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ORIGINAL ARTICLE

Primary parasitoids of red scale (*Aonidiella aurantii*) in Australia and a review of their introductions from Asia

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Abstract We used morphological and molecular differences to confirm the identities of red scale (*Aonidiella aurantii*) and yellow scale (*A. citrina*), and their primary parasitoids, in Australia. An extension to the distribution of yellow scale was confirmed. Six primary parasitoids of red scale were identified: *Aphytis chrysomphali*, *A. lingnanensis*, *A. melinus*, *Comperiella bifasciata*, *Encarsia citrina*, and *E. perniciosi*. With the exception of *A. lingnanensis*, these parasitoids, and a species of *Aphelinus*, were detected in association with red scale during studies in citrus orchards in coastal New South Wales between 2009 and 2012. Two races of *A. melinus* were recorded: one from the Indian Subcontinent, the other previously only recorded in China. The studies, and reviews of historical records, led us to conclude that 4 parasitoids, *A. lingnanensis*, *C. bifasciata*, and both species of *Encarsia*, were present in Australia before successful or unsuccessful formal introductions between 1902 and 1970. The *A. melinus* race previously recorded in China may also have been present before the Indian Subcontinent race was formally introduced in 1961. We suggest the possibility that the natural distribution of some of the parasitoids may include East and Southeast Asia, and parts of Australasia. We found no reports of native armored scales being recorded on species and hybrids of *Citrus* introduced to Australia, and no reports of introduced armored scales being recorded on native Rutaceae, including 6 species of *Citrus*. However, we subsequently recorded yellow scale on *Geijera parviflora*, a native rutaceous tree.

Key words *Aonidiella*; *Aphytis*; citrus; *Comperiella*; *Encarsia*; red scale

Introduction

Seven species of armored scales (Hemiptera: Sternorrhyncha: Diaspididae) of subtropical and tropical east Asian origin occur on introduced species and hybrids of *Citrus* cultivated in Australia: red scale *Aonidiella aurantii* (Maskell), yellow scale *A. citrina* (Coquillett), circular black scale *Chrysomphalus aonidum* (L.), purple scale *Lepidosaphes beckii* (Newman), Glover's scale *L. gloverii* (Packard), white louse scale *Unaspis citri* (Comstock), and chaff scale *Parlatoria pergandii* Comstock (Smith *et al.*, 1997). Red scale is a major pest in citrus orchards in all mainland states (Smith *et al.*, 1997). Yellow scale is a minor pest in citrus orchards in coastal New South Wales (NSW), in the Murray River regions of NSW, Victoria (VIC) and South Australia (SA) (Smith *et al.*, 1997), and in the Riverina region of NSW, where it was first recorded in 2006–2007 (Rob Weppler, Riverina IPM, pers. comm., 2012). Both scales are similar in appearance and difficult to distinguish *in situ* (Compere, 1961). Morphological determinations are generally based on the presence or absence of pygidial prevulvar scleroses in slide mounted specimens (McKenzie, 1937, 1938). Circular black scale is a minor pest in the Northern Territory (NT), Queensland (QLD), and the North Coast of NSW (Hely *et al.*, 1982; Smith *et al.*, 1997). Purple scale and white louse scale are occasional to major pests in the NT, QLD and coastal NSW. Glover's scale is a minor pest in QLD and coastal

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NSW, and chaff scale is a minor pest in coastal districts of southeast QLD and northern NSW (Smith *et al.*, 1997).

Six primary parasitoids of red scale occur in Australia: 3 ectoparasitoids, *Aphytis chrysomphali* (Mercet), *A. lingnanensis* Compere, *A. melinus* DeBach (Hymenoptera: Aphelinidae), and 3 endoparasitoids, *Encarsia citrina* (Craw), *E. perniciosi* (Tower) (Aphelinidae), and *Comperiella bifasciata* Howard (Encyrtidae). *Aphytis chrysomphali* is solitary or gregarious; hosts other than red scale include yellow scale and species of *Aspidiotus* and *Chrysomphalus* (Abdelrahman, 1974; Rosen & DeBach, 1978; Luck *et al.*, 1982; Malipatil *et al.*, 2000). *Aphytis lingnanensis* is gregarious, and hosts other than red scale include yellow scale, circular black scale, purple scale, Glover's scale, white louse scale and at least 7 other species of armored scales (Rosen & DeBach, 1979; Luck *et al.*, 1982; Malipatil *et al.*, 2000). *Aphytis melinus* is also gregarious (Abdelrahman, 1974). It parasitises red scale, yellow scale, and 7 other armored scale hosts, but not circular black scale, purple scale, Glover's scale or white louse scale (Abdelrahman, 1974; Rosen & DeBach, 1978, 1979). *Encarsia citrina* has host-specific races, the red scale and yellow scale races of which are morphologically indistinguishable (Flanders, 1950; Compere, 1961; Noyes, 2013). Three morphologically indistinguishable races of *E. perniciosi* are recognised (Rosen & DeBach, 1978; Stouthamer & Luck, 1991). *Comperiella bifasciata* is solitary, with distinct races that include a red scale race and a yellow scale race (Flanders, 1944; Compere, 1961; Rosen & DeBach, 1978; Noyes, 2013).

Molecular verification of the identities of the parasitoids, and records related to their presence in Australia, led us to conclude that most of the species were informally introduced to Australia through trade with, and migration from, Asia before they were formally introduced between 1902 and 1970. The records also suggest that the natural distibution of one or more of the species may include a geographical area encompassing East and Southeast Asia and parts of Australasia.

Materials and methods

Sources of specimens and general laboratory observations

Scale-infested leaves and/or fruit were collected from orchards in 5 states or territories of Australia and, for comparative purposes, from Java, Indonesia (Table 1). Host plants of armored scales in citrus orchards included Valencia, Washington (Bahia) navel and sweet oranges (*C.* × *aurantium* L.), grapefruit (*C.* × *aurantium* L.), mandarin (*C. reticulata* Blanco), and lemon (*C.* × *limon* (L.) Osbeck). Our nomenclature for *Citrus* species and varieties is based on Mabberley (2004) and Zhang *et al.* (2008).

Morphological identification of red scale and yellow scale

Fifty adult red scale and 70 yellow scale females were collected between 2008 and 2011 from the localities listed in Table 1. The general appearance of each scale was examined visually with and without the aid of stereomicroscopes from which photographs were taken.

Pygidial morphology was compared using fresh scales, or those preserved in 70% ethanol. Sandlant's (1978) method was superior to the procedure described by Wirth and Marston (1968) in yielding clearer specimens and was used for the majority of the work. With this method, specimens were placed in an excavated glass block with 50 drops of Essig's solution and heated in an oven at 60 °C for 3 h. The specimens were then carefully punctured with a fine needle and the body contents, including any embryos, teased out without destroying the taxonomic features. The specimens were then placed in 70% ethanol for 5–10 min and then in a solution comprising equal parts of 70% ethanol and chloroform for 5–10 min before dehydration in 100% ethanol for 5–10 min and mounting individually in Canada balsam on microscope slides.

Morphological identification of parasitoids

Specimens were collected from the locations given in Table 2. Specimens of *Aphytis* were initially separated into species by the presence or absence of characteristic pigmentation of thoracic and abdominal sternites in the "green-eye," pupal stage. During this stage, *A. lingnanensis* has a large, dark area on mid-thoracic sterna, *A. melinus* has a small, dark patch, and *A. chrysomphali* has no dark pigmentation but is readily identifiable by the presence of a longitudinal black line on the mesosternum (Rosen & DeBach, 1979). Once identified, each specimen was photographed and then set aside in 100% ethanol for molecular studies. For the *Encarsia* species, parasitised red scale, yellow scale and purple scale were kept individually in separate gelatine capsules until the adults emerged. Adults were then identified and set aside in 100% ethanol. *Comperiella bifasciata* adults were collected from parasitised hosts and stored in 100% ethanol.

Table 1 Host plants, host plant substrates and locations in New South Wales (NSW), Victoria (VIC), South Australia (SA), Western Australia (WA), the Northern Territory (NT), and Universitas Gadjah Mada (UGM), Yogyakarta, and Ngablak, Central Java, Indonesia, from which red scale (*Aonidiella aurantii*) and yellow scale (*Aonidiella citrina*) samples were collected for molecular studies: mandarin (M), sweet orange (SO), Valencia orange (VO), Washington navel orange (WNO), grapefruit (GF).

