# 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 $\mathbf{s p} . \mathbf{n}$. 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 $19^{\text {th }}$ and early $20^{\text {th }}$ 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 Łagowska (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 (SaakyanBaranova 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 reproduction in C. hesperidum s. s. and C. formicarii (a close relative) is asexual, so species concepts based on 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 Lagowska 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. GavrilovZimin) who described the species.

Insects collected in the field were killed and preserved in absolute ethanol ( $>99.5 \%$ ), and then stored at $4^{\circ} \mathrm{C}$. 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 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 ( $18 S 5$, region) and LSU ( $28 S$ D2 and D3 regions) rRNA genes, $E F-1 \alpha$ (nDNA), wingless (nDNA) and $C O I$ (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-1 $\alpha$ 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 praetermissus sp. n. |  |  |  |  |  |
| YPL00122 | Ficus irisana | Moraceae | Yilan County, TWN | 7.ii. 2009 | Y.-P. Lin |
| YPL00291 | Calophyllum inophyllum | Clusiaceae | Pingtung County, TWN | 24.viii. 2009 | Y.-P. Lin |
| YPL00465 | Ixora chinensis | Rubiaceae | Kuala Lumpur, MYS | 13.xii. 2010 | Y.-P. Lin |
| YPL00496 | Podocarpus costalis | Podocarpaceae | Chiayi City, TWN | 5.ii. 2011 | Y.-P. Lin |
| YPL00716 | Mangifera sp. | Anacardiaceae | Shelburne, QLD, AUS | 12.vii. 2013 | D. Pearce \& L Benson |
| YPL00732 | Rhizophora mucronata | Rhizophoraceae | Ranong, THA | iv. 2003 | J. Offenberg |
| C. hesperidum sensu stricto |  |  |  |  |  |
| YPL00009 | Lagerstroemia speciosa | Lythraceae | Chiayi City, TWN | 30.x. 2008 | Y.-P. Lin |
| YPL00076 | Morus sp. | Moraceae | Brisbane, QLD, AUS | 20.xi. 2008 | Y.-P. Lin |
| YPL00128 | Bauhinia variegata | Fabaceae | Brisbane, QLD, AUS | 15.iii. 2009 | Y.-P. Lin |
| YPL00247 | Heteromeles arbutifolia | Rosaceae | Davis, CA, USA | 1.iv. 2009 | Y.-P. Lin |
| YPL00286 | Tecoma stans | Bignoniaceae | Pretoria, ZAF | 4.v. 2009 | I. Miller |
| YPL00334 | Messerschmidia argentea | Boraginaceae | Pingtung County, TWN | 10.xii. 2009 | Y.-P. Lin |
| YPL00363 | Schefflera arboricola | Araliaceae | Taipei City, TWN | 19.ii. 2010 | Y.-P. Lin |
| YPL00377 | Ilex aquifolium | Aquifoliaceae | Berkshire, GBR | 4.iv. 2010 | Y.-P. Lin |
| YPL00380 | Camellia japonica | Theaceae | Crete, GRC | 6.iv. 2010 | Y.-P. Lin |
| YPL00388 | Eriobotrya japonica | Rosaceae | Crete, GRC | 9.iv. 2010 | Y.-P. Lin |
| YPL00390 | Elaeagnus thunbergii | Elaeagnaceae | London, GBR | 11.iv. 2010 | Y.-P. Lin |
| YPL00392 | Lonicera macrantha | Caprifoliaceae | London, GBR | 11.iv. 2010 | Y.-P. Lin |
| YPL00395 | Laurus nobilis | Lauraceae | London, GBR | 11.iv. 2010 | Y.-P. Lin |
| YPL00438 | Laurus nobilis | Lauraceae | Valencia, ESP | 15.v. 2010 | A. Beltrà-Ivars |
| YPL00441 | Agave sp. | Agavaceae | Brisbane, QLD, AUS | 7.x. 2010 | Y.-P. Lin |
| YPL00481 | Aphananthe aspera | Ulmaceae | Taitung County, TWN | 20.i. 2011 | Y.-P. Lin |
| YPL00506 | Celtis formosana | Ulmaceae | Miaoli County, TWN | 13.v. 2011 | Y.-P. Lin |
| YPL00517 | Monstera deliciosa | Araceae | Kuala Lumpur, MYS | 12.xii. 2010 | Y.-P. Lin |
| YPL00524 | Camellia sp. | Theaceae | Melbourne, VIC, AUS | 5.xii. 2011 | Y.-P. Lin |
| YPL00533 | Melaleuca sp. | Myrtaceae | Newnes, NSW, AUS | 12.xii. 2011 | Y.-P. Lin |
| YPL00541 | Phoradendron californicum | Santalaceae | Davis, CA, USA | 17.iii. 2004 | T. Kondo |
| YPL00546 | Arbutus unedo | Ericaceae | Canberra, ACT, AUS | 21.xii. 2011 | Y.-P. 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 |

