
Neosaissetia tropicalis Tao & Wong					
YPL00296	*Palaquium formosanum*	Sapotaceae	Pingtung County, TWN	25.viii.2009	Y.-P. Lin
Platylecanium sp.					
YPL00463	*Syzygium* sp.	Myrtaceae	Kuala Lumpur, MYS	13.xii.2010	Y.-P. Lin
Coccus hesperidum Linnaeus					
YPL00076	*Morus* sp.	Moraceae	Brisbane, QLD, AUS	17.xi.2008	Y.-P. Lin

Insects collected in the field were killed and preserved in 100% ethanol, and stored at 4°C. Genomic DNA was extracted from young adult females using a CTAB/chloroform protocol or a DNeasy Blood & Tissue kit (cat. no. 69504, Qiagen, Hilden, Germany). The protocols for both methods, which allow non-destructive extraction, have been described in detail in Lin *et al*. (2013). After DNA extraction, the cuticle of each specimen was slide-mounted as a voucher following the protocol of Ben-Dov & Hodgson (1997). The genomic DNA will be maintained at The University of Queensland (LGC Laboratory) and all slides will be deposited in the ANIC except for one specimen of *Austrolecanium* from *Cryptocarya* (LGC00831F2), which will be deposited in the Queensland Museum Insect Collection in Brisbane (QM).

All slide-mounted specimens were examined using a phase-contrast light microscope (Olympus BH-2 PH). The species identifications of *A. sassafras* were based on Gullan & Hodgson (1998). The four outgroup taxa from different genera were identified using the descriptions of Hodgson (1994) (*C. hesperidum*, *Neosaissetia tropicalis* Tao & Wong and *P. frenchii*) or Williams & Watson (1990) (*Platylecanium* sp.). Morphological terms follow those used by Hodgson (1994) and Gullan & Hodgson (1998).

We have registered the new species 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.

PCR reactions, clean-up, gel purification and cloning. Five genes from four independent loci and representing a range of different rates of evolution were amplified: SSU (*18S* 5' region) and LSU (*28S* D2 and D3 regions) nrRNA genes, *EF-1α* (nDNA), *wingless* (nDNA) and *COI* (mtDNA). We followed the same PCR conditions for amplifying *18S*, *28S*, *EF-1α* and *wingless* as Lin *et al*. (2013) (Table 2), and included a negative control for all reactions.

TABLE 2. Primers and PCR protocols used.

Gene region	Primer	Direction	Primer sequence 5' to 3'	Annealing temperature	Reference
28S D2/D3	S3660	F	GAGAGTTMAASAGTACGTGAAAC	55°C	Dowton & Austin 1998
	A335	R	TCGGARGGAACCAGCTACTA		Whiting *et al*. 1997
18S	2880	F	CTGGTTGATCCTGCCAGTAG	55°C	von Dohlen & Moran 1995
	B-	R	CCGCGGCTGCTGGCACCAGA		von Dohlen & Moran 1995
COI	PcoF1	F	CCTTCAACTAATCATAAAAATATYAG	45°C/51°C	Park *et al*. 2010
	HCO	R	TAAACTTCAGGGTGACCAAAAAATCA		Folmer *et al*. 1994
	LepR1	R	TAAACTTCTGGATGTCCAAAAAATCA		Hebert *et al*. 2004
EF-1α	scutA_F	F	ATTGTCGCTGCTGGTACCGGTGAATT	50°C	Hardy *et al*. 2008
	rcM52.6	R	GCYTCGTGGTGCATYTCSAC		Cho *et al*. 1995
wingless	scale_wg_F	F	CTGGTTCGTGCACGACGMGRACSTGYT GGATG	55°C	Hardy *et al*. 2008
	LEPWG2	R	ACTICGCARCACCARTGGAATGTRCA		Brower & DeSalle 1998

The PCR program from Park *et al*. (2010) was used for all amplifications of *COI*, but using two different primer pairs to try to amplify the *COI* barcode region (Table 2). Firstly, we used the primer pair PcoF1 and HCO. If the gene region was not able to be amplified, then the reverse primer (HCO) was replaced by LepR1 (Hebert *et al*. 2004). The PCR mixture consisted of 3 μL 5x PCR buffer A (including $MgCl_2$), 3 μL 5x Enhancer 1, 1.2 μL dNTP (2mM), 0.3 μL of each forward and reverse primer (10 μM), 0.1 μL (0.5 U) KAPA2G Robust Taq-polymerase (cat. no. KK5023, Kapa Biosystems, USA), 2 μL of template and 5.1 μL ddH_2O (UltraPure™ DNAse/RNAse-Free Distilled Water, cat. no. 10977, Invitrogen, Australia).

The successful PCR amplifications were checked by running samples on a 1% agarose gel. The clean-up and gel purification of successfully amplified PCR products for sequencing followed the protocols of Lin *et al*. (2013). All PCR products were sequenced in the forward direction using Sanger sequencing by Macrogen Inc. (Republic of Korea).