Scale	Location	Longitude, latitude and elevation	Host plant and substrate	Specimen code	Accession number		
					28S	EF	*CO*I
Red scale	New South Wales						
	Somersby	33.380°S, 151.274°E, 274 m asl	WNO leaf	*Aonidiella aurantii* SNSW1			
	Lower Portland	33.443°S, 150.884°E, 6 m asl	WNO fruit	*Aonidiella aurantii* LNSW	JQ434500		
	Richmond	33.610°S, 150.747°E, 24 m asl	WNO fruit	*Aonidiella aurantii* RNSW1	JQ434503		
			VO leaf	*Aonidiella aurantii* RNSW2			
			VO leaf	*Aonidiella aurantii* RNSW5	JQ434495		JQ765619
			VO leaf	*Aonidiella aurantii* RNSW7			JQ765618
	Griffith	34.287°S, 146.046°E, 130 m asl	VO fruit	*Aonidiella aurantii* GNSW1			
			VO fruit	*Aonidiella aurantii* GNSW2	JQ434498		
	Barham	35.630°S, 144.129°E, 84 m asl	VO fruit	*Aonidiella aurantii* BNSW1	JQ434497		
			VO leaf	*Aonidiella aurantii* BNSW2			
	Tocumwal	35.811°S, 145.567°E, 117 m asl	WNO leaf	*Aonidiella aurantii* ToNSW	JQ434502		
	Victoria						
	Sunraysia (Mildura)	34.191°S, 142.167°E, 52 m asl	GF leaf	*Aonidiella aurantii* MiVic1	JQ434494		
	Murrabit	35.530°S, 143.958°E, 74 m asl	VO leaf	*Aonidiella aurantii* MuVic	JQ434499	+	
	South Australia						
	Loxton	34.450°S, 140.570°E, 42 m asl	VO	*Aonidiella aurantii* LSA		+	
	Western Australia						
	Gingin	31.346°S, 115.905°E, 103 m asl	VO fruit	*Aonidiella aurantii* GWA	JQ434493		
	Northern Territory						
	Darwin	12.463°S, 130.842°E, 38 m asl	GF	*Aonidiella aurantii* DNT	JQ434501	+	

(to be continued)

Table 1 Continue.

Scale	Location	Longitude, latitude and elevation	Host plant and substrate	Specimen code	Accession number		
					28S	EF	*COI*
	Indonesia						
	Yogyakarta, Java	7.769°S, 110.382°E, 146 m asl	VO fruit	*Aonidiella aurantii* IND1	JQ434496	+	
	Ngablak, Java	7.384°S, 110.400°E, 1306 m asl	M fruit	*Aonidiella aurantii* IND2	+		
Yellow scale	New South Wales						
	Somersby		WNO leaf	*Aonidiella citrina* SNSW	+		+
			WNO leaf	*Aonidiella citrina* SNSW3	+		+
			WNO fruit	*Aonidiella citrina* SNSW2	+		
			WNO leaf	*Aonidiella citrina* SNSW6			+
	Lower Portland		WNO fruit	*Aonidiella citrina* LPNSW2			+
	Cornwallis	33.585°S, 150.817°E, 13 m asl	WNO leaf	*Aonidiella citrina* CoNSW1	+	+	+
	Richmond		VO leaf	*Aonidiella citrina* RNSW	JQ582399		+
			VO leaf	*Aonidiella citrina* RNSW1	+	+	+
			VO fruit	*Aonidiella citrina* RNSW2	JQ582396		
			VO leaf	*Aonidiella citrina* RNSW7	+		
	Castlereagh	33.642°S, 150.675°E, 19 m asl	WNO leaf	*Aonidiella citrina* CaNSW	JQ582398	+	
			WNO leaf	*Aonidiella citrina* CaNSW1	+		
			WNO leaf	*Aonidiella citrina* CaNSW2			+
	Griffith		GF leaf	*Aonidiella citrina* GNSW1	JQ582397	+	+
			GF leaf	*Aonidiella citrina* GNSW3	+	+	
			GF leaf	*Aonidiella citrina* GNSW4	JQ582395		+
	Victoria						
	Mildura		GF leaf	*Aonidiella citrina* MiVic2	+	+	+

Table 2 Host scales, host plants and locations from which parasitoids of armoured scales were collected for molecular studies: mandarin (M), Valencia orange (VO), Washington navel orange (WNO), Eureka lemon (EL), and PCI (pumpkin in commercial insectary). Geographical positions of locations other than Kulnura (33.230°S 151.211°E, 328 m asl) in NSW, Perth (31.950°S, 115.861°E, 16 m asl) in WA, and Mundubbera (25.591°S, 151.298°E, 139 m asl) in QLD are listed in Table 1.

Parasitoid and specimen code		Host scale	Host plant	Location
Aphelinus	sp. 1	*Aonidiella aurantii*	VO	Kulnura
	sp. 2	*Aonidiella aurantii*	VO	Kulnura
Aphytis	*chrysomphali* SNSW	*Aonidiella aurantii*	WNO	Somersby
	chrysomphali LNSW	*Aonidiella citrina*	WNO	Lower Portland
	lingnanensis culture1	*Aspidiotus nerii*	PCI	Mundubbera
	lingnanensis culture2	*Aspidiotus nerii*	PCI	Mundubbera
	melinus KNSW1	*Aonidiella aurantii*	EL	Kulnura
	melinus KNSW2	*Aonidiella aurantii*	EL	Kulnura
	melinus KNSW3	*Aonidiella aurantii*	VO	Kulnura
	melinus CaNSW	*Aonidiella aurantii*	WNO	Castlereagh
	melinus WA2	*Aonidiella aurantii*	VO	Perth
	melinus WA1	*Aonidiella aurantii*	VO	Gingin
	melinus WA3	*Aonidiella aurantii*	VO	Gingin
	melinus culture 1	*Aspidiotus nerii*	PCI	Loxton
	melinus culture 2	*Aspidiotus nerii*	PCI	Loxton
Comperiella	*bifasciata* CbS1	*Aonidiella aurantii*	WNO	Somersby
	bifasciata CbL1	*Aonidiella citrina*	WNO	Lower Portland
	bifasciata CbCa1	*Aonidiella citrina*	WNO	Castlereagh
	bifasciata CbG1	*Aonidiella aurantii*	VO	Griffith
	bifasciata CbG2	*Aonidiella citrina*	VO	Griffith
	bifasciata CbWA1	*Aonidiella aurantii*	VO	Gingin
Encarsia	*citrina* SNSW1C	*Aonidiella aurantii*	VO	Somersby
	citrina CaNSW1C	*Aonidiella citrina*	WNO	Castlereagh
	citrina LNSW1C	*Aonidiella citrina*	WNO	Lower Portland
	citrina CoNSW1C	*Aonidiella aurantii*	WNO	Cornwallis
	citrina RNSW1C	*Aonidiella citrina*	WNO	Richmond
	citrina CoNSW2C	*Aonidiella aurantii*	WNO	Cornwallis
	citrina CaNSW2C	*Lepidosaphes beckii*	VO	Castlereagh
	perniciosi CoNSW1P	*Aonidiella aurantii*	WNO	Cornwallis
	perniciosi CoNSW2P	*Unaspis citri*	WNO	Cornwallis
	perniciosi CaNSW2P	*Aonidiella aurantii*	WNO	Castlereagh

Molecular identification of scales and parasitoids

DNA extraction Specimens used for DNA extraction were either fresh or stored in 100% ethanol at 20 °C; single specimens were used for each extraction. For red scale and yellow scale, unparasitised third instar virgin and mated females were used (Table 1). In order to compare the morphology and molecular results for individual specimens, some red scale and yellow scale specimens were photographed, and their pygidium removed using a sterilised scalpel blade, before the remaining body was used for DNA extraction. *Aphytis chrysomphali* pupae or adults and *C. bifasciata*, *E. citrina*, and *E. perniciosi* adults that emerged from field-sampled hosts were used. *Aphytis melinus* and *A. lingnanensis* pupae or adults were obtained from both field-sampled hosts and cultures maintained on oleander scale (*Aspidiotus nerii* Bouché) on butternut pumpkin (*Cucurbita moschata* Duchesne (Cubcurbitales: Cucurbitaceae) (Table 2). DNA extraction was carried out using QIAamp DNA Micro Kit (QIAGEN Pty Ltd, Doncaster, VIC, Australia).