[^0]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 Clivia sp. | Amaryllidaceae | Taiyuan, Shanxi, CHN | 24.v. 2014 | Y.-P. 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 | Y.-P. Lin |
| YPL00707 Radermachera sinica | Bignoniaceae | Shanghai, CHN | 8.iii. 2015 | Y.-P. 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 | Y.-P. Lin |
| YPL00727 Unidentified plant | unidentified | London, GBR | 1.viii. 2015 | Y.-P. Lin |
| YPL00731 Laurus nobilis | Lauraceae | Exeter, Devon, GBR | iv. 2015 | C.J. Hodgson |
| YPL00735 Ocimum basilicum | Lamiaceae | Cairns, QLD, AUS | 27.ix. 2015 | Y.-P. Lin |
| YPL00739 Carica papaya | Caricaceae | Calavi, BEN | 12.v. 2015 | G. Goergen |
| YPL00758 Schefflera arboricola | Araliaceae | Amelup, WA, AUS | 29.xi. 2015 | Y.-P. Lin |
| YPL00777 Plumeria obtusa | Apocynaceae | Fremantle, WA, AUS | 11.xii. 2015 | Y.-P. 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 |
| OUTGROUPS |  |  |  |  |
| C. discrepans (Green) |  |  |  |  |
| YPL00710 Unidentified plant | unidentified | Ramu River Basin, Madang Province, PNG | 29.iii. 2007 | P. Klimeš |
| C. formicarii (Green) |  |  |  |  |
| YPL00094 Ficus microcarpa | Moraceae | New Taipei City, TWN | 21.i. 2009 | Y.-P. Lin |
| YPL00108 Litsea glutinosa | Lauraceae | Kinmen, TWN | 1.ii. 2009 | Y.-P. Lin |
| YPL00485 Machilus thunbergii | Lauraceae | Taitung County, TWN | 20.i. 2011 | Y.-P. Lin |
| YPL00488 Camellia chrysanthera | Theaceae | Guangzhou City, CHN | 25.i. 2011 | Y.-P. Lin |
| YPL00515 Ficus virgata | Moraceae | Taichung City, TWN | 4.vi. 2011 | Y.-P. Lin |
| TK0511 Mangifera indica | Anacardiaceae | Nakhorn Pathom, THA | 4.vi. 2007 | T. Kondo |
| C. penangensis Morrison |  |  |  |  |
| YPL00536 Macaranga bancana | Euphorbiaceae | Central Catchment, SGP | 18.iii. 2009 | P.S. Cranston |
| C. sulawesicus Gavrilov |  |  |  |  |
| YPL00571 Dicotyledonous shrub | unidentified | Kendari, Sulawesi, IDN | 11.xi. 2011 | I.A. Gavrilov- <br> 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 $E F-1 \alpha$ 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').

TABLE 2. Primers and PCR protocols used.

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

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 ( $18 S$ and $28 S$ ) were aligned manually. For the three protein-encoding regions (COI, EF-1 $\alpha$ 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 $E F-1 \alpha$ were detected by using the GT-AG rule (Rogers \& Wall 1980). All introns of $E F-1 \alpha$ can be unambiguously aligned with outgroups, and copies of $E F-1 \alpha$ obtained from cloning were included in our analyses.