For one sample, LGC00551, multiple copies of similar length were amplified for *EF-1α*. Copies were isolated following the cloning protocols described in Lin *et al*. (2013). The presence of the insertion of the target fragment in the vector was checked by PCR using 1 μL of the bacterial/plasmid solution and a universal primer pair, M13F (5'- GTAAAACGACGGCCAGT -3') and M13R (5'- GCGGATAACAATTTCACACAGG -3'). The PCR thermocycling was done with an initial denaturation of 3 min at 95°C, 35 cycles of 30 s at 95°C; 30 s at 47°C; 30 s at 72°C and a final extension of 10 min at 72°C. Ten clones that contained the target DNA fragments were sequenced at Macrogen with the universal primer, T7 Promoter (5'- TAATACGACTCACTATAGGG -3').

Sequence editing and alignment. Sequences were edited using MEGA5 (Tamura *et al*. 2011) and then imported and aligned manually in Se-Al v.2.0 (Rambaut 1998). Translation to amino acids was used to generate unambiguous alignments for the protein-coding genes (*COI*, *EF-1α* and *wingless*), which were also checked for the presence of stop codons. Intron-exon boundaries of *EF-1α* were detected using the GT-AG rule (Rogers & Wall 1980). All copies of *EF-1α* obtained from cloning were included in our analyses but introns were excluded because they could not be unambiguously aligned across all species. The lengths of *18S*, *28S*, *COI*, *EF-1α* and *wingless* alignments used in the following analyses were 562 bp, 698 bp, 579 bp, 462 bp and 321 bp respectively.

Phylogenetic analysis. Sequences of the different gene regions were analysed separately, except for the two nuclear rRNA genes (*18S* and *28S*) that are closely linked and were treated as a single locus, and as a concatenated dataset. Base composition was checked for bias among taxa using PAUP* 4.0b10 (Swofford 2003) because non-stationarity violates the assumptions of most methods of phylogeny estimation. Each codon position for the three protein-coding regions was tested separately and we also repeated all tests with invariant sites excluded. Two methods, maximum parsimony (MP) and Bayesian inference (BI), were used to estimate phylogenies because they have different underlying assumptions. The support for nodes from each dataset was assessed by bootstrapping (BS) and posterior probabilities (PP), with BS $\geq$ 70 (Hillis & Bull 1993) and PP $\geq$ 0.95 (Huelsenbeck & Rannala 2004) considered to be good support. Support values were also used to assess congruence among analyses of different gene partitions.

Maximum parsimony (MP). MP trees were estimated using PAUP* 4.0b10 (Swofford, 2003) with the heuristic searches applying TBR branch swapping, 1000 random sequence addition starting trees and no maxtrees restrictions. All sites were weighted equally for the rRNA genes. For the protein coding genes, *COI*, *EF-1α* and *wingless*, a weighting scheme for the three codon positions (first: second: third = 2: 3: 1) was applied. For each analysis, the strict consensus option was used to summarise MP trees and 1000 bootstrap pseudoreplicates were performed to assess the support for each node using the same parameters for the heuristic searches.

Bayesian inference (BI). The GTR (Tavaré 1986) + I (nst = 6, rates = propinv) model, specified by jModelTest (Darriba *et al*. 2012), was selected for all data partitions (each gene region but with *18S* and *28S* treated as a single partition). Each analysis comprised two independent runs (nruns = 2) of 30 million (*18S* + *28S*), 30 million (*COI*), 10 million (*EF-1α*), 10 million (*wingless*) or 30 million (concatenated) generations with the default settings of four Markov chains, temperature = 0.10, starting from a random tree and sampling trees each 1000 generations. All Bayesian analyses were run using MrBayes v.3.2.1.

The performance of each pair of runs was checked by examining the average standard deviation of split frequencies (should be less than 0.01) (Pedersen 2007), PSRF values (should be close to 1.00, shown in the output files of MrBayes v.3.2.1) (Ronquist & Huelsenbeck 2003), the absolute value of the difference between the harmonic means of the two runs (should be less than 2) (Kass & Raftery 1995) and the ESS (Effective Sample Size, should be > 200) (Drummond & Rambaut 2006) of each parameter shown in the output files of MrBayes v.3.2.1. The settings for the numbers of trees discarded from the burn-in period varied with each analysis, depending on when stationarity was reached. A maximum clade credibility tree with posterior probability values from the two runs of each analysis was computed by TreeAnnotator v.1.8.3 (Drummond & Rambaut 2007) using the trees sampled post-burnin.

Results and discussion

Adult female morphology. The morphologies of the four slide-mounted adult females (LGC00727f1, LGC00727f2, LGC00831f1 and LGC00831f2) collected from *Cryptocarya microneura* fit the descriptions of *Austrolecanium* by Gullan & Hodgson (1998) but differ from the two currently named species, *A. cappari* and *A. sassafras*. The morphological differences among the three species are listed in Table 3.

TABLE 3. Comparison of morphological features of adult females that differentiate the four specimens of *Austrolecanium cryptocaryae* **sp. n.** collected on *Cryptocarya microneura* in Queensland (QLD specimens) from specimens of *Austrolecanium sassafras* and *A. cappari*. The descriptions and measurements of *A. cappari* are based on Gullan & Hodgson (1998).

