



Parasitoids of the Australian citrus whitefly, *Orchamoplatus citri* (Takahashi) (Hemiptera, Aleyrodidae), with description of a new *Eretmocerus* species (Hymenoptera, Aphelinidae)

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Abstract

A field survey for potential biological control agents of the Australian citrus whitefly, *Orchamoplatus citri* (Takahashi), resulted in the discovery of three species of chalcid parasitoids (Hymenoptera, Chalcidoidea), viz.: *Cales orchamoplati* Viggiani & Carver, a species belonging to a genus that is currently unplaced to family within Chalcidoidea, and two species of Aphelinidae, *Encarsia iris* (Girault) and *Eretmocerus orchamoplati* sp. nov. *Orchamoplatus citri* is the first host record for *E. iris*. Morphological diagnoses are given, and DNA sequence data of the 28S rDNA and the cytochrome oxidase I genes are provided to define species at the molecular level.

Key words: Australia, parasitoid, biological control, taxonomy, survey

Introduction

The Australian citrus whitefly, *Orchamoplatus citri* (Takahashi, 1940) (Hemiptera: Aleyrodidae), is an endemic Australian whitefly that feeds only on species of citrus (Martin 1999). In Australia, the species is a minor pest (Cooper 1961, Hely 1968). In New Zealand, the whitefly was first detected in Auckland in 2000 and has since spread to Kerikeri, Gisborne, and Bay of Plenty, where it is causing damage to a range of citrus varieties. Prior to this study, the only definitive record of a parasitoid of the Australian citrus whitefly was *Cales orchamoplati* (Viggiani & Carver) from South Australia (Viggiani & Carver 1988). Earlier records of parasitoids of the Australian citrus whitefly are fragmentary. Parasitised *O. citri* have been recorded from Western Australia, but the parasitoids were not identified (Russell 1958). In 1978, two species of *Encarsia* (as *Encarsia* sp. and *Prospaltella* sp.) (Hymenoptera: Aphelinidae), which were presumably reared from *O. citri*, were introduced into the Cook Islands by E.W. Valentine for control of *O. mammaeferus* (Quaintance & Baker) (Walker & Deitz 1979).

Small parasitic wasps of the family Aphelinidae are among the most important natural enemies of whiteflies. Many species attack economically important hosts and are therefore potential biological control agents (Greathead 1986, Rosen & DeBach 1991, De Barro & Coombs 2009). Aphelinid wasps are primarily associated with Hemiptera, including whiteflies (Aleyrodidae), scale insects (Diaspididae), and aphids (Aphidoidea). The most important parasitoids of whiteflies are species of *Encarsia* Förster and *Eretmocerus* Haldeman (De Barro & Coombs 2009).

Encarsia is the largest genus of Aphelinidae, currently containing almost 400 described species (Noyes 2003). *Encarsia* species have been of particular interest to economic entomologists because several species have been used, or are currently being used, successfully for pest control (De Barro & Coombs 2009). In Australia, 94 *Encarsia* species have been recorded, although it is estimated that the number of species could be two to three times higher than currently known (Schmidt & Polaszek 2007).

Eretmocerus is a comparatively small genus with 74 species currently described worldwide (Noyes 2003), including 7 species from Australia. Several *Eretmocerus* species are considered to offer great potential as biological control agents (Goolsby *et al.* 1998, De Barro *et al.* 2000, De Barro & Coombs 2009).

Cales Howard is a very small genus with four described species, of which two occur in Australia (Viggiani & Carver 1988) and one in New Zealand (Mottern *et al.* 2011). The type species, *C. noacki* Howard, is Neotropical in origin, but has been successfully introduced into the Nearctic and Palaeartic regions as a biological control agent of the woolly whitefly, *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) (DeBach 1980). The taxonomic position of *Cales* is controversial and it is currently unplaced to family within Chalcidoidea (Mottern *et al.* 2011).

In addition to morphological diagnoses, DNA sequences data are used to provide additional evidence for taxonomy and to aid in the identification of species. In this study, the domain 2 gene region (D2) of the 28S rDNA gene, and the barcoding region of the mitochondrial cytochrome oxidase I (COI) gene, were sequenced to characterise species at the molecular level and to assess the intraspecific variation of species from locations across their known ranges in eastern Australia.