DNA amplification and sequencing The mitochondrial cytochrome oxidase subunit I (*CO*I) gene, the nuclear, protein-coding gene, elongation factor 1α (EF1α), and the 28S ribosomal RNA (28S) of the scales were

Table 3 Primers used to amplify different gene regions for red scale and yellow scale, and their parasitoids in this study.

Primer	Sequence (5′→3′)	Reference
Scale insects		
*CO*If (LCO1490)	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* (1994)
*CO*Ir (HCO2198)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* (1994)
EFf (EF1a)	GATGCTCCGGGACAYAGA	Morse and Normark (2006)
EFr (EF2)	ATGTGAGCGGTGTGGCAATCCAA	Morse and Normark (2006)
28Sf rDNA (28B)	TCGGAAGGAACCAGCTACTA	Morse and Normark (2006)
28Sr rDNA (S3660)	GAGAGTTMAASAGTACGTGAAAC	Morse and Normark (2006)
Encarsia spp. and *Aphytis* spp.		
28S rDNA (28S D-2F)	CGTGTTGCTTGATAGTGCAGC	Campbell *et al.* (1993)
28S rDNA (28S D-2R)	TTGGTCCGTGTTTCAAGACGG	Campbell *et al.* (1993)
*CO*I (C1-J-1718)	GGAGGATTTGGAAATTGATTAGTTCC	Simon *et al.* (1994)
*CO*I (C1-N-2191)	CCCGGTAAAATTAAAATATAAACTTC	Simon *et al.* (1994)
ITS (5.8S-F)	TGTGAACTGCAGGACACATGAAC	de León *et al.* (2010)
ITS (28S-R)	ATGCTTAAATTTAGGGGGTA	de León *et al.* (2010)
Comperiella bifasciata		
*CO*I (C1-J-1718)	GGAGGATTTGGAAATTGATTAGTTCC	de León *et al.* (2010)
*CO*I (C1-N-2191)	CCCGGTAAAATTAAAATATAAACTTC	de León *et al.* (2010)
28S rDNA (D2-3551F)	CGTGTTGCTTGATAGTGCAGC	Gillespie *et al.* (2005)
28S rDNA (D2-4057R)	TCAAGACGGGTCCTGAAA GT	Gillespie *et al.* (2005)

amplified. The 28S, internal transcribed spacer (ITS) regions of *Aphytis*, the 28S and *CO*I of *Encarsia* species, and the 28S and *CO*I genes of *C. bifasciata* were amplified. The reaction mixtures contained: 0.05 U/mL Taq polymerase (GoTaq® Flexi DNA Polymerase, Promega Corporation, Promega Corporation, Madison, Wisconsin, United States of America)); 1× manufacturer's buffer; 0.2 mmol/L dNTPs; and 0.4–0.8 μmol/L each primer; the primers used are listed in Table 3. For the scales, the reactions contained the following concentrations of $MgCl_2$: *CO*I, 3.5 mmol/L; EF1α and 28S, 25 mmol/L. For the parasitoids, the reactions contained: *CO*I, 1.6 mmol/L $MgCl_2$; ITS and 28S, 2 mmol/L $MgCl_2$. For *CO*I, the cycling parameters were: an initial denaturation for 5 min at 95 °C; 33 cycles of 45 s at 95 °C for denaturation, 90 s at 50 °C (scale insects) or 58 °C (parasitoids) for annealing, and 120 s at 72 °C for extension; and a final extension of 5 min at 72 °C. For the ITS region, the cycling conditions of de León *et al.* (2010) were followed. For the 28S rRNA gene from the *Aphytis* and *Encarsia* species, the same thermocycling conditions as for *CO*I were used but with an annealing temperature of 50 °C. However, for the 28S region and EF1α from the scale insects, the touch-down procedure of Morse and Normark (2006) was followed. The resulting amplicons were subjected to electrophoresis, visualized, and photographed. The amplicons were purified using either a Wizard® Genomic DNA Purification Kit (Promega) or EXoSAP (Exonuclease I [New England Biolabs® Inc., Ipswich, Massachusetts, USA] and shrimp alkaline phosphatase [Promega]). Purified amplicons were sequenced by Macrogen Inc., Seoul, Korea, using an ABI 3700 Sequencher.

Phylogenetic analysis The forward and reverse DNA sequences of both genes were assembled, edited and aligned using Sequencher™ 4.8 (Gene Codes Corporation Michigan, USA). Three phylogenetic analysis methods were used: maximum parsimony (MP) and maximum likelihood (ML) using PAUP* 4.0 (Phylogenetic Analysis Using Parsimony version 4.0 beta 10 WIN) (Swofford, 2002), and Bayesian inference (BI) using MrBayes version 3.0 for Windows (Hall, 2008). MP analysis was performed using a tree-bisection-reconnection branch swapping algorithm with a heuristic search with 1 000 bootstrap replicates. ForML and BI, prior to analysis, an evolutionary model was selected using MrModeltest 2.3 (Nylander, 2004).

Results

Morphology of red scale and yellow scale, and their distributions within tree canopies

The general appearance and pygidia of adult females of red scale and yellow scale (Fig. 1) resembled published accounts by McKenzie (1937, 1938), Ferris (1938),

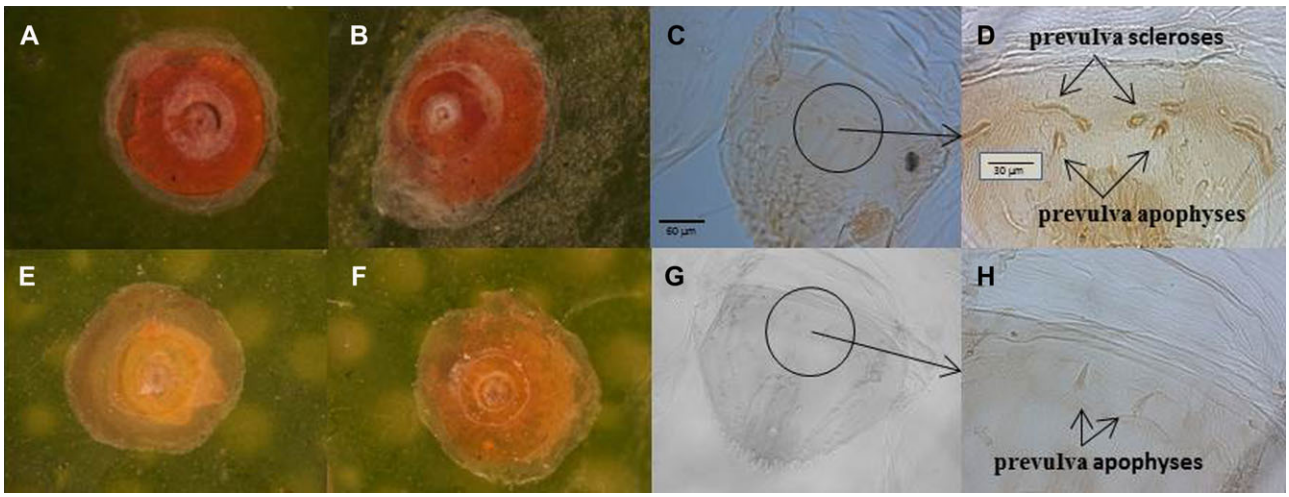

Fig. 1 Red scale third instar virgin (A) and mated (B) females on immature sweet orange fruit; red scale pygidium (C), prevulva scleroses and prevulva apophyses (D); yellow scale third instar virgin (E) and mated (F) females on immature sweet orange fruit; yellow scale pygidium (G); and prevulva apophyses (H).

Ebeling (1959), and OEPP/EPPO (2005). The appearance of red scale pygidial scleroses was variable in extent and, in some instances, they were barely visible or absent in cleared, slide-mounted specimens. For yellow scale, the scleroses were absent in all specimens (Fig. 1). Differences in the physical appearance of adult females of both species and their distributions within citrus trees are summarized in Table 4.