	A. cryptocaryae **sp. n.**	*A. sassafras*	*A. cappari*
Dorsal setae	Sharply spinose, 5–12 μm, long	Flagellate, 15–30 μm long	Bluntly spinose, 7–13 μm long
Marginal setae	Bluntly spinose, 11–29 μm long	Sharply spinose, 12–20 μm long	Bluntly spinose, 17–33 μm long
Shape of stigmatic cleft	Mushroom-shaped	Mushroom-shaped	Triangular
Number of stigmatic spines	6–12	6–12	2 (rarely 1, 3 or 4)
Anal plate length	294–354 μm	200–240 μm	153–172 μm
Anal plate setae	3 apical setae and 1 seta on inner margin of each anal plate	2 apical setae and 2 setae on posterior margin of each anal plate	2 apical setae and 1 seta on both inner and posterior margin of each anal plate
Anal ring width	96–116 μm	70–83 μm	60–73 μm
Anal ring setae	4 pairs, each 342–432 μm long	4 pairs, each 225–275 μm long	3 pairs, each 175–250 μm long
Anogenital fold setae	4, 23–30 μm long	4, 35–80 μm long	2, 15–25 μm long
Setae on apex of each supporting bar of anogenital fold	4, 50–68 μm long	6–10, 40–75 μm long	1, 12–15 μm long
Distribution of dorsal microducts	Along body margin	Evenly across dorsum	Evenly across dorsum
Body shape and colour in life	Symmetrical and bright green	Usually asymmetrical (when near mid vein) and bright green with yellow patches near body margin when mature	Usually asymmetrical and dark brown with lighter margins

Molecular phylogenetics. All sequence data are available in GenBank (Table 4). Base composition bias among taxa (non-stationarity) was detected in the third codon position of the *COI* dataset ($P < 0.001$). Therefore, only first and second codon positions of this gene region (386 bp) were used in phylogenetic analyses. There was no non-stationarity detected in the other three datasets (*18S+28S*, *EF-1α* and *wingless*), with *P* values ranging from 0.14 to 1.00.

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*)	GenBank accession no. (*wingless*)
Austrolecanium cryptocaryae **sp. n.**					
LGC00727f1	KY816390	KY816399	KY808493	KY824058	KY798545
LGC00727f2	KY816391	KY816400	KY808494	KY824059	KY798546
LGC00831f1	KY816392	KY816401	KY808495	KY824060	KY798547
LGC00831f2	KY816393	KY816402	KY808496	KY824061	KY798548
Austrolecanium sassafras Gullan & Hodgson					
coccid4	KY816394	KY816403	KY808497	KY824062	KY798543
LGC00551	KY816395	KY816404	KY808498	KY824063	KY798542
				KY824064	
LGC02279	KY816396	KY816405	KY808499	KY824065	KY798541
				KY824066	
YPL00691	KY816397	KY816406	KY808500	KY824067	KY798544
Paralecanium frenchii (Maskell)					
YPL00278	JX866684	JX866696	JX853910	JX965103	KY798539
Neosaissetia tropicalis Tao & Wong					
YPL00296	JX866685	JX866697	JX853911	JX965104	KY798538
Platylecanium sp.					
YPL00463	KY816398	KY816407	KY808501	KY824068	KY798540
Coccus hesperidum Linnaeus					
YPL00076	JX566902	JX627324	JX843722	JX945995	KY798537

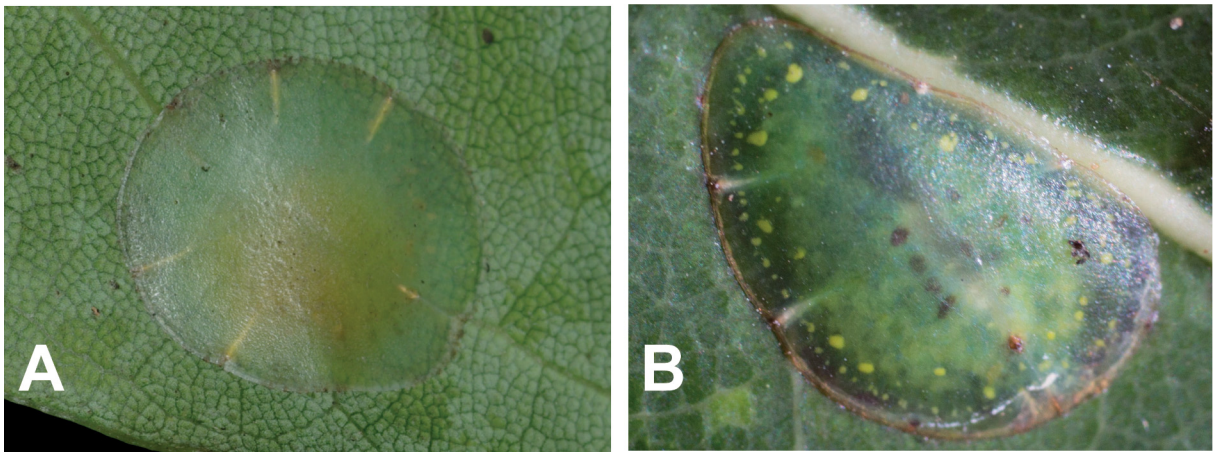

FIGURE 1. A. An adult female of *Austrolecanium cryptocaryae* **sp. n.** on a leaf of *Cryptocarya microneura* (Lauraceae) in Queensland, Australia. Photo by L.G. Cook. **B.** An adult female of *Austrolecanium sassafras* on a leaf of *Endiandra sieberi* (Lauraceae) from New South Wales, Australia. Photo by T.L. Semple.

