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A newly recognised species that has been confused with the global polyphagous pest scale insect, *Coccus hesperidum* Linnaeus (Hemiptera: Coccomorpha: Coccidae)

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Abstract

Coccus hesperidum L. (Hemiptera: Coccomorpha: Coccidae), the type species of the soft scale genus *Coccus* L., the family Coccidae and the whole of the scale insects (Coccoidea), is a cosmopolitan plant pest. Using DNA sequence data and morphological comparisons, we determine that there is a distinct species that is morphologically very similar to *C. hesperidum*. Here, we describe the species as *Coccus praetermissus* Lin & Tanaka **sp. n.**, based on adult female specimens from Australia, Malaysia and Thailand. The adult female of *C. praetermissus* **sp. n.** differs from *C. hesperidum* in having dorsal setae with bluntly rounded tips, whereas they are sharply pointed in *C. hesperidum*. A detailed description of the newly recognised species is provided, incorporating adult female morphology and DNA sequences from mitochondrial and nuclear loci. Our examination of slides from The Natural History Museum, London, and several Australian institutions indicates that *C. praetermissus* **sp. n.** has been confused sometimes with *C. hesperidum* s. s. These findings have potential relevance to plant biosecurity and quarantine because *C. hesperidum* is cosmopolitan whereas *C. praetermissus* **sp. n.**, which is also polyphagous and the two species can share many host plants, currently appears to be more geographically restricted. Additionally, there is deep genetic divergence within *C. praetermissus* **sp. n.** that might indicate that it is a cryptic species complex, but wider geographic sampling is required to test this possibility.

Key words: brown soft scale, Coccoidea, DNA barcode, asexual species, coalescent species delimitation

Introduction

Coccus hesperidum Linnaeus, 1758 (Hemiptera: Coccidae) (Fig. 1), the "brown soft scale" (Hamon & Williams 1984) or "Linnaeus' glasshouse scale", is the type species of the genus *Coccus* L., the family Coccidae (Melville 1985), and the whole of the scale insects, Coccomorpha. It is widespread throughout the world and is one of the most polyphagous species within Coccidae, feeding on host plants from more than 103 families (Lin *et al.* 2015; García Morales *et al.* 2016). In tropical and subtropical regions, *C. hesperidum* (as currently understood) is an economically important pest of many crops, especially citrus (Williams & Watson 1990): it can also damage ornamental plants in temperate areas (Hamon & Williams 1984; Gill 1988). It remains a challenge for entomologists to find effective management strategies in agriculture for this scale insect because of its wide host range, greater honeydew production than many other scale insects, and frequent invasion into indoor environments where the application of chemical or biological control is difficult (Golan *et al.* 2015). In addition, the invasion of *C. hesperidum* into some isolated and fragile ecosystems in the Galápagos Islands (Causton *et al.* 2006), south Florida (Zettler *et al.* 2012) and the Tristan da Cunha archipelago (Ryan *et al.* 2014) has threatened the native flora and indirectly threatened the endemic fauna of these areas.



FIGURE 1. Coccus hesperidum L. A. Mature adult females on papaya, Carica papaya, in Colombia. B. Young adult females and nymphs tended by Camponotus ants, in Brazil. Photographs by T. Kondo.

Linnaeus' (1758) original description of *Coccus hesperidum* was simply "The *Coccus* of the greenhouse; it lives on evergreen trees" (translated from Latin into English by Williams, 2007), which has made accurate species identification difficult if not impossible (Kondo *et al.* 2008). In addition, adult female specimens assigned to *C. hesperidum* by various authors collected from different hosts and geographic regions vary in body colour, size and other morphological features. Probably as a consequence, this scale insect was frequently redescribed under new names during the 19th and early 20th centuries; these were synonymised by Fernald (1903) and Sanders (1909) and are listed in García Morales *et al.* (2016). The only recognised subspecies, *C. hesperidum javaensis*, was found in Java and described briefly by Newstead (1908). The author stated that the adult female had much smaller marginal setae and a dorsal "longitudinal ridge", not seen in typical *C. hesperidum*. There have been no records of specimens matching the description of this subspecies since the original in 1908. The current concept of *C. hesperidum* has been built up from detailed descriptions and drawings by Tyrrell (1896), Newstead (1903), Thro (1903), Green (1904), Steinweden (1930), Zimmerman (1948), De Lotto (1959), Saakyan-Baranova (1964), Gill *et al.* (1977), Hamon & Williams (1984), Williams & Watson (1990), Avasthi & Shafee (1991), Tang (1991), Hodgson (1967; 1994) and Lin *et al.* (2013).

In the past century, most studies on *C. hesperidum* have focused on the life history, population dynamics, parasitoid wasps and management (listed in Ben-Dov 1993, and García Morales *et al.* 2016). There are few works regarding the intraspecific morphological variation of this scale insect. Morrison (1929) mentioned that some specimens collected from Ancón in Panama and attended by ants (*Azteca* sp.) had an "abnormal" body shape and smaller size. However, the author did not express doubt about the conspecific status of these specimens with *C. hesperidum*. Blair *et al.* (1964) and Hodgson (1967) found that some adult females sampled from the same host plant had considerable differences in morphology, but the authors did not address whether these variations indicated the possibility of cryptic species within *C. hesperidum*. Williams & Watson (1990) examined some of the specimens labelled as *C. hesperidum* collected from the tropical South Pacific regions and found that the dorsal setae of several specimens (particularly from Papua New Guinea) had bluntly rounded apices, in contrast with the spine-like and pointed dorsal setae typically seen in this species (see the drawings in Hodgson 1994, and Lin *et al.* 2013). Individuals with setae with bluntly rounded apices have also been observed by Łagowska (1999) in four Polish populations of *C. hesperidum* collected from various hosts.

Recently, Lin *et al.* (2013) found that three adult female specimens resembling *Coccus hesperidum*, collected from Taiwan and Malaysia, formed a sister clade to an obligate myrmecophilous species of coccid, *C. formicarii* (Green). These insects are morphologically distinct from *C. formicarii* but almost identical to *C. hesperidum*, with the exception that their dorsal setae which have bluntly rounded apices similar to specimens reported from Papua New Guinea by Williams & Watson (1990) and from Poland by Łagowska (1999). Tao *et al.* (1983) illustrated *C. hesperidum* from Taiwan as having bluntly rounded apices, and Lin *et al.* (2013) suggested that this drawing might be the unusual taxon discussed here rather than *C. hesperidum*. Lin *et al.* (2013) did not deal with the taxonomic

status of the three specimens due to a lack of certainty about the identity of the Asian specimens and the lack of resolution in molecular analyses of some gene regions.

In this study, we seek to determine whether the adult females of *Coccus* with the bluntly rounded dorsal setae in Lin *et al.* (2013) merit distinct species status. We include the specimens from that study and additional samples of *C. hesperidum* and morphologically similar specimens, and DNA sequence data from additional loci, to test species boundaries. Because the specimens with roundly blunt dorsal setae are otherwise so similar morphologically to *C. hesperidum*, we also examined slides labelled as *C. hesperidum* in important insect collections in Australia, The Natural History Museum (London) (BMNH) and the Laboratory of Professor Lagowska (Department of Entomology, University of Life Sciences, Lublin, Poland) to determine whether there has been widespread inclusion of both morphologies under *C. hesperidum*, and to determine the geographic distribution of each form.