Phylogenetic analysis. 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 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 $\mathrm{BS} \geq 70$ (Hillis \& Bull 1993) and $\mathrm{PP} \geq 0.95$ (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 $E F-1 \alpha$. For the three proteincoding genes, $C O I, E F-1 \alpha$ 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 $18 S$ and $28 S$ 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 $E F-1 \alpha$ because of their unequal base compositions and numerous variable sites. For the $18 S+28 S$ 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 ( $18 S+28 S$; wingless; concatenated) or 60 million (COI; EF-1 $\alpha$ ) 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 $29(\mathrm{CI}=0.97, \mathrm{RI}=0.99)$ for the $18 S+28 S$ dataset, 73 trees of length $317(\mathrm{CI}=0.67, \mathrm{RI}=0.92)$ for the $C O I, 217$ trees of length $143(\mathrm{CI}=0.81, \mathrm{RI}=0.96)$ for the $E F-1 \alpha$, six trees of length $22(\mathrm{CI}=0.91, \mathrm{RI}=0.97)$ for the wingless and 13 trees of length $463(\mathrm{CI}=0.70, \mathrm{RI}=0.93)$ for the concatenated datasets. The required burn-in proportions for Bayesian runs were $95 \%$ ( $C O I$ and wingless), $90 \%$ ( $18 S+28 S$ ), $83 \%(E F-1 \alpha)$ 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 $\alpha$ 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 $C O I$ (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 $C O I$ (Fig. S2), $E F-1 \alpha$ (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.

TABLE 3. Data of the synonyms of Coccus hesperidum studied.

| Synonym | Author (year, page) | Collecting data |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Depository | Country (area) | Host | Type of dorsal setae | Examiner |
| Primary type (s) examined |  |  |  |  |  |  |
| Lecanium alienum | Douglas (1886, 77$)$ | $\mathrm{BMNH}^{1}$ | 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) | $\mathrm{MNHN}{ }^{3}$ | Cyprus | Ceratonia siliqua | pointed | $\mathrm{DM}^{8} / \mathrm{JG}^{9}$ |
| L. nanum | Cockerell (1896, 19) | USNM | Trinidad | Manilkara bidentata | pointed | SS |
| L. flaveolum | Cockerell (1897, 52) | USNM | U.S.A. <br> (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) | Begonia 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 (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 | ? | $\mathrm{HT}^{10}$ |
| Primary type (s) unable to examine |  |  |  |  |  |  |
| L. maculatum | Signoret (1873, 400) | NMW ${ }^{5}$ | France | Hedera helix | ? | - |

${ }^{1}$ BMNH: The Natural History Museum, London, U.K.
${ }^{2}$ USNM: U.S. National Museum of Natural History, District of Columbia, U.S.A.
${ }^{3}$ MNHN: Museum National d'Histoire Naturelle, Paris, France
${ }^{4}$ NIAES: National Institute of Agricultural Environmental Sciences, Tsukuba, Japan
${ }^{5}$ NMW: Naturhistorisches Museum Wien, Austria
${ }^{6}$ YPL: Yen-Po Lin
${ }^{7}$ SS: Scott A. Schneider
${ }^{8}$ DM: Danièle Matile-Ferrero
${ }^{9}$ JG: Jean-François Germain
${ }^{10} \mathrm{HT}$ : 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 National Insect Collection, Canberra, Australia) |  |  |  |  |  |  |
|  | Mangifera indica | Anacardiaceae | PHL | 26.viii. 1985 | B.J. Read | 1 |
| ASCU (Agricultural Scientific Collections Unit, Orange, Australia) |  |  |  |  |  |  |
| ASCTHE101667 | Mangifera indica | Anacardiaceae | PHL | 26.viii. 1985 | B.J. Read | 1 |
| BMNH (The Natural History Museum, London, 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, <br> Phoenix Islands, KIR | 9.xi. 1964 | R. Holuray | 1 |
|  | Hibiscus tiliaceus | Malvaceae | N. Male Atoll, Baros, MDV | 19.iii. 1993 | D.S./G.W. <br> 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 |
|  | Timonius sp. | Rubiaceae | Buso, Morobe <br> 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 <br> 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. Lagowska | 8 |
|  | Schefflera actinophylla | Araliaceae | Lublin, POL | 26.ix. 1995 | B. Łagowska | 6 |
| QDPC (Queensland Primary Industries Insect Collection, Brisbane, Australia) |  |  |  |  |  |  |
| 0-063660 to 62 | Mangifera indica | Anacardiaceae | Cape Don, NT, AUS | 17.vi. 2002 | G. Bellis | 3 |