The sequences of all amplified gene regions from the two specimens of *A. cryptocaryae* **sp. n.** are identical. *Austrolecanium cryptocaryae* **sp. n.** differed from *A. sassafras* by 24.1%–25.5% in *COI* (uncorrected p distance). There was sequence divergence in *COI* among populations of *A. sassafras* collected from different localities ranging up to 4.3%, with genetic distance being related to geographic distance between samples, i.e., the southern-most and northern-most individuals were the most divergent from each other. This pattern was not replicated in the nuclear genes, which showed varying relationships among alleles from the four populations. The genetic variation among populations of *A. sassafras* warrants investigation to determine whether the incongruence between

geographical patterns of mitochondrial and nuclear loci is the result of incomplete lineage sorting, in which case the populations might represent distinct species, or is the result of recent or ongoing gene flow (in which case the populations would represent a single species).

MP analyses resulted in one tree of length 296 (CI = 0.90, RI = 0.85) for the *18S+28S*, one tree of length 397 (CI = 0.82, RI = 0.82) from the *COI*, one tree of length 221 (CI = 0.83, RI = 0.78) from the *EF-1α*, one tree of length 203 (CI = 0.83, RI = 0.79) from the *wingless* and two trees of length 1123 (CI = 0.84, RI = 0.82) from the concatenated dataset. The two independent runs of all Bayesian analyses converged after burn-ins of 66% (*18S+28S*), 50% (*wingless*), 33% (*COI* and concatenated) and 10% (*EF-1α* dataset) of generations.

Austrolecanium cryptocaryae **sp. n.** and *A. sassafras* formed a well-supported clade in phylogenies estimated from rDNA, *EF-1α* and *wingless* (Fig. 2) and the concatenated (Fig. 3) datasets. Although *A. cryptocaryae* **sp. n.** and *A. sassafras* were each strongly supported as monophyletic in analyses of *COI* (Fig. 2; Fig. 3), the pair formed a clade in the Bayesian analysis of the *COI* dataset (Fig. 2B) but with a PP value of only 0.55.

Considering (i) a series of fixed morphological differences (Table 3), (ii) its reciprocal monophyly in analyses of multiple gene regions, (iii) the level of DNA differentiation between it and other species, and (iv) different host plant use, we conclude that *A. cryptocaryae* **sp. n.** clearly represents a distinct biological species, which we describe below.