Molecular differences between red scale and yellow scale

The 28S rDNA, EF1α, and *CO*I sequences of red scale and yellow scale were successfully amplified. The sequences of the 28S rDNA region comprised 665 base pairs, and among all the sampled taxa including the outgroups, 37 characters were parsimony-informative. The EF1α sequences comprised 828 base pairs with 94 characters being parsimony-informative, and the *CO*I sequences comprised 590 base pairs of which 72 were parsimony-informative. Genetic analyses of these data sets were undertaken using MP, ML, and BI. In most cases, the phylogenetic trees derived from these 3 methods were identical, and the trees derived from maximum likelihood analysis or Bayesian analysis are shown in Figures. 2–4. These analyses consistently separated red scale from yellow scale. Within each of these species, the cladograms separated accessions into various groups. However, there was no consistency in the accessions comprising these groups, and this is reflected in the low bootstrap values associated with the groups, particularly in the 28S and *CO*I analyses.

Morphological and molecular identification of species of Aphytis

The majority of *Aphytis* pupae were readily discerned on the basis of the pigmentation of thoracic and abdominal tergites during the green-eye stage of development. They were either *A. chrysomphali* (Fig. 5), with a thin, conspicuous, longitudinal black line on the mesoscutum (see Prinsloo, 1984), or *A. melinus* (Fig. 5), with dark, thoracic tergites (see Rosen & DeBach, 1978). However, some pupae had dark thoracic and abdominal tergites that resembled pupal pigmentation in *A. lingnanensis* (see Rosen & DeBach, 1978). Red-eye and yellowish brown pupae were less readily discerned. These observations caused uncertainty about the number of species of *Aphytis* associated with red scale and yellow scale in the study orchards. This was resolved by molecular studies that showed that the pupae represented *A. chrysomphali*, 2 races of *A. melinus* (Fig. 5), and an undetermined and previously unrecorded aphelinid species (Fig. 5). These groups were based on the successful amplification of the 28S rDNA of 7 *A. melinus*, 3 *A. lingnanensis* and 3 *A. chrysomphali* pupae, and 2 pupae of the unidentified parasitoid, and ITS regions of 7 *A. melinus*, 1 *A. lingnanensis* and 2 *A. chrysomphali* pupae, and 1 pupae of the unidentified parasitoid.

The 28S rDNA sequences of each species comprised 443 base pairs and among all the sampled taxa including the outgroup, 138 were parsimony-informative. The ML and BI analyses were performed using the GTR+G model. All accessions of *Aphytis* are in 1 clade, and the unidentified aphelinid from Kulnura groups in a second clade

Table 4 Differences in habitat and appearance of red scale and yellow scale observed and sampled in citrus orchards in New South Wales between 2009 and 2011: colors based on Munsell® Soil Color Diagrams.

Character	Red scale	Yellow scale
Color of scale cover	Brownish yellow/yellowish brown	Yellow
Color of scale body	Opaque white or dark yellow to brown	Lemon yellow
Shape	Convex	Relatively flat
Scale cover thickness and transparency	Thicker, relatively opaque	Thinner, relatively transparent, easily seen through
Habitat within orchards/blocks	Evenly distributed with preference for sunlight, higher densities adjacent to windbreaks, common on both young and mature trees, more so on young trees	More common in shaded and dense canopies within orchard; more common on old trees
Habitat within trees	Common on outer and upper parts of trees, more abundant on the lately mature leaves; also common on twigs and branches	Commonly found in parts of trees not exposed to direct sunlight such as lower inner canopies, lower side of leaves; more abundant on the old leaves found on outer parts on trees adjoining trees; rare on twigs and branches
Distribution on fruit and leaves	Common on fruit surfaces exposed to the sunlight and on upper surfaces of leaves	More commonly found between touching surfaces of fruit and/or leaves and on lower surfaces of leaves
Chlorosis	Chlorosis around scales on green fruit uncommon	Chlorosis around scales on green fruit noticeable

Fig. 2 Pupae of *Aphytis chrysomphali* (A), *Aphytis melinus* WA1 (B), *Aphytis melinus* KNSW1 (C), *Aphelinus* sp. 2 (D), *Aphytis lingnanensis* culture1 (E), *Aphytis melinus* WA2 (F), *Aphytis melinus* KNSW2 (G), and *Aphelinus* sp. 2 (H).

with *Aphelinus* accessions found in molecular databases (Fig. 6). This unidentified parasitoid occurred at low levels in association with red scale in the orchards. The results from a BLAST search of molecular databases using the 28S rDNA sequence of this unidentified species show it is closely related (93% similarity) to *Aphel. varipes* Förster (HQ599562) and *Aphel. asychis* (Walker) (DQ350482). Within the *Aphytis* clade, accessions of *A. chrysomphali* form a subclade as do the accessions of *A. lingnanensis*. The sequences of *A. lingnanensis* are identical to accession AY635333 of *A. lingnanensis* collected by RF Luck, UCR, in south China (Gillespie *et al.*, 2005), and the sequences of *A. chrysomphali* to accession AY635330 of *A. chrysomphali* from an unrecorded location (Kim

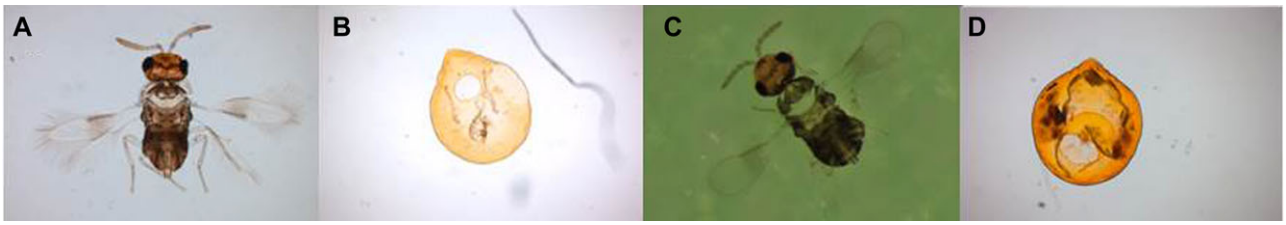

Fig. 3 *Encarsia citrina* adult with long marginal wing hairs (A), *E. citrina* exit hole in mummified second instar red scale (B), *E. perniciosi* adult with short wing hairs (C), and E. perniciosi exit hole in mummified second instar red scale (D). *Encarsia citrina* meconia normally line edges of mummified scale; in this case, they were removed during clearing of the specimen.

& Heraty, unpublished direct submissions). Accessions of *A. melinus*, however, form 2 separate subclades with 100% bootstrap support. One of these comprises 3 accessions, AY635342 of *A. melinus* (Gillespie *et al.*, 2005) collected by RF Luck at Futian (22.520°N, 114.053°E, 35 m asl), Shenzhen, on the Guangdong/Hong Kong border (Lisa Forster, UCR, pers. comm., August 2013), and KNSW1 and KNSW3 from Kulnura. The second comprises 4 accessions: culture 1, culture 2 (from *A. melinus* cultures established in SA from an Indian subcontinent-race imported to VIC from California in 1961; Furness *et al.*, 1983; Rosen & DeBach, 1978), WA1 from Perth, and CaNSW1 from Castlereagh, the latter being sister to the other 3. Pupae of these accessions (Fig. 5) resemble published accounts of pupae of *A. melinus* (Rosen & DeBach, 1978). In contrast, the pupae of KNSW1 and KNSW3 (Fig. 5) resemble published accounts of pupae of *A. lingnanensis* (Rosen & DeBach, 1978).

The ITS region of the *Aphytis* species comprised 415 base pairs. The phylogenetic tree derived from ML analysis is shown in Fig 7. Among taxa used for the outgroup and the in-groups, 214 were parsimony-informative. As for the 28S rDNA region, the accessions of *A. melinus* are separated into 2 groups that are separate from the accessions of *A. lingnanensis* and *A. chrysomphali*. The unknown species of *Aphelinus* forms part of a clade that includes *Aphel. asychis* and *Aphel. varipes*; however, within this clade the accessions of the unknown species form a subclade separate from the other two species.