Material and methods

Representatives of all species from each sample were examined morphologically and genetically. DNA was extracted from single, whole specimens using a non-destructive DNA extraction protocol. Specimens were placed in a mixture of ATL lysis buffer containing 10% proteinase-K (Qiagen) and incubated at 56°C for several hours. After lysis the specimens were washed by rinsing in distilled water and transferred to 100% ethanol where they remained until slide preparation. The lysates were processed for subsequent molecular analysis using Qiagen's DNeasy Tissue Kit according to the manufacturer's protocol.

Primer sequences and cycling conditions are given in Table 1. Polymerase chain reaction was performed on 1 µl genomic DNA extracts using Bioline MangoTaq DNA Polymerase. Each 25 µl reaction mix contained 5 µl (10x) Mango buffer (Bioline), 2.5 µl dNTP (2.5 mM each), 1.5 µl MgCl₂ (50mM), 1 µl forward and reverse primer each, and 0.5 µl MangoTaq (5u/µl, Bioline). Sequences were edited using Sequencher version 4.9 (Gene Codes Corporation). Sequences have been deposited in GenBank, with accession numbers given as part of the species treatments. Genetic distances of the COI region were calculated using the Kimura 2-parameter method (Kimura 1980).

TABLE 1. Primer sequences and cycling conditions.

Primer	Sequence	Cycling conditions			
		Denaturation	Annealing	Extension	Cycles
28S-D2	J. Heraty, UC Riverside	94°C	55°C	72°C	
D2F	5' CGGGTTGCTTGAGAGTGCAGC 3'	(1 min)	(1 min)	(1 min)	35x
D2Ra	5' CTCCTTGGTCCGTGTTTC 3'				
COI	Folmer <i>et al.</i> (1994)	94°C	47°C	72°C	
LCO1490	5' GGTCAACAAATCATAAAGATATTGG 3'	(30 s)	(40 s)	(1 min)	5x
HCO2198	5' TAAACTTCAGGGTGACCAAAAAATCA 3'	94°C	52°C	72°C	
		(30 s)	(40 s)	(1 min)	30x

For morphological examination, all specimens, including specimens used for sequencing, were slide mounted as described by Noyes (1982). The microphotographs were obtained using a digital camera (TCA-9.1 Tucsen Kamera, Fuzhou Tucsen Imaging Technology Co., Ltd) and processed using Helicon Focus, version 5.1 (Helicon Soft Ltd). The digital images were enhanced using Adobe Photoshop. Terminology generally follows Schmidt & Polaszek (2007), with the abbreviation "F" referring to the flagellomere number.

Acronyms refer to the following institutions: ANIC, Australian National Insect Collection, Canberra, Australia; NZAC, New Zealand Arthropod Collection, Auckland, New Zealand; ZSM, Zoologische Staatssammlung, Munich, Germany.

Systematics

Cales orchamoplati Viggiani & Carver

(Figs 1–4)