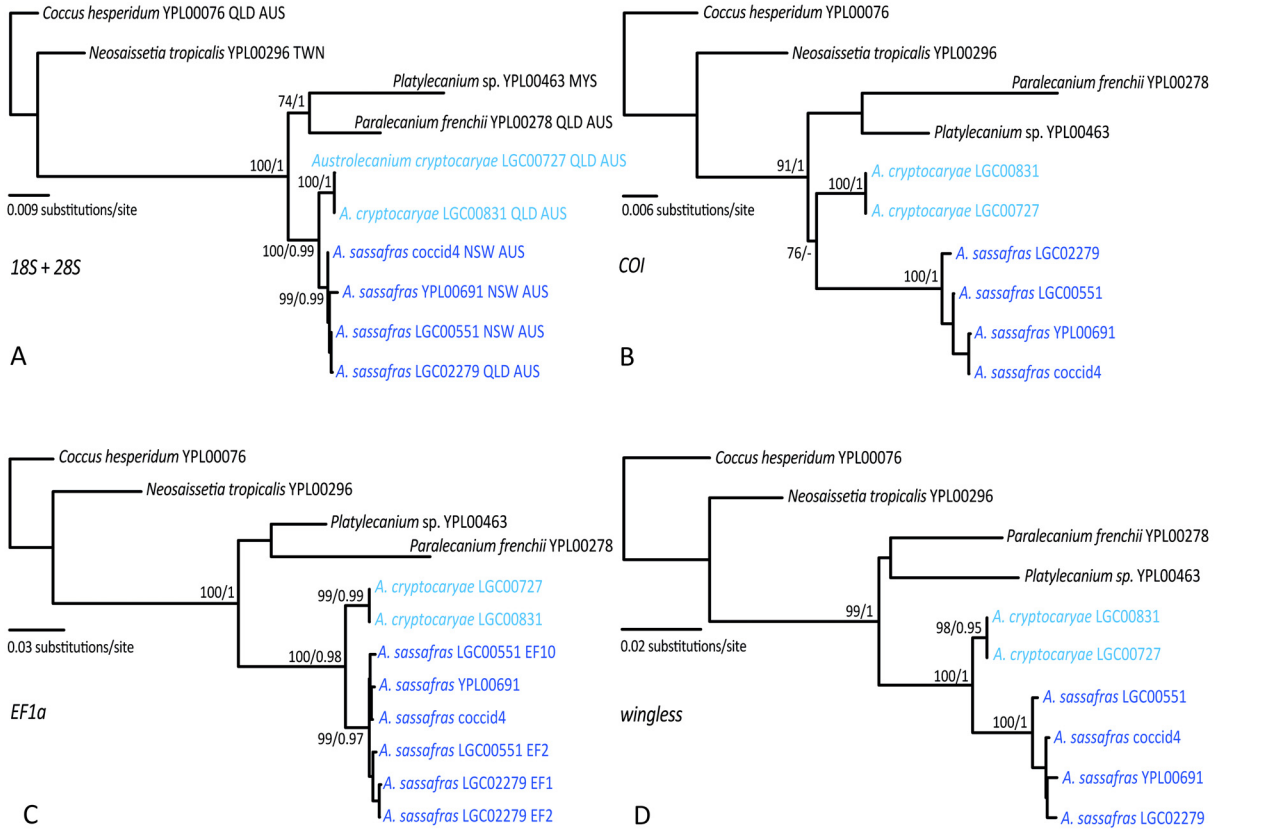

FIGURE 2. The Maximum Clade Credibility (MCC) trees from MrBayes analyses of four datasets: **A.** rDNA (*18S+28S*), **B.** *COI*, **C.** *EF-1α* and **D.** *wingless*. The tree was rooted using sequences from *Coccus hesperidum*. Branch support is indicated on internal branches (MP bootstrap/Bayesian posterior probability). Only bootstrap values ≥ 70% and posterior probabilities ≥ 0.95 are shown.