Morphological and molecular identification of species of Encarsia

We observed the differences in the length of forewing fringes as described by Craw (1891) and Tower (1913) and illustrated in Smith *et al.* (1997) (Fig. 8). As noted by Rosen and DeBach (1979), mummified scales parasitised by *E. perniciosi* were darker and contained more exuviae than those parasitised by *E. citrina*. The *E. perniciosi* exit holes we observed were larger and more irregular than those of *E. citrina* (Fig. 8). They were also common in parasitised, adult, virgin females in contrast to *E. citrina*, which did not complete its development in adult females.

DNA was extracted from 7 *E. citrina* and 3 *E. perniciosi* adults. The 8 successfully amplified sequences of the 28S rDNA gene each comprised 571 base pairs. MP analysis shows that the *E. perniciosi* and *E. citrina* clades are separated with 100% bootstrap support (Fig. 9) but there is no variation in the 28S rDNA sequences of the 5 *E. citrina* specimens from armored scale species in the orchards in mid-coastal NSW. There are differences in 25 base pairs between *E. citrina* in this study and the accession AF254236 of *E. citrina* from an armored scale found at Riverside, California (Babcock & Hearty, 2001). In contrast to *E. citrina*, there is variation among the *E. perniciosi* specimens from mid-coastal NSW; the sequences from *E. perniciosi* CaNSW1 from red scale at Castlereagh and *E. perniciosi* CoNSW1P from red scale at Cornwallis are identical to each other and to accession AF254235 of *E. perniciosi* from California (University of California, Riverside [UCR] culture). The sequence of *E. perniciosi* CoNSW2P from white louse scale at Cornwallis differs in 8 bases and was sister to the above.

The *CO*I sequences of 5 specimens of *E. citrina* and 2 of *E. perniciosi* were amplified successfully. Sequences comprise 651 base pairs of which 50 characters were parsimony informative. No sequences of *CO*I of *E. citrina* or *E. perniciosi* were available from molecular databases. MP analysis of the *CO*I sequences (Fig. 10) also separated *E. perniciosi* and *E. citrina* into 2 clades both with 100% bootstrap support. The sequences of the 2 accessions of *E. perniciosi* (both from red scale at Castlereagh) are identical. Although variation was found among the accessions of *E. citrina*, there was no consistency in the groups formed with those occurring from the analysis of the 28S rRNA gene.

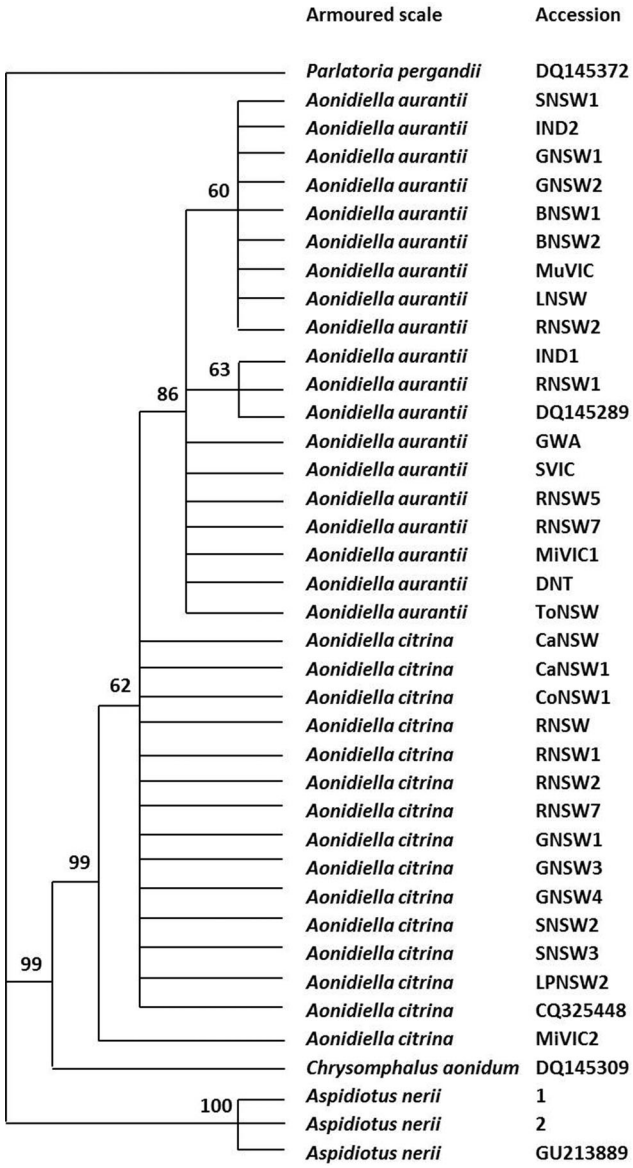

Fig. 4 Fifty percent majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of red and yellow scale derived from maximum likelihood analysis. *Parlatoria pergandii* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.

Comperiella bifasciata

DNA was successfully extracted from 6 *C. bifasciata* adults, 3 from red scale and 3 from yellow scale, and the *CO*I genes and the 28S rDNA and ITS regions successfully amplified. The sequences of the 28S rDNA gene comprised 600 base pairs and the 6 sequences are identical to each other and to *C. bifasciata* accession AY599317 from Riverside County, California (see Gillespie *et al.*, 2005; Supplementary Material 1). The sequence of the *CO*I gene comprises 484 base pairs and the 6 sequences are again identical to each other. There were no available datasets in the databases for *CO*I and the ITS region of *C. bifasciata*. As no variation among sequences from different specimens for each of the 3 gene regions was found, no further analysis was undertaken.

Discussion

The presence of red scale in Australia before 1840 (Koebele, 1892; Compere, 1961) suggests that it was

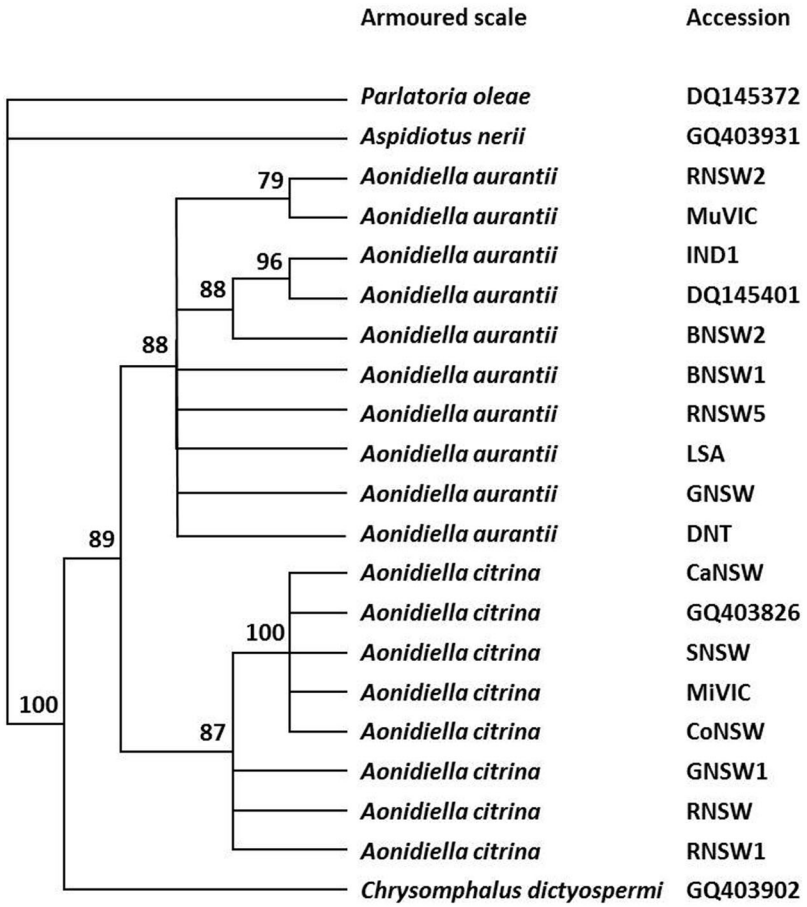

Fig. 5 Bayesian inference tree resulting from analysis of EF1α of red scale and yellow scale. *Parlatoria oleae* was used as the out-group and posterior probabilities are shown below each branch.