Materials and methods

Species concept. Although implementing different species concepts to delineate species could affect the number of species recognised, very few taxonomists have reported which species concept they use (Yeates *et al.* 2011). The use of the biological species concept (BSC) (Mayr 1942) might be inappropriate for this group of scale insects because they are thought to be mostly asexual and there is no direct evidence for their reproducing sexually. It is widely accepted that, in most cases, *C. hesperidum* s. s. (adult females with pointed dorsal setae) reproduces parthenogenetically and is ovoviviparous (Hamon & Williams 1984; Gill 1988; Tang 1991). Two studies (Saakyan-Baranova 1964; Giliomee 1967), however, have reported males of *C. hesperidum* s. s. but, in both cases, no direct evidence was provided to show that the males definitely belong to that species. Even if males occasionally occur, most reproductive isolation are inappropriate. Similarly, phylogenetic species concepts are also inappropriate for asexual lineages because mutation in the absence of sex leads to a pattern of independent lineages and reciprocal monophyly across multiple genes (see discussion in Lin *et al.* 2017a). Here, we apply a species concept for asexual lineages in which species are considered to be independently evolving genetic lineages that are differentiated in additional ways from other such lineages (e.g., ecologically, behaviourally or morphologically) (as per Lin *et al.* 2017a).

Strategies and criteria to morphological examination of slide-mounted specimens. Because adult females with pointed setae and with bluntly rounded setae might occur on the same host, all specimens mounted together on a slide were checked. All specimens were scored for at least 10 dorsal setae per individual, and assigned to one type or the other if more than 90% of the observed setae could be clearly scored as either pointed or bluntly rounded.

Examination of primary types of synonyms and subspecies of **C. hesperidum.** It is possible that our target taxon has already been described as one of the current synonyms and subspecies of *C. hesperidum* but without knowledge of the setal tip character. However, many primary types listed in García Morales *et al.* (2016) are lost. We examined all available specimens of types that are deposited in Museum National d'Histoire Naturelle, Paris, France (MNHN), National Institute of Agricultural Environmental Sciences, Tsukuba, Japan (NIAES), The Natural History Museum, London, U.K. (BMNH), and the U.S. National Museum of Natural History, Smithsonian Institution, District of Columbia, U.S.A. (USNM).

Slide-mounted specimens examined in Australian collections. All slides labelled as "*Coccus hesperidum*" in six important Australian insect collections were examined: Australian National Insect Collection, CSIRO National Facilities and Collections, Canberra, ACT, Australia (ANIC) (92 slides); Agricultural Scientific Collections Unit, NSW Department of Primary Industries, Orange Agricultural Institute, NSW, Australia (ASCU) (50 slides); Northern Territory Economic Insect Reference Collection, Darwin, NT, Australia (NTEIC) (25 slides); Queensland Primary Industries Insect Collection, Queensland Department of Agriculture, Fisheries and Forestry, Brisbane, QLD, Australia (QDPC) (385 slides); Tasmanian Agricultural Insect Collection, Tasmanian Department of Primary Industries, Parks, Water and Environment, Hobart, TAS, Australia (TASAGRIC) (41 slides); and Waite Insect and Nematode Collection, University of Adelaide, Adelaide, SA, Australia (WINC) (40 slides).

Slide-mounted specimens examined in BMNH. There are more than 1000 slides labelled as "Coccus hesperidum" deposited in The Natural History Museum, London. We examined 339 of them, focusing on 1) the

slides listed in Williams & Watson (1990) because the authors noted observations of adult females with dorsal setae with rounded tips and 2) representative samples from different countries and different continents in order to have geographically broad sampling. There are four slides labelled "*Coccus hesperidum*" that include male specimens from Brazil, India and Kenya (Appendix 1, Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4ps2t) deposited in the BMNH. The species identifications are uncertain because all are nymphs and the morphology of the associated adult females is too poor to allow identification to species level. There are no slides of males labelled as *C. hesperidum* deposited in any of the Australian collections checked in this study.

Slide-mounted specimens examined in the Department of Entomology, University of Life Sciences (Lublin, Poland). Thirty slides labelled as *C. hesperidum* deposited in the Łagowska Laboratory (Department of Entomology, University of Life Sciences, Lublin, Poland) were examined. These greenhouse specimens were collected on *Citrus limon* (10 slides), *Ficus benjamina* (10 slides) and *Schefflera actinophylla* (10 slides) in 1995 and were part of the examined materials listed in Łagowska (1999).

Taxon sampling and DNA extraction. In addition to the three adult females of Lin *et al.* (2013) that are morphologically similar to *C. hesperidum* s. s. but have bluntly rounded dorsal setae, another three adult females with the same morphology were collected from Taiwan (YPL00291), Australia (YPL00716) and Thailand (YPL00732) (Table 1). *Coccus hesperidum* s. s. is represented in this study by 45 adult female specimens from at least 34 different host plant species (belonging to 29 families) and 41 localities (Table 1). The samples included the 15 used by Lin *et al.* (2013) and 30 newly sequenced specimens. Among them, YPL00009, YPL00334 and YPL00517 were from Taiwan (Chiayi City and Pingtung County) and Malaysia (Kuala Lumpur), and were sympatric with the three specimens with bluntly rounded dorsal setae (YPL00496, YPL00291 and YPL00465 respectively). YPL00568, which has pointed dorsal setae, was collected from the same host species and locality (Lublin, Poland) as the specimens studied by Łagowska (1999), which have bluntly rounded dorsal setae.

Coccus penangensis is included as one of the outgroups (Table 1), based on the phylogeny of Lin *et al.* (2013), and we also included *C. discrepans*, *C. sulawesicus* and *C. formicarii* from that study because they are closely related to *C. hesperidum* and the adult females are morphologically similar (Tao *et al.* 1983; Avasthi & Shafee 1991; Gavrilov-Zimin 2013). The species identification of *C. discrepans* was confirmed by YPL, who examined the primary types deposed in BMNH. The specimen of *C. sulawesicus* was sent to YPL by the author (I. Gavrilov-Zimin) who described the species.

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 young adult females using either a CTAB/chloroform protocol or a DNeasy Blood & Tissue kit (cat. no. 69504, Qiagen, Hilden, Germany) as outlined 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 is stored at The University of Queensland (LGC Laboratory) and all the voucher slides are deposited in the Australian National Insect Collection, Canberra, Australia.

The morphology of slide-mounted adult females was examined under a phase-contrast compound light microscope (Olympus BH-2 PH). Particular attention was paid to the following morphological features that can vary among *Coccus* species: (i) shape and length of dorsal setae; (ii) shape and length of marginal setae; (iii) number and distribution of dorsal tubular ducts; (iv) number and distribution of dorsal tubular ducts; (iv) number and distribution of dorsal tubercles; (v) shape and number of stigmatic spines in each stigmatic cleft; (vi) shape of the anal plates; (vii) number of antennal segments; (viii) distribution of ventral tubular ducts; and (ix) the presence or absence of tibio-tarsal sclerosis. The species identifications of outgroup taxa were based on Avasthi & Shafee (1991) (*C. discrepans*), Lin *et al.* (2013) (*C. formicarii*), Hodgson (1994) (*C. hesperidum* s. s.), Morrison (1921) (*C. penangensis*) and Gavrilov-Zimin (2013) (*C. sulawesicus*). The morphological terms used follow those in Hodgson (1994).

PCR reactions, clean-up, gel purification and cloning. Five genes from four 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-1a* (nDNA), *wingless* (nDNA) and *COI* (mtDNA). A negative control was used for all PCR reactions. We used the same primer pairs, Taq-polymerase (MangoTaq, cat. no. BIO-21083, Bioline, Australia), PCR thermocycles and volumes of template DNA for amplifying *18S*, *28S*, *EF-1a* and *wingless* as per Lin *et al.* (2017b) (Table 2).

The PCR program from Park *et al.* (2010) was used for all amplifications of *COI*, but using three 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 by using that primer pair, then the reverse primer (HCO) was

replaced by JerryR (the reverse compliment of CI-J-2183 from Simon *et al.* 1994). Finally, because *C. hesperidum* s. s. was not readily amplified with either of these primer pairs, we used PcoF1 and a newly designed reverse primer HCOCh26 (Table 2), which overlaps HCO, to amplify *COI* from all the samples of *C. hesperidum* s. s. The settings of PCR mixture and used Taq-polymerase were the same as Lin *et al.* (2017b).