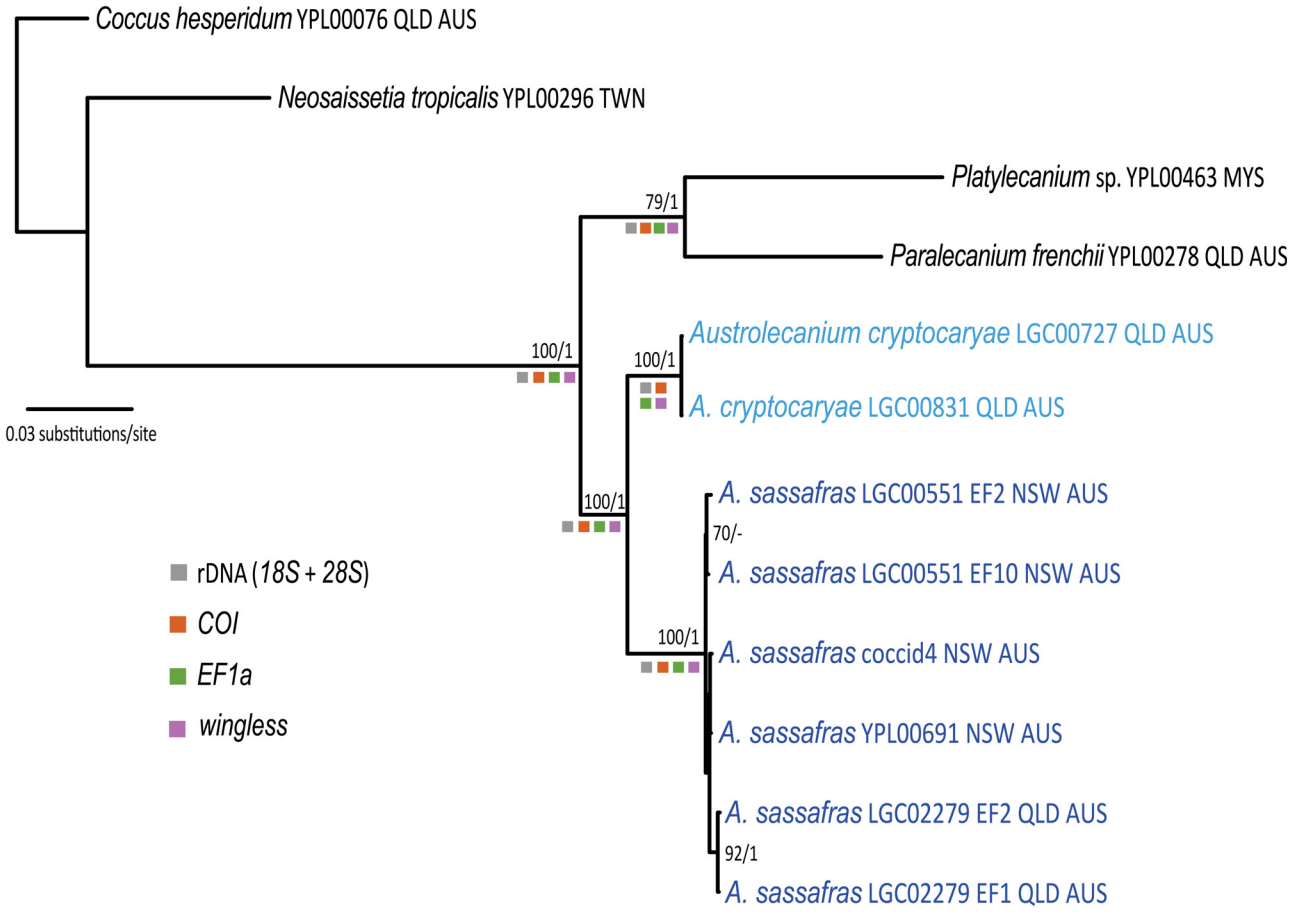

FIGURE 3. The Maximum Clade Credibility (MCC) tree from analysis of the concatenated dataset (2429 bp). The tree was rooted using sequences from *Coccus hesperidum*. Branch support is indicated on internal branches (MP bootstrap/Bayesian posterior probability). Only bootstrap values ≥ 70% and posterior probabilities ≥ 0.95 are shown. The coloured squares under branches indicate that the branch was present in analyses of that gene. Abbreviations as per Table 1.

Taxonomy

***Austrolecanium cryptocaryae* Lin & Cook sp. n.**
(Fig 4)
urn:lsid:zoobank.org:act:C52B76D7-35E0-4767-92EA-E2978C484BEB

Material examined. *Holotype*. Adult female (ID: LGC00727f2). Australia: Mt Glorious, D'Aguilar National Park, Queensland, -27.33° S, 152.75° E, on *Cryptocarya microneura*, 28.iv.2007, L. G. Cook (ANIC: 1/1 female). GenBank accession numbers: *18S*: KY816391; *28S*: KY816400; *COI*: KY808494; *EF-1α*: KY824059; *wingless*: KY798546.

Paratype. Adult female (ID: LGC00727f1). Same data as for holotype (ANIC: 1/1 female). GenBank accession numbers: *18S*: KY816390; *28S*: KY816399; *COI*: KY808493; *EF-1α*: KY824058; *wingless*: KY798545.

Paratype. Adult female (ID: LGC00831f1). Australia: Mt Glorious, D'Aguilar National Park, Queensland, -27.33° S, 152.75° E, on *C. microneura*, 15.iii.2008, L. G. Cook (ANIC: 1/1 female). GenBank accession numbers: *18S*: KY816392; *28S*: KY816401; *COI*: KY808495; *EF-1α*: KY824060; *wingless*: KY798547.

Paratype. Adult female (ID: LGC00831f2). Same data as for paratype LGC00831f1 (ANIC: 1/1 female). GenBank accession numbers: *18S*: KY816393; *28S*: KY816402; *COI*: KY808496; *EF-1α*: KY824061; *wingless*: KY798548.

Diagnosis. Adult females of *A. cryptocaryae* can be identified and distinguished from *A. sassafras* and *A. cappari* by the following combination of morphological character states (the contrasting states for the other two

species are given in Table 3); (i) dorsal setae sharply spinose; (ii) dorsal microducts present along body margin; (iii) marginal setae bluntly spinose; (iv) each anal plate with three apical setae and one seta on inner margin; (v) each supporting bar of anal plate with four setae; and (vi) anal ring with four pairs of setae, each longer than 300 μm.