initially introduced to Sydney shortly after European settlement in 1788, possibly before 1828 on mandarin trees from China or, based on our sequence data, on a pomelo (*C. maxima* L.) tree from Java (Fraser, 1829; Bowman, 1955). Plants, including the Washington navel orange, were also introduced from Brazil during this interval (Bowman, 1955) but Brazil would have been an unlikely source of the scale, as records suggest that red scale was not present in South America until the late 1800s (Compere, 1961). Maskell's (1878) description of the scale, as *Aspidiotus aurantii*, was based on scale infested lemon and orange fruit imported to NZ from Sydney, and the common name was first used in 1870 by James Pye, a prominent Sydney citrus grower (The Sydney Morning Herald, 13 May 1870). Yellow scale may have also been introduced in the 1800s but it was not recognised as morphologically distinct from red scale until 1880 (Lelong, 1890), and not formally recorded in Australia (at Gosford) until the 1930s. It was not until the 1930s that morphological differentiation based on the presence or absence of pygidial scleroses of third instar females (McKenzie, 1937, 1938) could be used to distinguish the scales. However, the extent of sclerosis of these structures in red scale is variable, and they can be absent (Ferris, 1938). DeBach *et al.* (1978) noted that this variation led to about a 5% margin of error in correct identification of the scales. In some instances in this study, the scleroses were either absent or barely visible in red scale virgin females. However, the molecular techniques used in this study clearly confirmed the identities of the 2 species, including specimens of red scale with indistinct or absent scleroses, and allowed us to confirm the presence of yellow scale in the Riverina district of NSW.

Our molecular results also confirmed McKenzie's (1938) view that the red scale and yellow scale are genetically distinct, and do not intergrade or hybridise. Greater genetic divergence between the 2 species was evident in

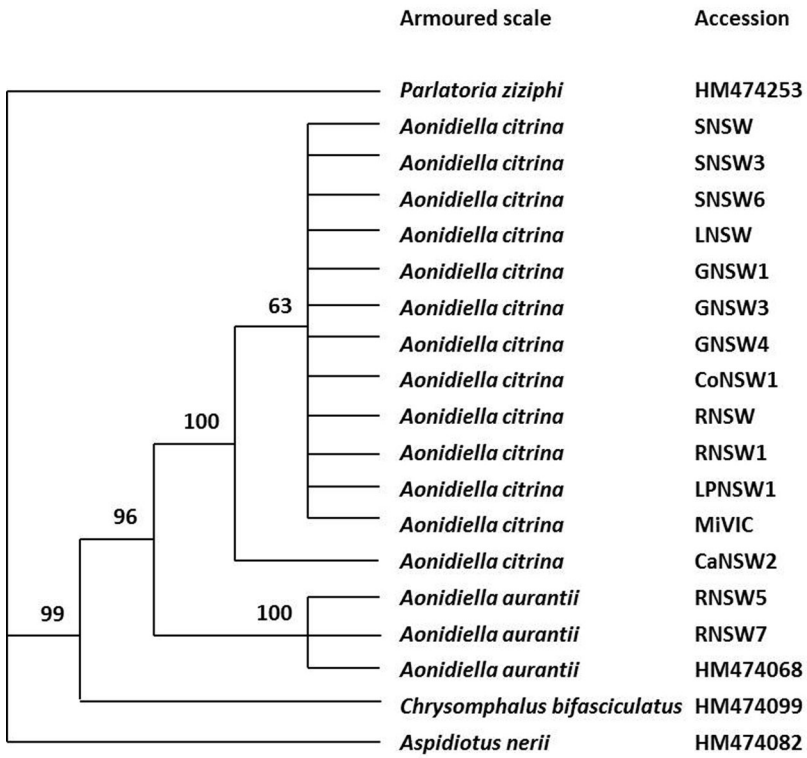

Fig. 6 Fifty percent majority-rule bootstrap consensus tree of the *CO*I region of accessions of red scale and yellow scale derived from maximum likelihood analysis. *Parlatoria ziziphi* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.

*CO*I than for EF1α and the 28S rDNA region, and genetic variability among red scale specimens was greater than among specimens of yellow scale. The sequences of both the EF1α and 28S rDNA regions showed no obvious association between their placement in clades or subclades with the locations within Australia from which accessions were collected. Our studies also showed that the scales occupy different habitats within orchards or blocks and within trees; these ecological differences can also aid identification of the 2 species.

Our molecular results clearly distinguished between *A. chrysomphali*, *A. lingnanensis*, and *A. melinus* and showed that *A. lingnanensis*-like pupae from the study orchards were either *A. melinus* or an unidentified *Aphelinus* species, the latter rare and possibly a seasonal aberration as *Aphelinus* species are parasitoids of aphids (Wilson, 1960; Hely *et al.*, 1982; Viggiani, 1984; Smith *et al.*, 1997). Thus, the study confirmed that *A. chrysomphali* and *A. melinus*, but not *A. lingnanensis*, parasitise red scale in orchards on the Central Coast of NSW. Surprisingly, 2 races of *A. melinus* were recorded. One race was molecularly identical to *A. melinus* from commercial insectary cultures established in SA in 1968, following the official introduction of the parasitoid to Australia from the Indian Subcontinent via UCR in 1961 (Smith *et al.*, 1997). It was found in orchards at Richmond, Cornwallis and Castlereagh in NSW but not on the Somersby Plateau (Dao, 2012). This suggested limited spread from where it was released at Richmond in 1991. The other race was molecularly identical to a race of *A. melinus* collected by Robert Luck (UCR) from Futian ("Futhi," 23.523°N, 114.055°E, 24 m asl) in southern Guangdong and subsequently identified by Gordon Gordh on the basis of the morphology of 5 males and 5 females (UCR Quarantine Database, Single Record Listing, 90.05.04, *Aphytis* sp.) from yellow scale on a species within the Magnoliacae. We recommend that the Futian specimens at UCR be re-examined to confirm that they are morphologically identical to the race from the Indian Subcontinent. The distribution of the Futian race in Southeast Asia and in Australia should also be determined.

Records related to the introduction of species of *Aphytis* to Australia for the control of red scale are ambiguous. Reviews by Wilson (1960), Compere (1961), Furness *et al.* (1983), and Smith *et al.* (1997) indicate that the first parasitoid to be officially introduced for control of

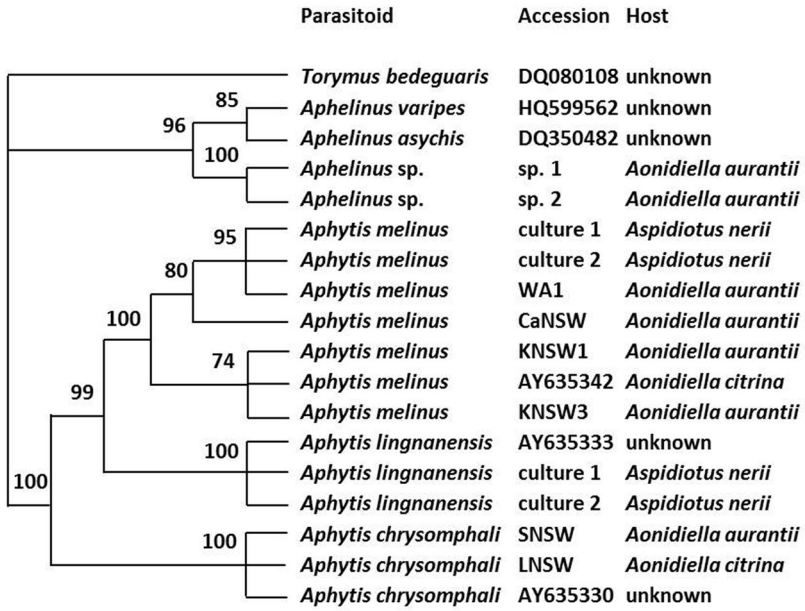

Fig. 7 Fifty percent of majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of *Aphytis* species and unidentified species, red scale and yellow scale parasitoids, from maximum likelihood analysis. *Torymus bedeguaris* was used as the outgroup and bootstrap values are provided as percentages from 1000 replications.