TABLE 1. Samples of Coccidae used in this study. Abbreviations: ACT: Australian Capital Territory; AUS: Australia; BEN; Benin; CA: California, U.S.A.; CHN: China; COL: Colombia; ESP: Spain; GBR: United Kingdom; GHA: Ghana; GRC: Greece; IDN: Indonesia; JPN: Japan; MYS: Malaysia; NSW: New South Wales, Australia; PNG: Papua New Guinea; POL: Poland; QLD: Queensland, Australia; SA: South Australia; SGP: Singapore; THA: Thailand; TWN: Taiwan; USA: United States of America; VIC: Victoria, Australia; WA: Western Australia; ZAF: South Africa.

Code	Host	Host plant family	Locality	Date	Collector
Coccus prae	termissus sp. n.				
YPL00122	Ficus irisana	Moraceae	Yilan County, TWN	7.ii.2009	YP. Lin
YPL00291	Calophyllum inophyllum	Clusiaceae	Pingtung County, TWN	24.viii.2009	YP. Lin
YPL00465	Ixora chinensis	Rubiaceae	Kuala Lumpur, MYS	13.xii.2010	YP. Lin
YPL00496	Podocarpus costalis	Podocarpaceae	Chiayi City, TWN	5.ii.2011	YP. Lin
YPL00716	<i>Mangifera</i> sp.	Anacardiaceae	Shelburne, QLD, AUS	12.vii.2013	D. Pearce & L. Benson
YPL00732	Rhizophora mucronata	Rhizophoraceae	Ranong, THA	iv.2003	J. Offenberg
C. hesperidi	ım sensu stricto				
YPL00009	Lagerstroemia speciosa	Lythraceae	Chiayi City, TWN	30.x.2008	YP. Lin
YPL00076	Morus sp.	Moraceae	Brisbane, QLD, AUS	20.xi.2008	YP. Lin
YPL00128	Bauhinia variegata	Fabaceae	Brisbane, QLD, AUS	15.iii.2009	YP. Lin
YPL00247	Heteromeles arbutifolia	Rosaceae	Davis, CA, USA	1.iv.2009	YP. Lin
YPL00286	Tecoma stans	Bignoniaceae	Pretoria, ZAF	4.v.2009	I. Miller
YPL00334	Messerschmidia argentea	Boraginaceae	Pingtung County, TWN	10.xii.2009	YP. Lin
YPL00363	Schefflera arboricola	Araliaceae	Taipei City, TWN	19.ii.2010	YP. Lin
YPL00377	Ilex aquifolium	Aquifoliaceae	Berkshire, GBR	4.iv.2010	YP. Lin
YPL00380	Camellia japonica	Theaceae	Crete, GRC	6.iv.2010	YP. Lin
YPL00388	Eriobotrya japonica	Rosaceae	Crete, GRC	9.iv.2010	YP. Lin
YPL00390	Elaeagnus thunbergii	Elaeagnaceae	London, GBR	11.iv.2010	YP. Lin
YPL00392	Lonicera macrantha	Caprifoliaceae	London, GBR	11.iv.2010	YP. Lin
YPL00395	Laurus nobilis	Lauraceae	London, GBR	11.iv.2010	YP. Lin
YPL00438	Laurus nobilis	Lauraceae	Valencia, ESP	15.v.2010	A. Beltrà-Ivars
YPL00441	Agave sp.	Agavaceae	Brisbane, QLD, AUS	7.x.2010	YP. Lin
YPL00481	Aphananthe aspera	Ulmaceae	Taitung County, TWN	20.i.2011	YP. Lin
YPL00506	Celtis formosana	Ulmaceae	Miaoli County, TWN	13.v.2011	YP. Lin
YPL00517	Monstera deliciosa	Araceae	Kuala Lumpur, MYS	12.xii.2010	YP. Lin
YPL00524	Camellia sp.	Theaceae	Melbourne, VIC, AUS	5.xii.2011	YP. Lin
YPL00533	<i>Melaleuca</i> sp.	Myrtaceae	Newnes, NSW, AUS	12.xii.2011	YP. Lin
YPL00541	Phoradendron californicum	Santalaceae	Davis, CA, USA	17.iii.2004	T. Kondo
YPL00546	Arbutus unedo	Ericaceae	Canberra, ACT, AUS	21.xii.2011	YP. Lin
YPL00557	Ficus sp.	Moraceae	Nuwerus, Worcester, ZAF	11.ii.2012	J.H. Giliomee
YPL00568	Ficus benjamina	Moraceae	Lublin, POL	14.ix.2012	B. Łagowska

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TABLE 1. (Continued)

Code	Host	Host plant family	Locality	Date	Collector
YPL00642	Unidentified plant	unidentified	Brisbane, QLD, AUS	6.ii.2014	L.G. Cook
YPL00661	<i>Clivia</i> sp.	Amaryllidaceae	Taiyuan, Shanxi, CHN	24.v.2014	YP. Lin
YPL00666	Platycerium sp.	Polypodiaceae	Itami, Hyogo, JPN	15.iii.2014	H. Tanaka
YPL00675	Ruellia brittoniana	Acanthaceae	Ekimae, Kouchi, JPN	29.iii.2014	H. Tanaka
YPL00696	Laurus nobilis	Lauraceae	Adelaide, SA, AUS	21.xi.2014	YP. Lin
YPL00707	Radermachera sinica	Bignoniaceae	Shanghai, CHN	8.iii.2015	YP. Lin
YPL00713	Sarcocephalus coadunatus	Rubiaceae	Ramu River Basin, Madang Province, PNG	30.v.2007	P. Klimeš
YPL00725	Olea europaea	Oleaceae	London, GBR	25.vii.2015	YP. Lin
YPL00727	Unidentified plant	unidentified	London, GBR	1.viii.2015	YP. Lin
YPL00731	Laurus nobilis	Lauraceae	Exeter, Devon, GBR	iv.2015	C.J. Hodgson
YPL00735	Ocimum basilicum	Lamiaceae	Cairns, QLD, AUS	27.ix.2015	YP. Lin
YPL00739	Carica papaya	Caricaceae	Calavi, BEN	12.v.2015	G. Goergen
YPL00758	Schefflera arboricola	Araliaceae	Amelup, WA, AUS	29.xi.2015	YP. Lin
YPL00777	Plumeria obtusa	Apocynaceae	Fremantle, WA, AUS	11.xii.2015	YP. Lin
YPL00785	Euonymus japonicus	Celastraceae	Winters, CA, USA	23.vi.2015	A. Klein & T. Nobhaft
YPL00786	Citrus sp.	Rutaceae	Fallbrook, CA, USA	19.vi.2015	J. Le
YPL00787	Orchid leaf	Orchidaceae	Los Osos, CA, USA	30.iv.2015	C. Kirkland
YPL00788	Citrus limon	Rutaceae	Antioch, CA, USA	13.iv.2015	Williamson & Mendoza
TK0051	Carica papaya	Caricaceae	Bunso Arboretum, GHA	19.vi.2005	T. Kondo
TK0193	Unidentified weed	unidentified	Cali, Valle, COL	15.i.2006	T. Kondo
TK0214	Ficus benjamina	Moraceae	Cali, Valle, COL	31.xii.2005	T. Kondo
OUTGROU	PS				
C. discrepan	us (Green)				
YPL00710	Unidentified plant	unidentified	Ramu River Basin, Madang Province, PNG	29.iii.2007	P. Klimeš
C. formicari	ii (Green)				
YPL00094	Ficus microcarpa	Moraceae	New Taipei City, TWN	21.i.2009	YP. Lin
YPL00108	Litsea glutinosa	Lauraceae	Kinmen, TWN	1.ii.2009	YP. Lin
YPL00485	Machilus thunbergii	Lauraceae	Taitung County, TWN	20.i.2011	YP. Lin
YPL00488	Camellia chrysanthera	Theaceae	Guangzhou City, CHN	25.i.2011	YP. Lin
YPL00515	Ficus virgata	Moraceae	Taichung City, TWN	4.vi.2011	YP. Lin
TK0511	Mangifera indica	Anacardiaceae	Nakhorn Pathom, THA	4.vi.2007	T. Kondo
C. penanger	<i>ısis</i> Morrison				
YPL00536	Macaranga bancana	Euphorbiaceae	Central Catchment, SGP	18.iii.2009	P.S. Cranston
C. sulawesic	cus Gavrilov				
YPL00571	Dicotyledonous shrub	unidentified	Kendari, Sulawesi, IDN	11.xi.2011	I.A. Gavrilov- Zimin

The successful PCR amplifications were checked by doing 1% agarose gel electrophoresis. The clean-up, gel purification and preparation 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).