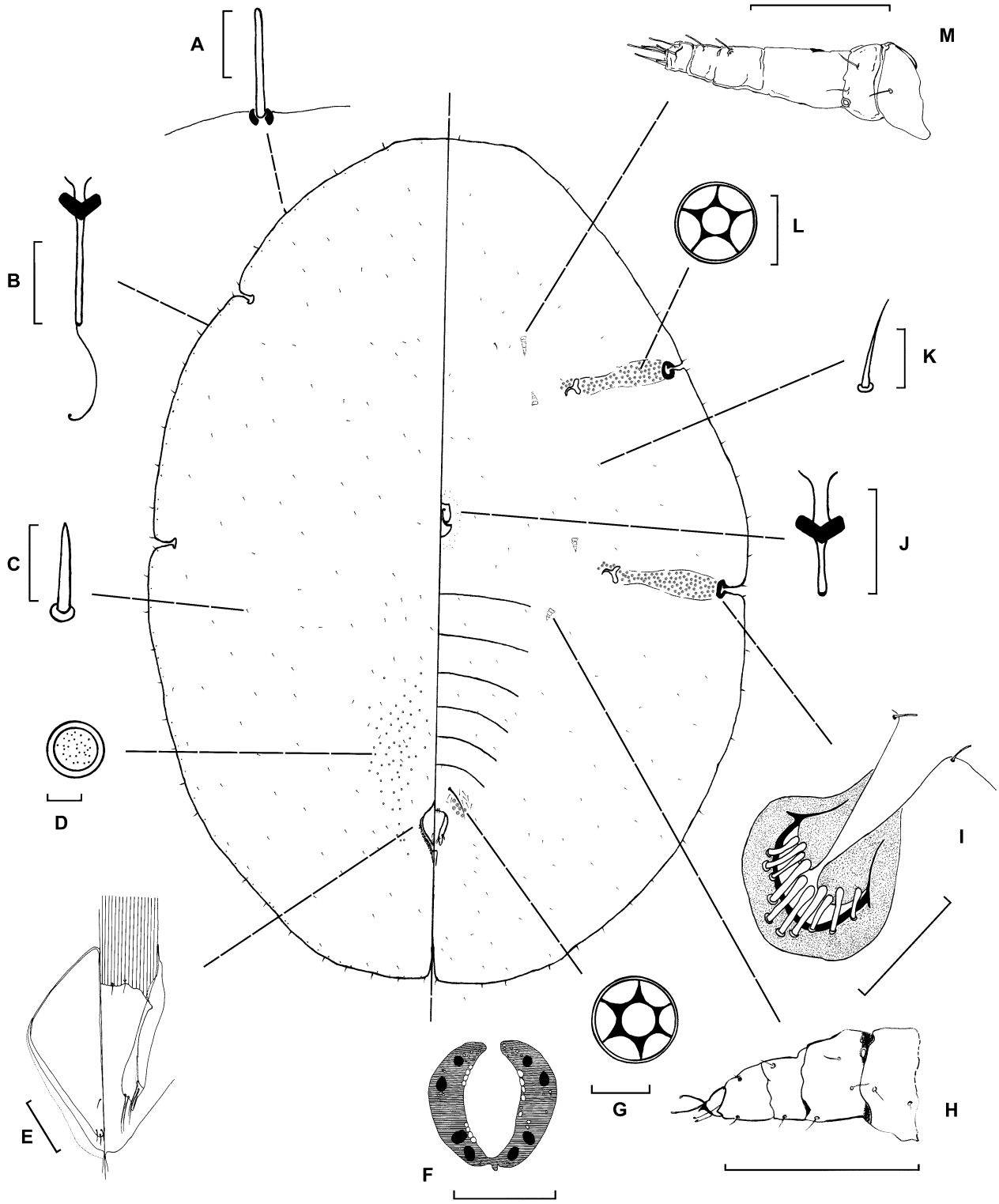

FIGURE 4. Adult female of *Austrolecanium cryptocaryae* Lin & Cook, **sp. n.** A: marginal seta; B: dorsal microductule; C: dorsal seta; D: preopercular pore; E: anal plate and anogenital fold; F: anal ring; G: pregenital disc-pore; H: leg; I: stigmatic area; J: ventral microduct; K: ventral seta; L: spiracular disc-pore; M: antenna. The scale lines against each structure are: E, F, H, I and M = 100 μm; A, B and K = 10 μm; C, D, G, J and L = 5 μm.

Austrolecanium cryptocaryae differs from *A. sassafras* at the following DNA sequence positions (mapped to the GenBank reference sequence listed for each gene):

18S: Reference sequence: KY816394. Site# 22 (A), 309 (-).

28S: Reference sequence: KY816403. Site# 9 (C), 29 (G), 49 (G), 87 (A), 89 (A), 90 (C), 143 (T), 154 (T), 166 (C), 167 (T), 175 (T), 255 (T), 381 (C), 454 (-), 476 (T), 514 (G), 544 (T).

COI: Reference sequence: KY808497. Site# 3 (A), 12 (A), 18 (T), 24 (T), 27 (C), 33 (C), 36 (C), 41 (A), 43–45 (TTA), 47–48 (CT), 51 (T), 61 (G), 63 (T), 69 (T), 72 (T), 75 (C), 79 (C), 87 (C), 93 (C), 105 (T), 108 (T), 120 (G), 123 (T), 126 (A), 128 (T), 141 (T), 144 (A), 151–154 (AGAT), 156–157 (TT), 159 (A), 165 (A), 168 (G), 174 (C), 177 (A), 183 (T), 187 (T), 189 (A), 192 (A), 195 (T), 198 (A), 206 (A), 213–214 (TT), 222–223 (CT), 225 (A), 227 (T), 240 (T), 243 (T), 255–258 (TTTC), 261 (T), 271 (C), 273 (T), 283 (T), 290–291 (CA), 294 (G), 297–298 (TT), 300 (A), 301–303 (ACA), 309 (C), 312–313 (TT), 328 (A), 330 (C), 336 (G), 339 (C), 343 (A), 345 (A), 348 (T), 354 (T), 360 (C), 363 (A), 366 (C), 369 (T), 373 (T), 375–376 (AA), 378 (T), 381–382 (AT), 385 (G), 387 (T), 389–391 (GAC), 396 (C), 398 (A), 405–406 (AA), 410–412 (TTT), 417 (T), 419 (A), 421 (A), 423–424 (TT), 426 (T), 429 (T), 435 (C), 450 (A), 456 (T), 459 (T), 462 (T), 465 (T), 468–469 (TC), 471 (T), 474 (A), 477 (C), 480 (C), 483–484 (TT), 489 (A), 492 (A), 495 (A), 501 (T), 505 (A), 507–508 (AT), 510 (T), 513 (C), 528 (C), 537 (T), 547 (A), 554–555 (AT), 558 (A), 565 (G), 567 (T), 570 (C), 572 (A), 579 (T).