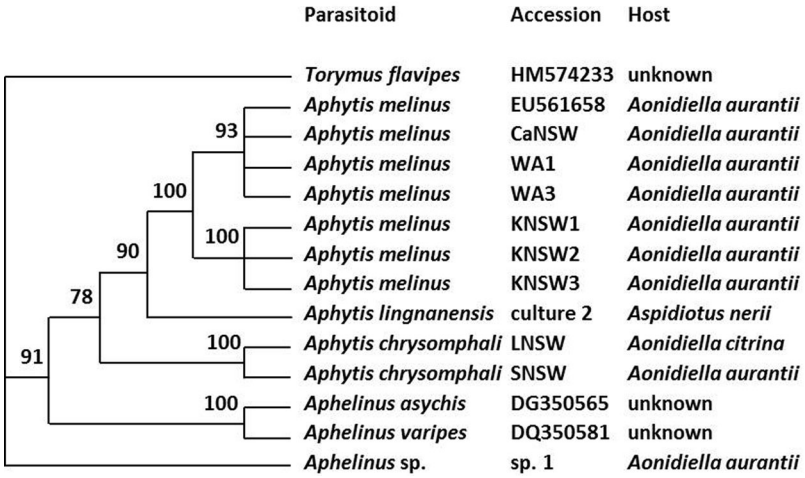

Fig. 8 Fifty percent of majority-rule bootstrap consensus tree of the ITS region of accessions of *Aphytis* species and unidentified species, red scale and yellow scale parasitoids, from maximum parsimony analysis *Torymus flavipes* was used as the outgroup and bootstrap values are provided as percentages from 1000 replications.

red scale was either *A. chrysomphali* or *A. lingnanensis* from China, introduced by George Compere in 1905 (Compere, 1905; Despeissis, 1906) and 1907 (Newman, 1907; Jenkins, 1946). On both occasions, material was released in Government Gardens in Perth on plants severely infested with red scale (Newman, 1907) and successful establishment occurred in orchards near Perth (Anon, 1906). Subsequently, Newman (1922) referred to the parasitoid as "*Aphelinus fusipennis*" (*A. diaspidis* [Howard]), a name incorrectly applied at the time to *A. chrysomphali* (Wilson, 1960; Compere, 1961; Rosen & DeBach, 1979). Jenkins (1946) referred to it as *A. chrysomphali*, but circumstantial evidence led Harold Compere (1955, 1961) to suggest that it was *A. lingnanensis*. However, all specimens of *Aphytis* received at UCR before 1960 from red scale in Australia were *A. chrysomphali* (Rosen & DeBach, 1978).

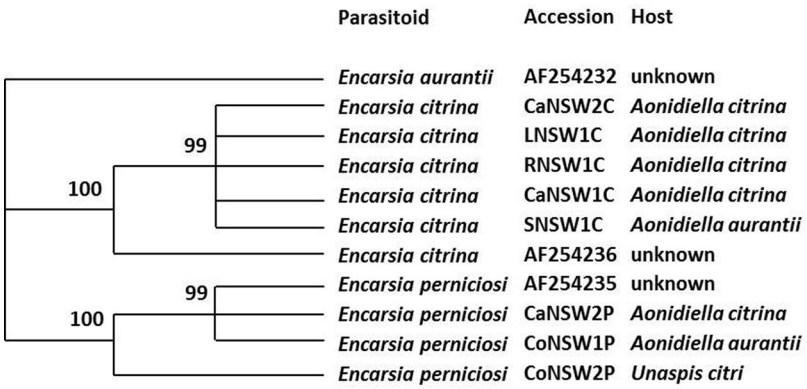

Fig. 9 Fifty percent of majority-rule bootstrap consensus tree of the 28S region of accessions of *Encarsia citrina* and *Encarsia perniciosi*, red scale and yellow scale parasitoids, from maximum parsimony analysis. *Encarsia aurantii* was used as the outgroup and bootstrap values are provided as percentages from 1000 replications.

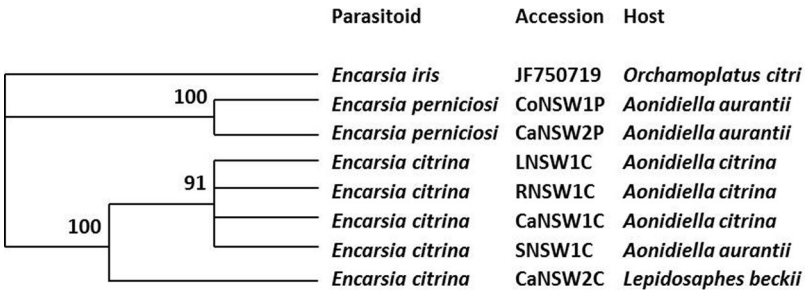

Fig. 10 Fifty percent of majority-rule bootstrap consensus tree based on maximum parsimony analysis of the *CO*I region of accessions of *Encarsia citrina* from red scale and yellow scale and *Encarsia perniciosi* from red scale. *Encarsia iris* was used as the outgroup and bootstrap values are provided as percentages from 1000 replications.

Moreover, *A. chrysomphali* became established near Gosford and in inland districts of NSW after it was introduced from WA during 1925–1926 (Wilson, 1960; Hely, 1968; Smith *et al.*, 1997). These records indicate that *A. chrysomphali* was introduced to WA by George Compere in 1905 or 1907, possibly both, and was subsequently introduced from WA to other states. If *A. lingnanensis* was introduced to WA in the early 1900s it may have been the unidentified "second parasite" that, according to Wilson (1960), became established in WA after it was introduced with 'another parasitoid' in 1907 (Wilson, 1960; Jenkins, 1946). There is no record of it being formally introduced to QLD, where it was present before 1930 (Rosen & DeBach, 1979; Smith *et al.*, 1997). An attempt to introduce it to VIC from UCR in 1962 failed (Furness *et al.*, 1983). Rosen and DeBach (1979) raised the possibility that Australia may have formed part of the original range of *A. lingnanensis*. We think this unlikely, given the Oriental origins of the lingnanensis species group to which *A. lingnanensis* belongs (Rosen & DeBach, 1979). A more plausible explanation would be that the species was unintentionally introduced on plants before plant quarantine was implemented, possibly in conjunction with Chinese immigration linked to the gold-rush years from the 1850s to the 1890s.

All ~150 *Aphytis* pupae from parasitised red scale in Perth and nearby locations in WA were pupae of the Indian Subcontinent race of *A. melinus*. Thus, if *A. chrysomphali* and/or *A. lingnanensis* were introduced to WA in the early 1900s, they may have been displaced by *A. melinus*, thereby replicating events reported to have occurred in the hot-dry lower Murray River regions of SA and VIC in the 1970s (Smith *et al.*, 1997; Furness *et al.*, 1983), and in Riverside and other regions of California (DeBach & Sundby, 1963; Luck & Podoler, 1985; Murdoch *et al.*, 1996). However, displacement of *A. chrysomphali* by *A. melinus* was not evident during surveys in the late 1970s and early 1980s of Murray River and Riverina orchards in inland NSW where releases of *A. melinus* commenced in the mid-1970s (Beattie, unpublished) and, in our study

orchards, *A. chrysomphali* coexisted with both racesrace of *A. melinus*. Both parasitoid species also co-exist in the Mediterranean (Sorribas *et al.*, 2012) and *A. melinus* and *A. lingnanensis* may still coexist in the Riverside region of California (Vasquez, 2010).

We detected no genetic variation in the sequences of the 28S rDNA, ITS and *CO*I regions of *C. bifasciata*, regardless of the host or location from which specimens were collected in Australia. Our 28S rDNA sequences of the parasitoid are identical to those of the red scale race of *C. bifasciata* from cultures at UCR (UCR code D0599, AY599317; Gillespie *et al.*, 2005). It was officially introduced to Australia via UCR from China in the 1940s (Wilson, 1960; Furness *et al.*, 1983; Smith *et al.*, 1997). We also detected no evidence to indicate that the yellow scale race of *C. bifasciata*, the eggs and larvae of which are killed and encapsulated by red scale (Compere & Smith, 1927; Flanders, 1944; Rosen & DeBach, 1978), occurs in Australian orchards. This conclusion is supported by high levels of parasitism (≈ 80%) reported in SA by Furness *et al.* (1983) and rare encapsulation in thousands of red scale adult females examined in state-wide surveys in NSW from the late 1970s to the early 1980s (Beattie, unpublished data) and studies in coastal NSW between 2009 to 2012 (Dao, 2012). However, if observations reported by Brewer (1971) and Snowball and Sands (1971) were erroneously based on the red scale race then the yellow scale race may be present. Alternatively, it may have been displaced by the red scale race since the early 1970s.