The sequenced PCR products of the *EF-1a* gene region of YPL00524 and YPL00568 included multiple amplified copies of almost identical length. To separate the multiple copies, we followed the cloning protocols described in Lin *et al.* (2017b). Ten clones that contained the target DNA fragments were sequenced at Macrogen with the universal primer, T7 Promoter (5'- TAATACGACTCACTATAGGG -3').

Gene	Primer	F or R	Primer sequence 5' to 3'	Annealing temperature	Alignment length (bp)	Reference
28S D2/ D3	S3660	F	GAGAGTTMAASAGTACGTGAAAC	55°C	745	Dowton & Austin 1998
	A335	R	TCGGARGGAACCAGCTACTA			Whiting et al. 1997
18S	2880	F	CTGGTTGATCCTGCCAGTAG	55°C	557	von Dohlen & Moran 1995
	B-	R	CCGCGGCTGCTGGCACCAGA			von Dohlen & Moran 1995
COI	PcoF1	F	CCTTCAACTAATCATAAAAATATYAG	45°C/51°C	579	Park et al. 2010
	НСО	R	TAAACTTCAGGGTGACCAAAAAATC A			Folmer et al. 1994
	CI-J-2183 (Jerry)	R	CCAAAAAATCAAAATAAATGTTG			Simon <i>et al</i> . 1994
	HCOCh26	R	TATACTTCTGGATGCCCGAAGAATCA			This study
EF-1α	scutA_F	F	ATTGTCGCTGCTGGTACCGGTGAATT	50°C	625	Hardy et al. 2008
	rcM52.6	R	GCYTCGTGGTGCATYTCSAC			Cho et al. 1995
wingless	scale_wg_ F	F	CTGGTTCGTGCACGACGMGRACSTG YTGGATG	55°C	321	Hardy et al. 2008
	LEPWG2	R	ACTICGCARCACCARTGGAATGTRCA			Brower & DeSalle 1998

TABLE 2. Primers and PCR protocols used.

Sequence editing and alignment. The computer software packages and methods using for sequence editing and alignment were as per Lin *et al.* (2017b). Sequences of the two rRNA genes (*18S* and *28S*) were aligned manually. For the three protein-encoding regions (*COI*, *EF-1a* and *wingless*), 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-1a* were detected by using the GT-AG rule (Rogers & Wall 1980). All introns of *EF-1a* can be unambiguously aligned with outgroups, and copies of *EF-1a* obtained from cloning were included in our analyses.

Phylogenetic analysis. Maximum parsimony (MP) and Bayesian inference (BI) were used to estimate phylogenetic analyses, the same methods used by Lin *et al.* (2017b) were used for checking the presence of base composition bias among taxa. The support for particular nodes from each dataset and the congruence among the hypotheses of relationships across different gene regions were assessed using bootstraps (BS) or posterior probabilities (PP), with BS \geq 70 (Hillis & Bull 1993) and PP \geq 0.95 (Huelsenbeck & Rannala 2004) considered to be good support. All phylogenies were rooted using *C. penangensis*, based on relationships estimated by Lin *et al.* (2013).

Maximum parsimony (MP). MP trees were estimated using PAUP* 4.0b10 (Swofford 2003) with the heuristic searches. All sites were weighted equally for the rRNA genes and the introns of *EF-1a*. For the three protein-coding genes, *COI*, *EF-1a* and *wingless*, a weighting scheme for the three codon positions (first: second: third = 2: 3: 1) was applied. All other settings including the method of branch swapping (TBR), algorithm of tree starting (random-addition-sequence method with 1000 replicates), maximum number of kept trees (no restriction), option of summarising MP trees (strict consensus) and the number of bootstrap pseudoreplicates (1000) 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 trialled more complicated partition

schemes, such as treating *18S* and *28S* as separate partitions or partitioning each protein-coding gene region by codon position. However, these trials rarely reached stationarity so a more restrictive partitioning scheme was applied. A single partitioning scheme was used for all datasets. The GTR (Tavaré 1986) model was chosen for *COI* and *EF-1* α because of their unequal base compositions and numerous variable sites. For the *18S+28S* and *wingless* datasets, which has only 28 (2.1%) and 16 (5.0%) variable sites respectively, the K2P (Kimura 1980) model was selected. The partition schemes and substitution models applied in the analyses of the concatenated dataset were the same as that used in the single gene analyses. Each analysis comprised two independent runs (nruns = 2) of 100 million (*18S+28S*; *wingless*; concatenated) or 60 million (*COI*; *EF-1* α) 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 1000 generations (samplefreq = 1000).

The criteria and methods to check the performance of each Bayesian analysis were the same as per Lin *et al.* (2017b). 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 computed by TreeAnnotator v.1.8.3 (Drummond & Rambaut 2007) using the trees sampled post-burnin.

Results

Morphological examination of primary types of synonyms of **C. hesperidum.** There are 14 synonyms of *C. hesperidum* currently listed in ScaleNet (García Morales *et al.* 2016) with primary types being recorded as available (Table 3). We examined 11 of them and the dorsal setae were pointed in all of them (Table 3). Of the other three types, those of *L. assimile amaryllidis* and *L. hesperidum pacificum* are lost (Table 3): the latter was probably destroyed by the Great Kantō earthquake in 1923. The synonymy of *Lecanium maculatum* with *C. hesperidum* was claimed by Ben-Dov (1976), but he did not mention the shape of dorsal setae. It cannot be denied that our target might be one of the synonyms for which the types are lost but this is impossible to determine.

Morphological examination of other slide-mounted specimens. Specimens with bluntly rounded dorsal setae and labelled as *C. hesperidum* were found on 27 of the 342 slides examined from the BMNH, 44 of the 633 slides from Australian institutions, and 16 of the 30 slides examined from Lublin (Poland). They are listed in Table 4.

Specimens with the bluntly rounded setae were mainly collected from tropical Eastern Asia, Australia and some Pacific and Indian Ocean islands: within Australia, all such specimens were from the Top End (Northern Territory) and Cape York Peninsula (Queensland). The Polish populations examined were a mix of specimens with the bluntly rounded setae and *C. hesperidum* s. s. Other examined slides with specimens with the pointed dorsal setae and recognised as *C. hesperidum* s. s. are listed in Appendix 2 (Australian institutes) (Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4ps2t) and Appendix 3 (BMNH) (Dryad Digital Repository: http:// dx.doi.org/10.5061/dryad.4ps2t) respectively.

Molecular phylogenetics. All sequence data are available in GenBank (Table 5). No premature stop codons or base composition biases among taxa were detected in any dataset, with P values for tests of non-stationarity ranging from 0.90 to 1.00.

Uncorrected genetic distances (p-distances) in *COI* between specimens of *Coccus hesperidum* s. s. and *C. hesperidum*-like specimens with bluntly rounded dorsal setae ranged from 12.0–13.0%. The sequence divergence among samples with the same form of dorsal setae ranged from 1.0–5.0% (bluntly rounded) and 0–2.0% (pointed apices) respectively.