TABLE 4. (Continued)

| 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 |
| $\begin{aligned} & 0-063675 \text { to } 76,78 \text { to } \\ & 79 \end{aligned}$ | Carica papaya | Caricaceae | Doomadgee, QLD, AUS | 23.iii. 1999 | J.F. Grimshaw | 4 |
| 0-063689, 91 to 93 | Mangifera indica | Anacardiaceae | Katherine <br> 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 $\times$ 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 $\times$ 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 <br> Junction, QLD, AUS | 12.vii. 2013 | D. Pearce \& L. <br> Benson | 2 |
| WINC (Waite Insect and Nematode Collection, Adelaide, Australia) |  |  |  |  |  |  |
| 11252 | Musa sp. | Musaceae | Kununurra, WA, AUS | 17.x. 1978 | S.E. <br> Learmouth | 1 |
| 11255 | Mangifera indica | Anacardiaceae | Darwin, NT, AUS | 13.v. 1982 | R. Lawrence | 1 |

## Discussion

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 $C O I$ ) 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 $\mathbf{s p}$. $\mathbf{n}$. (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.

TABLE 5. Sequences used in this study.

| Species and Code | GenBank accession no. (18S) | GenBank accession no. $(28 S)$ | GenBank accession no. (COI) | GenBank accession no. (EF-I $\alpha$ ) | GenBank accession no. (wingless) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Coccus praetermissus 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 (Green) |  |  |  |  |  |
| YPL00710 | MF579697 | MF579700 | MF579681 | MF594380 | MF579622 |
| C. formicarii (Green) |  |  |  |  |  |
| 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 sensu 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 |

......continued on the next page

TABLE 5. (Continued)

| Species and Code | GenBank accession no. (18S) | GenBank accession no. (28S) | GenBank accession no. (COI) | GenBank accession no. (EF-1 $\alpha$ ) | 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 Morrison |  |  |  |  |  |
| YPL00536 | MF579695 | MF579698 | MF579679 | MF594378 | MF579620 |
| C. sulawesicus Gavrilov |  |  |  |  |  |
| 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 $\mathbf{s p}$. $\mathbf{n}$. 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 $\mathbf{s p}$. 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 $E F-1 \alpha$ (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:Isid:zoobank.org:act:68DA1396-37BF-4C1A-8126-562AE4CC1BFC

Material examined. Holotype. Adult female (ID: YPL00716). Australia: Queensland, Shelburne, Bramwell Junction, $-12.09^{\circ} \mathrm{S}, 142.56^{\circ} \mathrm{E}$, on Mangifera indica (Anacardiaceae), 12.vii.2013, D. Pearce and L. Benson (ANIC: $1 / 1$ female). GenBank accession numbers: $18 S$ : MF594280; 28S: MF594316; COI: MF579677; EF-1 $\alpha$ : 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-1 : 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-1 $\alpha$ : MF594370; wingless: MF579619.

The three specimens from Taiwan, which are morphologically similar to Coccus praetermissus $\mathbf{s p}$. 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-1 $\alpha$ : 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-1 $\alpha$ : 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$1 \alpha$ : 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),

EF-1 $\alpha$ : 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, $3.0-3.8 \mathrm{~mm}$ long, $1.5-3.0 \mathrm{~mm}$ 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 $\mu \mathrm{m}$ long, $1-1.5 \mu$ wide on shaft with a well-developed basal socket. Preopercular pores small, 3-4 $\mu \mathrm{m}$ 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 $90-105 \mu \mathrm{~m}$ and posterior margin $93-108 \mu \mathrm{~m}$ long; each plate with well-developed supporting bar and 4 fine apical setae; length of plates 130-203 $\mu \mathrm{m}$; maximum width of single plate $70-87 \mu \mathrm{~m}$. 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 $12-51 \mu \mathrm{~m}$ 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, $40-55 \mu \mathrm{~m}$ 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 $30-45 \mu \mathrm{~m}$, posterior 38-59 $\mu \mathrm{m}$. 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 $\mu \mathrm{m}$, tibia $82-132 \mu \mathrm{~m}$, and tarsus $67-100 \mu \mathrm{~m}$. Antennae each with 7 segments; total length 230-357 $\mu \mathrm{m}$. Labium approximately $71-118 \mu \mathrm{~m}$ wide, $135-165 \mu \mathrm{~m}$ 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 pores; SP: spiracular pore; SSP: stigmatic spines; VMD: ventral microduct; VTD: ventral tubular duct. Scale bars: $200 \mu \mathrm{~m}$ for ANT, AP; $100 \mu \mathrm{~m}$ for DA, LG; $50 \mu \mathrm{~m}$ for SSP; $10 \mu \mathrm{~m}$ 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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