Comperiella bifasciata was recorded by Flanders (1934) at Marmor (23.683°S, 50.712°E, 18 m asl) in coastal QLD in 1931, a decade before it was officially introduced to Australia (Wilson, 1960). Flanders (1934, 1944) reared it from what he considered to be yellow scale on Australian desert lime (*C. glauca* [Lindl.] Burkill, syn. *Eremocitrus glauca* [Lindl.] Swing.). Sands and Snowball (1980) presumed that Flander's (1934) observations were related to *Comperiella pia* (Girault) on *Ch. aonidum*; however, the scale was subsequently described as *Aonidiella eremocitri* by McKenzie (1937). The native host of *C. pia* is not known. Whether the parasitoid recorded by Flanders evolved in association with *A. eremocitri* or whether it stemmed from an unofficial introduction of *C. bifasciata* from Asia with red scale, yellow scale, or another host, before 1930, needs to be resolved. There are no records of the red scale or yellow scale races of *C. bifasciata* parasitising *A. eremocitri*, or of an "*A. eremocitri* race" parasitising red scale or yellow scale, and Flander's record is the only record of *C. bifasciata* parasitising *A. eremocitri*. There are no records of other parasitoids. If *A. eremocitri* evolved in Australasia, then the region may fall within the natural distribution of *C. bifasciata*. Host records for *A. eremocitri* are restricted to *C. glauca*, *Maytenus* (*Celastrus*) *bilocularis* (F. Muell.) Loes. (Celastrales: Celastraceae) and *Owenia venosa* F. Muell. (Sapindales: Meliaceae) in Australia (McKenzie 1937, 1938; Brimblecombe, 1962), coconut (*Cocos nucifera* L. [Arecales: Palmae]), *Barringtonia* sp. (Ericales: Lecythidaceae), Asiatic species and hybrids of *Citrus*, in South Pacific islands (Beardsley, 1966; Williams & Butcher, 1987; Williams & Watson, 1988), and *Coelogyne asperata* Lindl. (Aparagales: Orchidaceae) in Thailand (McKenzie, 1946).

All records for *E. citrina* and *E. perniciosi* as parasitoids of diaspidids in Australia are related to introduced hosts (Schmidt & Polaszek, 2007). Our molecular results distinguished between the 2 parasitoids and confirmed their presence in coastal NSW. Our 28S rDNA sequences of 2 *E. perniciosi* accessions from red scale are identical to accession AF254235 of *E.* nr. *perniciosi* (DNA voucher D132; Babcock *et al.*, 2001) from a UCR culture (R Luck, UCR, pers. comm., July 2013). However, these 3 sequences differed from our accession of *E. perniciosi* from *U. citri*, a known host (Malipatil *et al.*, 2000; Noyes, 2013). We assume that accession AF254235 represents the thelytokous red scale form of *E. perniciosi* from China that appears to have been introduced from California to WA between 1960 and 1963 (Sproul, 1981). It was the only form of *E. perniciosi* that we observed in association with hundreds of red scale that we examined on heavily infested fruit from WA during our study. Smith *et al.* (1997) regarded it as a useful parasitoid of red scale in lower Murray River orchards in the early 1990s following its introduction from California to VIC in 1970. However, *E. perniciosi* was recorded on red scale and "*A. citri*" (presumably yellow scale, *A. citrina*) at Kulnura, NSW in the 1960s, and in QLD at Palmwoods in 1978 and Mundubbera in 1998 (Schmidt & Polaszek, 2007). It was a common parasitoid of red scale on the Somersby Plateau in the 1980s (Beattie, unpublished). Thus, the race of *E. perniciosi* we recorded in association with red scale and yellow scale in our study orchards was probably introduced from Asia to coastal NSW and QLD prior to 1900, before it was officially introducted to Australia.

Our 28S rDNA sequences of *E. citrina* accessions from red, yellow and purple scales were identical, but differed from UCR accession AF254236 (DNA voucher 272; Babcock *et al.*, 2001) (R Luck, UCR, pers. comm., July 2013). This could, in part, explain the relatively high levels of *E. citrina* parasitism of red scale in our study orchards in contrast to low levels of parasitism of the scale in California, as reported by Compere (1961) and Rosen and DeBach (1978). Our *CO*I sequences showed no variation

between our accessions from red scale and yellow scale, but variation occurred between these accessions and the accession from purple scale. ITS sequences (cladogram not presented) also showed variation between the accession from purple scale and the 2 species of *Aonidiella*. The ITS and *CO*I sequence data may suggest the presence of *Aonidiella*- and *Lepidosaphes*-specific races of *E. citrina* in our study orchards.

Encarsia citrina was introduced into WA by George Compere in 1902 (Despeissis, 1903b) but the record of its introduction was overlooked by Jenkins (1946), Wilson (1960), Compere (1961), and Smith *et al.* (1997), despite reference to an illustration of it in Despeissis (1903b) by Anonymous (1906). Thus, *E. citrina*, not a species of *Aphytis*, was the first parasitoid officially introduced to Australia for the control of red scale. However, whether the parthenogenetic species was successfully released is not clear in reports by Despeissis (1903a), Despeissis (1903b; text by George Compere) and Wilson (1960), and in articles in the The West Australian (16 January 1903) and Western Mail (24 January 1903). Despite this uncertainty, *E. citrina* was present in association with diaspidids on citron (*C. medica* L.) and orange trees near Cairns (16.892°S, 145.651°E) in northeast coastal QLD between 1911 and 1913 (Girault, 1913; Hayat, 1989). It was also reared from red scale at the Sydney Botanic Gardens between June 1931 and October 1933 (Compere, 1961). There is no record of the red-scale-inhabiting race being introduced intentionally from overseas, or from WA, to QLD or NSW, and we did not observe it in hundreds of scales on heavily infested fruit received from WA during our study. This suggests that the form of *E. citrina* that parasitises armored scales on citrus on the Central Coast of NSW may be native to Australia or unintentionally introduced. Further studies are required to determine the differences between these races and those that occur overseas, particularly those in Asia and California.

Notwithstanding probable overseas origins of all of the primary parasitoids associated with red scale and yellow scale in Australia it is possible that one or more of the species may be native to Australia or to a geographical area encompassing Australia. Diaspidids appear to have originated in western Gondwana (Kozár, 1990) and some 10% of the 2500 species occur in Australia (Hardy & Henderson, 2011). Molecular phylogenies derived by Andersen (2010) indicated that the ancestral diaspidid was of Australasian origin, with the subfamily Diaspidinae of Paleartic origin, and the subfamily Aspidiotinae of Australasian origin. Four of 17 species of *Chrysomphalus* and 20 of the 164 species of *Lepidosaphes* appear to be native to Australia (ScaleNet, accessed 16 September 2013; Smith-Pardo *et al.*, 2012). *Aonidiella eremocitri*, 1 of 32 species of *Aonidiella*, may be native to Australia and other parts of Australasia (ScaleNet, accessed October 2013; Williams & Watson, 1988). There are no apparent records of parasitoids associated with the native species of *Chrysomphalus* and *Lepidosaphes*, and with the exception of *C. bifasciata* (Flanders, 1934), there are no records of parasitoids associated with *A. eremocitri*. In addition, there are no records of parasitoids associated with 9 other native diaspidid species known to occur on Rutaceae in Australia (Froggatt, 1914; Brimblecombe, 1956, 1959; Takagi, 1984; Hardy & Henderson, 2011). In reviewing the host records for armored scales on Australian Rutaceae, it became apparent that the number of armored scales associated with these plants may be much greater than currently known. In addition, virtually nothing is known about the parasitoids or entomopathogens associated with the scales. Surprisingly, no native diaspidids have been recorded on introduced species and hybrids of *Citrus*, and, with the exception of yellow scale, which was recorded on *Geijera parviflora* Lindl. (Rutoideae) on the UWS Hawkesbury campus on 9 September 2013, no introduced diaspidids associated with introduced species and hybrids of *Citrus* have been recorded on native Rutaceae. Given the economic and evolutionary consequences of the above, it is important that future work concentrates on these areas.

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Disclosure

All the authors disclose that there are no potential conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of this manuscript and agree with the submission to *Insect Science*.

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