MP analyses resulted in four trees of length 29 (CI = 0.97, RI = 0.99) for the *18S*+28S dataset, 73 trees of length 317 (CI = 0.67, RI = 0.92) for the *COI*, 217 trees of length 143 (CI = 0.81, RI = 0.96) for the *EF*-1 α , six trees of length 22 (CI = 0.91, RI = 0.97) for the *wingless* and 13 trees of length 463 (CI = 0.70, RI = 0.93) for the concatenated datasets. The required burn-in proportions for Bayesian runs were 95% (*COI* and *wingless*), 90% (*18S*+28S), 83% (*EF*-1 α) and 50% (concatenated dataset).

Coccus hesperidum s. s. was supported as a monophyletic group in analyses of all the genes, and the concatenated dataset, to the exclusion of *C. hesperidum*-like specimens with bluntly rounded dorsal setae (Fig. 2; Figs S1-S4, Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4ps2t). There were two clades of adult females with bluntly rounded dorsal setae recovered in analyses of *COI*, *EF*-1 α and the concatenated datasets (Fig. 2; Figs S2 & S3). All six adult females with bluntly rounded dorsal setae formed a well-supported clade only in

phylogenies estimated from *COI* (Fig. S2) and the concatenated datasets (Fig. 2). A clade including specimens of *C. formicarii*, *C. discrepans*, *C. sulawesicus* and the "bluntly rounded setae clade" was well supported in the results of *COI* (Fig. S2), *EF-1a* (Fig. S3) and concatenated datasets (Fig. 2), but was not present in analyses of the ribosomal genes (Fig. S1) and *wingless* (Fig. S4), which had very little variation across the datasets.

Synonym Author (year, page)			Collecting data			
		Depository	Country (area)	Host	Type of dorsal setae	Examiner
Primary type (s) exami	ined					
Lecanium alienum	Douglas (1886, 77)	BMNH ¹	U.K. (England)	Asplenium bulbiferum	pointed	YPL ⁶
L. minimum	Newstead (1892, 141)	BMNH	U.K. (England)	Areca sp.	pointed	YPL
L. terminaliae	Cockerell (1893b, 254)	USNM ²	Jamaica (Kingston)	Terminalia catappa	pointed	SS^7
L. ceratoniae	Gennadius (1895, cclxxvii)	MNHN ³	Cyprus	Ceratonia siliqua	pointed	DM ⁸ /JG ⁹
L. nanum	Cockerell (1896, 19)	USNM	Trinidad	Manilkara bidentata	pointed	SS
L. flaveolum	Cockerell (1897, 52)	USNM	U.S.A. (New Mexico)	Pilea sp.	pointed	SS
L. minimum pinicola	Maskell (1897, 310)	USNM	South Africa (Cape of Good Hope)	Pinus insignis	pointed	SS
L. ventrale	Ehrhorn (1898, 245)	USNM	U.S.A. (California)	'tuberous plant'	pointed	SS
L. signiferum	Green (1904, 197)	BMNH	Sri Lanka (Pundaluoya)	<i>Begonia</i> sp.	pointed	YPL
L. punctuliferum	Green (1904, 205)	BMNH	Sri Lanka (Paradeniya)	Michelia champaca	pointed	YPL
L. mauritiense	Mamet (1936, 96)	MNHN	Mauritius (Rose Hill and Ebene)	Furcraea foetida	pointed	DM
Primary type (s) lost (c	confirmed by this study)					
L. assimile amaryllidis	Cockerell (1893a, 53)	USNM	Antigua	Amaryllis sp.	?	SS
L. hesperidum pacificum	Kuwana (1902, 30)	NIAES ⁴	Ecuador (Galápagos Islands)	12 species	?	HT^{10}
Primary type (s) unable	e to examine					
L. maculatum	Signoret (1873, 400)	NMW ⁵	France	Hedera helix	?	-

FABLE 3. Data of the synonyms	s of Coccus hesperidum studied.
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¹BMNH: The Natural History Museum, London, U.K.

²USNM: U.S. National Museum of Natural History, District of Columbia, U.S.A.

³MNHN: Museum National d'Histoire Naturelle, Paris, France

⁴NIAES: National Institute of Agricultural Environmental Sciences, Tsukuba, Japan

⁵NMW: Naturhistorisches Museum Wien, Austria

⁶YPL: Yen-Po Lin

⁷SS: Scott A. Schneider

⁸DM: Danièle Matile-Ferrero

⁹JG: Jean-François Germain

¹⁰HT: Hirotaka Tanaka

TABLE 4. The list of examined slides that are currently labelled as "*Coccus hesperidum*" but which have bluntly pointed dorsal setae as per *C. praetermissus* **sp. n.** Abbreviations: AUS: Australia; COK: Cook Islands; KIR: Kiribati; MDV: Maldives; MUS: Mauritius; MYS: Malaysia; NT: Northern Territory, Australia; PHL: Philippines; PNG: Papua New Guinea; POL: Poland; QLD: Queensland, Australia; TKL: Tokelau; TON: Tonga; TUV: Tuvalu; VUT: Vanuatu; WA: Western Australia, Australia; WSM: Samoa.

Depository	Host	Host plant family	Locality	Date	Collector	Number of slides
ANIC (Australian Nati	ional Insect Collection	, Canberra, Australi	a)			
	Mangifera indica	Anacardiaceae	PHL	26.viii.1985	B.J. Read	1
ASCU (Agricultural S	cientific Collections U	nit, Orange, Austral	ia)			
ASCTHE101667	Mangifera indica	Anacardiaceae	PHL	26.viii.1985	B.J. Read	1
BMNH (The Natural H	listory Museum, Lond	on, U.K.)				
	Acacia holosericea	Fabaceae	Darwin, NT, AUS	3.xi.1915	G.F. Hill	12
	Plumeria rubra	Apocynaceae	Avarua, Rarotonga, COK	10.iii.1975	P.A. Maddison	1
	Unidentified plant	unidentified	Enderbury, Phoenix Islands, KIR	9.xi.1964	R. Holuray	1
	Hibiscus tiliaceus	Malvaceae	N. Male Atoll, Baros, MDV	19.iii.1993	D.S./G.W. Watson	1
	Cocos nucifera	Arecaceae	Planti, Serdang, Selangor, MYS	8.ii.1985	J.H. Martin	1
	Ardisia crenata	Primulaceae	Les Mares, MUS	v.1934	R. Mamet	1
	Calophyllum inophyllum	Calophyllaceae	Buso, Morobe Province, PNG	10.x.1979	J.H. Martin	1
	<i>Timonius</i> sp.	Rubiaceae	Buso, Morobe Province, PNG	9.ix.1979	J.H. Martin	1
	Anisoptera thurifera	Dipterocarpaceae	Buso, Morobe Province, PNG	12.x.1979	J.H. Martin	1
	Elmerrillia papuana	Magnoliaceae	Buso, Morobe Province, PNG	13.xi.1979	J.H. Martin	1
TT1388	Citrus sinensis	Rutaceae	Tongatapu, TON	23.x.1974		1
	Carica papaya	Caricaceae	Nokunonu, TKL	1.vi.2002	S.N. Lal	1
	Pisonia grandis	Nyctaginaceae	Niulakita, TUV	29.ii.1972	P.D. Manser	1
	Alpinia purpurata	Zingiberaceae	Port Vila, Efate, VUT	12.xi.1983	P.A. Maddison	1
	Carica papaya	Caricaceae	Leauvaa, Upolu, WSM	16.v.2001	P. Makalavea	1
IIE 24221	Mangifera indica	Anacardiaceae	Nuu, Upolu, WSM	6.vii.2000		1
Łagowska Laboratory (Department of Entomology, University of Life Sciences, Lublin, Poland)						
	Citrus limon	Rutaceae	Lublin, POL	22.v.1995	B. Łagowska	2
	Ficus benjamina	Moraceae	Lublin, POL	26.ix.1995	B. Łagowska	8
	Schefflera actinophylla	Araliaceae	Lublin, POL	26.ix.1995	B. Łagowska	6
QDPC (Queensland Pr	imary Industries Insec	t Collection, Brisba	ne, Australia)			
0-063660 to 62	Mangifera indica	Anacardiaceae	Cape Don, NT, AUS	17.vi.2002	G. Bellis	3