EF-1α: Reference sequence: KY824062. Site# 9 (T), 18 (C), 24 (G), 165 (C), 171 (G), 183 (A), 189 (T), 216 (C), 249 (G), 292 (T), 321 (C), 324 (A), 412 (T), 459 (C).

wingless: Reference sequence: KY798543. Site# 33 (A), 123 (G), 126 (C), 129 (G), 156 (T), 159 (G), 171 (C), 183 (A), 201 (C), 205 (T), 207 (G), 215–216 (GT), 247 (C), 255 (A), 276 (T).

Description. Adult female (Fig. 4) (drawing and measurements based on four specimens: LGC00727f1, LGC00727f2, LGC00831f1 and LGC00831f2, all in good condition).

Unmounted specimens. Live insects (Fig. 1A) bright green with a shiny dorsum, partially transparent near body margin. All specimens were found on the underside of leaves of the host plant.

Mounted specimens. Body oval and symmetrical, 5.3–6.5 mm long, 4.6–5.2 mm wide.

Dorsum. Dorsum mostly membranous but sclerotized around inner margin of each stigmatic cleft and on dorsal derm around anal plates. Dorsal setae sharply spinose, each 5–12 μm long, scattered throughout dorsum. Dorsal pores of 2 kinds: (i) microductules, each about 1 μm in diameter, with inner ductule tubular, 8–15 μm long, filamentous distally, frequent along margin; and (ii) preopercular pores, each 3–8 μm in diameter, scattered over dorsum, but most situated in a broad area anterior to anal plates, forming a cluster of 528–692 pores. Anal plates each triangular with anterior and posterior margins subequal in length, 294–354 μm long, 96–126 μm wide; with 3 setae apically on each plate plus 1 seta on inner margin anterior to apical setae. Anogenital fold with 2 pairs of setae along anterior margin, each 23–30 μm long; also with a distinct supporting bar on each margin, each bar with 4 setae (50–68 μm long), 2 marginally and 2 posteriorly. Anal tube shorter than length of anal plates, 213–300 μm long; anal ring 96–116 μm in diameter, bearing 4 pairs of setae, each seta 342–432 μm long.

Margin. Each marginal seta with a rounded apex, longer than a dorsal seta, 11–29 μm long, arranged in a single marginal row, with 16–22 setae on head between stigmatic clefts, 4–10 on each side between anterior and posterior stigmatic clefts, and 11–18 on each side of abdomen; marginal setae on anal lobes not differentiated from others. Each stigmatic cleft mushroom-shaped, with narrow cleft broadening into a wide space; each with a well-defined area of sclerotization along inner margin of cleft and with 6–12 stigmatic spines; each spine 12–48 μm long, parallel-sided, with a rounded to clavate apex.

Venter. Venter membranous; segmentation only visible on abdomen. Ventral setae hair-like, each 5–7 μm long, sparsely scattered across venter. Pregenital segment (VII) with a single pair of long pregenital setae, each seta 114–144 μm long. A group of 11–15 setae, each 12–39 μm long, present near each pregenital seta and just anterior to each group of pregenital disc-pores. Pregenital disc-pores each 5–8 μm in diameter, mostly each with 6 loculi, present in 2 groups each of 6–16 pores on either side of anogenital fold. Each stigmatic furrow with a band of spiracular disc-pores, each pore mostly with 5 loculi and about 6 μm in diameter, with 87–124 pores present between each spiracle and stigmatic cleft. Ventral microducts minute, each with outer ductule 3–6 μm long and inner ductule inconspicuous; sparsely scattered throughout venter but concentrated around mouthparts. Ventral tubular ducts absent. Spiracles well developed: anterior spiracle plus peritreme 108–162 μm long, 60–132 μm wide; posterior spiracle plus peritreme 126–162 μm long, 108–144 μm wide. Legs much reduced, each 91–130 μm long, trochanter fused with femur; tarsal digitules narrower than claw digitules, each 9–17 μm long, with minute

apical knob; claw digitules each 15–23 μm long, with minute apical knob; claw without a denticle. Antennae each with 6 segments but segmentation often obscure, each antenna 132–192 μm long in total; all setae on terminal 3 segments fleshy, each 8–27 μm long. A pair of interantennal setae present, each 27–33 μm long. Mouthparts positioned at centre of body. Clypeolabral shield 174–180 μm long, 120–150 μm wide. Labium 84 μm long, 90–120 μm wide.

Etymology. The other two species of *Austrolecanium* are named after their host plants, so we continue this tradition here. The species epithet *cryptocaryae* is derived from the genus name of the host plant, *Cryptocarya microneura*, and means "of *Cryptocarya*".

Biological notes. No natural enemies, including parasitoids or predators, of *A. cryptocaryae* were found in this study.

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