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Depository	Host	Host plant family	Locality	Date	Collector	Number of slides	
0-063665	Ichnocarpus frutescens	Apocynaceae	Cape Tribulation, QLD, AUS	30.iv.2001	N. Bluthgen	1	
0-063675 to 76, 78 to 79	Carica papaya	Caricaceae	Doomadgee, QLD, AUS	23.iii.1999	J.F. Grimshaw	4	
0-063689, 91 to 93	Mangifera indica	Anacardiaceae	Katherine Research Station, NT, AUS	11.ii.1997	E.S.C. Smith	4	
0-063792 to 96	Ganophyllum falcatum	Sapindaceae	Pormpuraaw, QLD, AUS	25.iii.1999	G.N. Maynard	5	
0-063797 to 99	Psychotria poliostemma	Rubiaceae	Punsand Bay, QLD, AUS	22.ii.2002	J.F. Grimshaw	3	
0-063885	Unidentified plant	unidentified	Weipa, QLD, AUS	19.iii.1989		1	
0-063886 & 90	Pinus sp.	Pinaceae	AUS	5.xi.2002		2	
0-073618	Citrus × aurantifolia	Rutaceae	Lockhart, QLD, AUS	24.ix.2003	J. Grimshaw & B. Waterhouse	1	
0-074302 to 03 & 05	Pouteria sapota	Sapotaceae	Cape Tribulation, QLD, AUS	28.xi.2000	J. Grimshaw	3	
0-074350 to 55	Citrus × aurantifolia	Rutaceae	Yorke Island, QLD, AUS	27.v.2002	J. Grimshaw	6	
0-135251 & 52	Unidentified plant	unidentified	Bay, WA, AUS	4.iv.2006	L. Halling	2	
0-169692 & 94	Mangifera indica	Anacardiaceae	Bramwell Junction, QLD, AUS	12.vii.2013	D. Pearce & L. Benson	2	
WINC (Waite Insect and Nematode Collection, Adelaide, Australia)							
11252	Musa sp.	Musaceae	Kununurra, WA, AUS	17.x.1978	S.E. Learmouth	1	
11255	Mangifera indica	Anacardiaceae	Darwin, NT, AUS	13.v.1982	R. Lawrence	1	

Discussion

TABLE 4. (Continued)

Specimens of adult females resembling *Coccus hesperidum*, but in which the dorsal setae are bluntly rounded, clearly represent a distinct species from *C. hesperidum* s. s. They are genetically distinct from *C. hesperidum* s. s. (> 12.0% in *COI*) and appear to be more closely related to three ant-associated species of *Coccus* (*C. discrepans*, *C. formicarii* and *C. sulawesicus*) than they are to *C. hesperidum* s. s. (Fig. 2; Figs S2 & S3). The level of *COI* divergence between *C. hesperidum* s. s. and the specimens with bluntly rounded dorsal setae far exceeds the 2% threshold for species delimitation under the DNA-barcoding criterion suggested by Hajibabaei *et al.* (2006). More compellingly, the close relationship between specimens with bluntly rounded dorsal setae and the ant-associated species, rather than with *C. hesperidum* s. s., clearly indicates that they have been isolated from a common ancestor with *C. hesperidum* s. s. for a long time in spite of the otherwise very similar morphologies of the adult females.

Considering the level of DNA differentiation, combined with a fixed morphological difference (shape of the apices of dorsal setae), we conclude that specimens with the bluntly rounded dorsal setae represent a distinct species, *Coccus praetermissus* Lin & Tanaka **sp. n.**, under the species concept for asexual species explained by Lin *et al.* (2017a), that species are independently evolving genetic lineages that are differentiated in additional ways from other such lineages (here, morphologically).



FIGURE 2. The Maximum Clade Credibility (MCC) tree from analysis of the concatenated dataset (2827 bp). Specimen codes of *Coccus hesperidum* s. s. (apices of dorsal setae pointed) are in dark blue and those of *C. praetermissus* **sp. n.** (apices of dorsal setae bluntly rounded) are in light blue. The tree was rooted using *C. penangensis*. Branch support is indicated on internal branches (MP bootstrap/Bayesian posterior probability). Only bootstrap values \geq 70% and posterior probabilities \geq 0.95 are shown. The coloured squares under branches indicate that the branch was present in analyses of that gene. Branch support from individual genes are not shown within *C. formicarii* (Chinese and Taiwanese populations) and *C. hesperidum* s. s. Abbreviations as per Table 1.

Species and Code	GenBank accession no. (18S)	GenBank accession no. (28S)	GenBank accession no. (COI)	GenBank accession no. (<i>EF-1α</i>)	GenBank accession no. (wingless)
Coccus praeterm	issus sp. nov.				
YPL00122	MF594276	MF594312	MF579673	MF594365	MF579614
YPL00291	MF594277	MF594313	MF579674	MF594366	MF579615
YPL00465	MF594278	MF594314	MF579675	MF594367	MF579616
YPL00496	MF594279	MF594315	MF579676	MF594368	MF579617
YPL00716	MF594280	MF594316	MF579677	MF594369	MF579618
YPL00732	MF594281	MF594317	MF579678	MF594370	MF579619
C. discrepans (G	reen)				
YPL00710	MF579697	MF579700	MF579681	MF594380	MF579622
C. formicarii (Gr	een)				
YPL00094	JX566901	JX866687	JX853902	MF594371	MF579623
YPL00108	JX853914	JX866688	JX845483	MF594372	MF579624
YPL00485	JX853915	JX866689	JX853903	MF594373	MF579625
YPL00488	JX853916	JX866690	JX853904	MF594374	MF579626
YPL00515	JX853917	JX866691	JX853905	MF594375	MF579627
TK0511	JX853918	JX866692	JX853906	MF594376	MF579628
				MF594377	
C. hesperidum se	nsu stricto				
YPL00009	MF594246	MF594282	MF579682	MF594318	MF579629
YPL00076	JX566902	JX627324	JX843722	MF594319	KY798537
YPL00128	MF594247	MF594283	MF579683	MF594320	MF579630
YPL00247	MF594248	MF594284	MF579684	MF594321	MF579631
YPL00286	JX566903	JX627325	MF579685	MF594322	MF579632
				MF594323	
YPL00334	MF594249	MF594285	MF579686	MF594324	MF579633
YPL00363	MF594250	MF594286	MF579687	MF594325	MF579634
YPL00377	JX566904	JX627326	JX843723	MF594326	MF579635
YPL00380	MF594251	MF594287	MF579688	MF594327	MF579636
YPL00388	JX566905	JX627327	JX843724	MF594328	MF579637
YPL00390	JX566906	JX627328	JX843725	MF594329	MF579638
YPL00392	JX566907	JX627329	JX845472	MF594330	MF579639
YPL00395	MF594252	MF594288	MF579689	MF594331	MF579640
YPL00438	JX566908	JX627330	JX845473	MF594332	MF579641
YPL00441	MF594253	MF594289	MF579690	MF594333	MF579642
YPL00481	JX566909	JX627331	JX845474	MF594334	MF579643
YPL00506	JX566910	JX627332	JX845475	MF594335	MF579644
YPL00517	MF594254	MF594290	MF579691	MF594336	MF579645
YPL00524	MF594255	MF594291	MF579692	MF594337	MF579646
YPL00533	JX566911	JX627333	MF579693	MF594338	MF579647
YPL00541	JX566912	JX645346	MF579694	MF594339	MF579648
YPL00546	JX566913	JX645347	JX845476	MF594340	MF579649

TABLE 5. Sequences used in this study.

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TABLE 5. (Continued)
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Species and Code	GenBank accession no. (18S)	GenBank accession no. (28S)	GenBank accession no. (COI)	GenBank accession no. (<i>EF-1α</i>)	GenBank accession no. (wingless)
YPL00557	JX566914	JX645348	JX845477	MF594341	MF579650
YPL00568	MF594256	MF594292	MF594224	MF594342	MF579651
				MF594343	
YPL00642	MF594257	MF594293	MF594225	MF594344	MF579652
YPL00661	MF594258	MF594294	MF594226	MF594345	MF579653
YPL00666	MF594259	MF594295	MF594227	MF594346	MF579654
YPL00675	MF594260	MF594296	MF594228	MF594347	MF579655
YPL00696	MF594261	MF594297	MF594229	MF594348	MF579656
YPL00707	MF594262	MF594298	MF594230	MF594349	MF579657
YPL00713	MF594263	MF594299	MF594231	MF594350	MF579658
YPL00725	MF594264	MF594300	MF594232	MF594351	MF579659
YPL00727	MF594265	MF594301	MF594233	MF594352	MF579660
YPL00731	MF594266	MF594302	MF594234	MF594353	MF579661
YPL00735	MF594267	MF594303	MF594235	MF594354	MF579662
YPL00739	MF594268	MF594304	MF594236	MF594355	MF579663
YPL00758	MF594269	MF594305	MF594237	MF594356	MF579664
YPL00777	MF594270	MF594306	MF594238	MF594357	MF579665
YPL00785	MF594271	MF594307	MF594239	MF594358	MF579666
YPL00786	MF594272	MF594308	MF594240	MF594359	MF579667
YPL00787	MF594273	MF594309	MF594241	MF594360	MF579668
YPL00788	MF594274	MF594310	MF594242	MF594361	MF579669
TK0051	JX566915	JX645349	MF594243	MF594362	MF579670
TK0193	JX566916	JX645350	MF594244	MF594363	MF579671
TK0214	MF594275	MF594311	MF594245	MF594364	MF579672
C. penangensis N	Aorrison				
YPL00536	MF579695	MF579698	MF579679	MF594378	MF579620
C. sulawesicus G	avrilov				
YPL00571	MF579696	MF579699	MF579680	MF594379	MF579621

Specimens currently labelled as *Coccus hesperidum* in the BMNH, multiple Australian institutions and the Łagowska Laboratory comprise a mix of *C. hesperidum* s. s. and *C. praetermissus* **sp. n.** Those in the BMNH have not been fully enumerated here because not all the slides were studied. It appears from samples of *C. praetermissus* **sp. n.** collected during this study, and from those slide-mounted specimens held in Australian state and national collections, that this species might be restricted to tropical regions of eastern Asia and Oceania including Australia, whereas *C. hesperidum* s. s. is much more widespread. The Polish specimens examined in Łagowska (1999) were collected on exotic plants in an artificial environment (a greenhouse) and included three species, namely *C. hesperidum*, *C. praetermissus* **sp. n.** and *C. moestus* De Lotto. The shape of the dorsal setae of *C. moestus* is similar to that of *C. praetermissus* **sp. n.**, but the former species has many dorsal tubular ducts that are always larger than those on the venter (Williams & Watson 1990). The occurrence of these species in Poland probably resulted from human transportation of infested plants. The other species of *Coccus* that are closest relatives of *C. praetermissus* **sp. n.** (Fig. 2) also are restricted to Asia and Oceania (García Morales *et al.* 2016) so it is possible that the group, including *C. hesperidum* s. s., has its biogeographic origins in this region. This hypothesis needs to be tested with phylogenetic analyses that include more species of *Coccus* from a broad geographic sampling before strong conclusions can be drawn.

There is considerable genetic differentiation within *C. praetermissus* **sp. n.** and, in our analyses of *COI*, specimens fall into two clades that are about 4.0-5.0% divergent. These two clades are also supported in analyses of *EF*-1 α (Fig. S3). If applying only a general lineage species concept (De Queiroz 1998), these two clades could be argued to represent distinct species. We do not do that here because, currently, specimens in one clade are all from Taiwan and in addition there is not yet evidence of differentiation other than genetic (see arguments for this criterion for asexual lineages in Lin *et al.* 2017a). We exclude the specimens from Taiwan from the formal description of *C. praetermissus* **sp. n.** due to the uncertainty of their distinctness from the specimens from Australia, Malaysia and Thailand.

Taxonomy

Coccus praetermissus sp. n. Lin & Tanaka

(Fig. 3) urn:lsid:zoobank.org:act:68DA1396-37BF-4C1A-8126-562AE4CC1BFC

Material examined. *Holotype*. Adult female (ID: YPL00716). Australia: Queensland, Shelburne, Bramwell Junction, -12.09° S, 142.56° E, on *Mangifera indica* (Anacardiaceae), 12.vii.2013, D. Pearce and L. Benson (ANIC: 1/1 female). GenBank accession numbers: *18S*: MF594280; *28S*: MF594316; *COI*: MF579677; *EF-1α*: MF594369; *wingless*: MF579618.

Paratype. Adult female (ID: YPL00465). Malaysia: Kuala Lumpur, on *Ixora chinensis* (Rubiaceae), 13.xii.2010, Y.-P. Lin (ANIC: 1/1 female). GenBank accession numbers: *18S*: MF594278; *28S*: MF594314; *COI*: MF579675; *EF-1a*: MF594367; *wingless*: MF579616.

Paratype. Adult female (ID: YPL00732). Thailand: Ranong, on *Rhizophora mucronata* (Rhizophoraceae), iv.2003, J. Offenberg (ANIC: 1/1 female). GenBank accession numbers: *18S*: MF594281; *28S*: MF594317; *COI*: MF579678; *EF*-1α: MF594370; *wingless*: MF579619.

The three specimens from Taiwan, which are morphologically similar to *Coccus praetermissus* **sp. n.** but for which morphological measurements are not included in the species description, are as follows:

Adult female (ID: YPL00122). Taiwan: Suao Port, Suao, Yilan County, on *Ficus irisana* (Moraceae), 7.ii.2009, Y.-P. Lin (ANIC: 1/1 female). GenBank accession numbers: *18S*: MF594276; *28S*: MF594312; *COI*: MF579673; *EF-1α*: MF594365; *wingless*: MF579614.

Adult female (ID: YPL00291-1). Taiwan: Kenting National Park, Hengchuen, Pingtung County, on *Calophyllum inophyllum* (Clusiaceae), 24.viii.2009, Y.-P. Lin (ANIC: 1/1 female). GenBank accession numbers: *18S*: MF594277; *28S*: MF594313; *COI*: MF579674; *EF-1a*: MF594366; *wingless*: MF579615.

Adult female (ID: YPL00496). Taiwan: Chiayi City, on *Podocarpus costalis* (Podocarpaceae), 24.viii.2009, Y.-P. Lin (ANIC: 1/1 female). GenBank accession numbers: *18S*: MF594279; *28S*: MF594315; *COI*: MF579676; *EF*-*1α*: MF594368; *wingless*: MF579617.

Other specimens examined that are considered to be Coccus praetermissus sp. n. are listed in Table 4.

Diagnosis. Adult females of Coccus praetermissus **sp. n.** can be identified by the following combination of morphological character states; (i) dorsal setae with blunt, rounded apices; (ii) dorsal tubular ducts, if present, situated around submarginal areas; (iii) anal plates together quadrate, with anterior margin subequal in length to posterior margin; (iv) antennae each with 7 segments; (v) ventral tubular ducts present on medial area near mesocoxae; (vi) each leg with a weak tibio-tarsal sclerosis. The only character of adult females that can be used to differentiate *C. praetermissus* **sp. n.** from *C. hesperidum* s. s. is the shape of the dorsal setae.

DNA sequence-based diagnoses (fixed differences between *C. praetermissus* **sp. n.** and *C. hesperidum* s. s., mapped to the GenBank reference sequence listed) are as follows:

18S: Reference sequence: *Coccus hesperidum* s. s. (ID: YPL00377): GenBank accession number: JX566904. No fixed difference existed.

28S: Reference sequence: *Coccus hesperidum* s. s. (ID: YPL00377): GenBank accession number: JX627326. Site# 120 (C), 141 (C), 165 (C), 180 (A), 186 (G), 201 (C), 593 (C).

COI: Reference sequence: *Coccus hesperidum* s. s. (ID: YPL00377): GenBank accession number: JX843723. Site# 10 (T), 12 (A), 18 (T), 41 (G), 51 (C), 66 (A), 69 (C), 72 (C), 75–76 (CC), 79 (C), 87 (C), 99 (A), 105 (A),

108 (C), 114 (C), 133 (T), 135 (A), 138 (C), 147 (A), 151 (G), 165 (A), 174 (A), 186 (T), 213–214 (AC), 216 (T), 229 (C), 249 (C), 279 (A), 282 (T), 300–301 (AA), 309 (C), 318 (C), 324 (A), 327–328 (TT), 330 (A), 334 (T), 339 (T), 343 (C), 369 (C), 381 (A), 399 (T), 411–412 (TA), 415 (A), 427 (C), 432 (T), 447 (C), 456 (T), 460 (T), 477 (T), 480 (T), 489 (T), 507 (C), 513 (G), 516 (C), 528 (T), 537 (C), 543 (C), 561 (T), 579 (T).

EF-1a: Reference sequence: *Coccus hesperidum* (ID: YPL00377): GenBank accession number: MF594326. Site# 18 (C), 99 (G), 105 (G), 114 (T), 121 (T), 160 (G), 162–166 (-), 184 (T), 199 (G), 241 (T), 298 (T), 326 (T), 334 (G), 355 (T), 391 (G), 394 (C), 427 (C), 443 (A), 453 (A), 456–457 (CC), 462 (T), 464–467 (ATTG), 471 (A), 477 (C), 492 (G), 498 (G), 503 (T).

wingless: Reference sequence: *Coccus hesperidum* (ID: YPL00377): GenBank accession number: MF579635. Site# 174 (T), 192 (C), 207 (A), 306 (G).

Description. Adult female (Fig. 3): description based on three specimens, each on a separate slide, the holotype (YPL00716) in good condition and two paratypes (YPL00465 and YPL00732) in fair condition.

Slide-mounted material. Body elongate oval, 3.0-3.8 mm long, 1.5-3.0 mm wide, margin with a shallow indentation at each stigmatic cleft; anal cleft 1/5-1/7 body length.

Dorsum. Derm membranous throughout when young. Dermal areolations well developed in old females, indicating slight sclerotisation. Dorsal setae frequent throughout, relatively short and with blunt apices, each 5–10 μ m long, 1–1.5 μ wide on shaft with a well-developed basal socket. Preopercular pores small, 3–4 μ m in diameter, barely sclerotised, present in diffuse group of 4–17 pores anterior to anal plates. Dorsal tubular ducts each with a thin outer ductule, shallow cup-shaped invagination, and fine inner ductule with a small terminal gland, found only on submarginal areas of abdomen. Dorsal microducts relatively evenly and sparsely distributed throughout dorsum. Dorsal tubercles present submarginally, each tubercle simple; with 1 pair on head, 0 or 1 pair between stigmatic clefts, 0 or 1 pair on abdomen. Anal plates together quadrate, with anterior margin 90–105 μ m and posterior margin 93–108 μ m long; each plate with well-developed supporting bar and 4 fine apical setae; length of plates 130–203 μ m; maximum width of single plate 70–87 μ m. Ano-genital fold with 2 or 3 pairs of setae along anterior margin and 2 or 3 pairs laterally. Anal ring bearing 6 setae. Eye spot present on margin.

Margin. Marginal setae spinose, each 12–51 μ m long, with a well-developed basal socket and typically with a fimbriate apex but can appear pointed; 10–18 setae present on each side between stigmatic clefts; 38–45 setae on head between anterior stigmatic clefts of each side; 24–38 setae on each side of abdomen posterior to posterior stigmatic cleft. Stigmatic clefts shallow but indented, each cleft containing 3 stigmatic spines, median spine much the longest, 40–55 μ m long, about 3–4 times as long as a lateral spine.

Venter. Derm entirely membranous. Pregenital disc-pores each with 9–11 loculi (mostly 10), present around genital opening and medio-lateral area of preceding two segments. Spiracular disc-pores each with 5 loculi, present between margin and each spiracle in band 1–3 pores wide; anterior bands each containing 13–33 pores, posterior bands each containing 16–41 pores. Ventral microducts relatively evenly and sparsely distributed throughout venter; 0 or 1 preantennal pores present near base of each antenna. Ventral tubular ducts of one type, each with long narrow outer ductule, fine inner ductule and well-developed terminal gland; present in medial area near mesocoxae in a group of 2 or 3. Ventral setae: with 3 pairs of long pregenital setae and 1–3 pairs of long setae between antennae; other setae short and fine. Spiracles each composed of a sclerotised, funnel-shaped outer peritreme, which leads through spiracular opening into tracheae; width of each peritreme: in anterior spiracle 30–45 μ m, posterior 38–59 μ m. Legs well developed, each with small tibio-tarsal articulation and small articulatory sclerosis; claw without denticle; both claw digitules rather broad and slightly shorter than thin tarsal digitules; trochanter + femur 148–191 μ m, tibia 82–132 μ m, and tarsus 67–100 μ m. Antennae each with 7 segments; total length 230–357 μ m. Labium approximately 71–118 μ m wide, 135–165 μ m long.

Etymology. The species epithet *praetermissus* is Latin for "overlooked", and refers to the specimens of this species having long been considered conspecific with *Coccus hesperidum* s. s.

Remarks. *Coccus praetermissus* **sp. n.** is morphologically very similar to *C. hesperidum* and it might be difficult to distinguish them if good slide mounts of adult females are not available. Therefore, we recommend that both morphological (having dorsal setae with blunt apices) and molecular (COI DNA) data should be used when an authoritative identification is required.

Among the examined slides labelled/identified as "Coccus hesperidum" deposited in the BMNH, there are five with a manuscript name from E.E. Green on the labels, "Lecanium holosericeae" (citation of this manuscript name here is not intended to be for nomenclatural purposes; it is not an available name). These specimens were collected



FIGURE 3. Adult female of *Coccus praetermissus* Lin & Tanaka, **sp. n.** ANT: antenna; AP: anal plate; DA: dorsal areolations; DMD: dorsal microduct; DS: dorsal seta; DT: dorsal tubercle; DTD: dorsal tubular duct; LG: leg; MP: multilocular pore; MS: marginal setae; PAP: preantennal pore; POP: preopercular pore; SP: spiracular pore; SSP: stigmatic spines; VMD: ventral microduct; VTD: ventral tubular duct. Scale bars: 200 µm for ANT, AP; 100 µm for DA, LG; 50 µm for SSP; 10 µm for other details.

in Darwin (Northern Territory, Australia) on *Acacia holosericea* by G.F. Hill on 3.xi.1915. They are morphologically indistinguishable from *C. praetermissus* **sp. n.**, having dorsal setae with bluntly rounded apices (noted on one slide label in D.J. Williams's handwriting as "cylindrical setae"). Dr D.J. Williams mounted some specimens from this sample in Green's collection (seven slides) and identified them as *C. hesperidum* on 30.iii.1954. Green's manuscript name was never published.

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