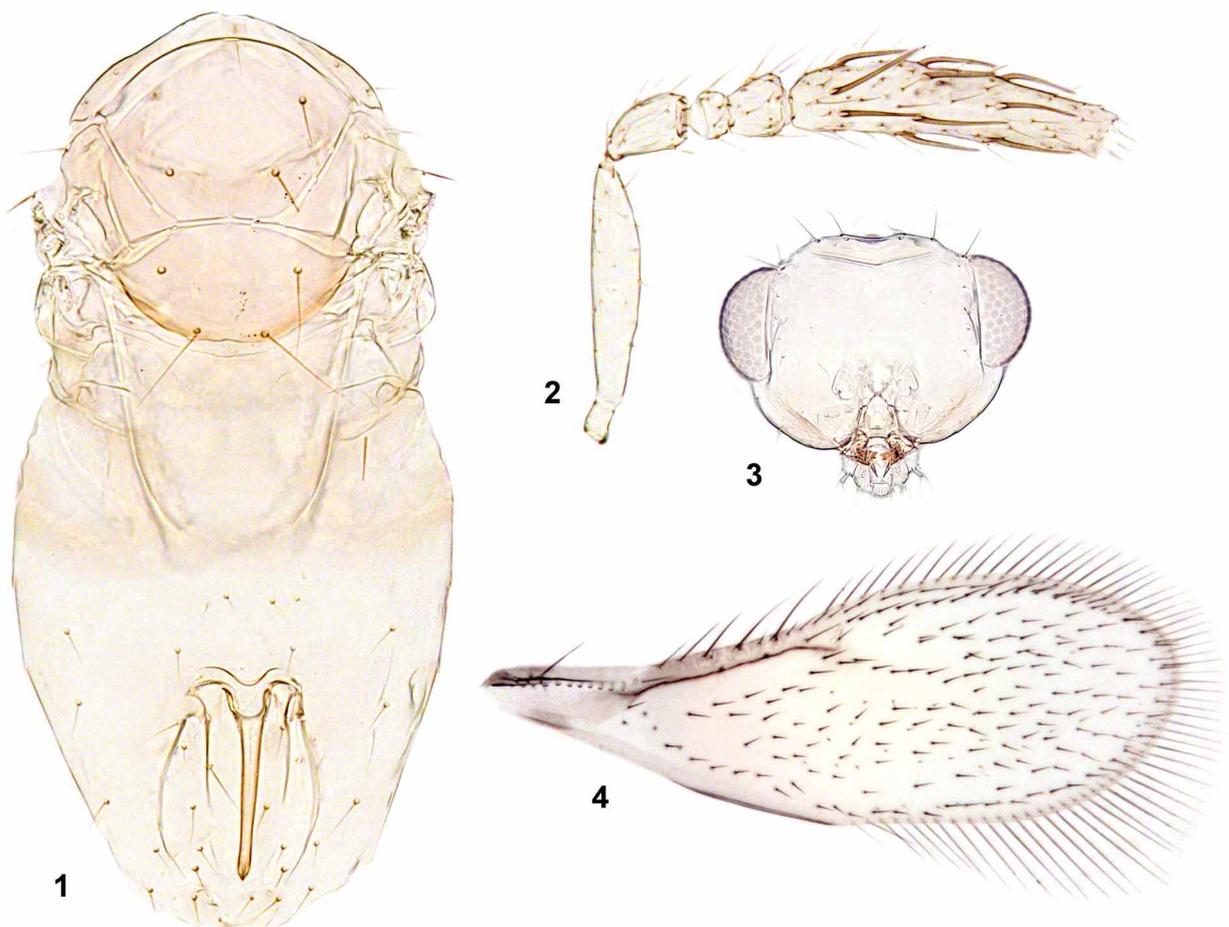
Cales orchamoplati Viggiani & Carver 1988: 44–45. Holotype ♀, Australia, South Australia, Waite Agricultural Research Institute, Adelaide, 30.xi.1976, H.M. Brookes and M. Carver, ex *Orchamoplatus citri* on lemon (ANIC, not examined).

Cales orchamoplati Viggiani & Carver: Mottern *et al.* 2011: 282 (diagnosis).

Diagnosis. FEMALE. Colour: yellow, head and mesosoma slightly darker, wings hyaline.

Morphology. Stemmaticum with reticulate surface sculpture. Antennal formula 1,1,3,1 (Fig. 2). Scape 2.9–3.1X as long as pedicel and 0.7–0.8X as long as clava. Pedicel slightly shorter than combined length of F1 and F2 (0.8–0.9X). F1 1.3X as long as its maximum width, shorter than F2 (0.6X). F2 subequal in length to its maximum width. Clava 4.3–4.5X as long as its maximum width, equipped with 10 or 11 hair-like sensilla, apex truncate with 3 terminal peg-like sensilla (Fig. 2). Midlobe of mesoscutum with 2 pairs of setae, arranged symmetrically, side lobes with 2 setae each (Fig. 1). Distance between anterior pair of scutellar setae distinctly greater than distance between posterior pair (Fig. 1). Fore wing 2.8X as long as width of disc (Fig. 4). Marginal fringe 0.5X as long as width of disc (Fig. 4). Basal cell without setae. Submarginal vein with 1 seta, marginal vein anteriorly with 5 setae. Apical spur of midtibia distinctly longer than half length of corresponding basitarsus (0.8X). Ovipositor distinctly shorter than midtibia (0.6–0.7X) and slightly shorter than clava (0.7–0.8X).

MALE. No males of the species were collected as part of the present study. For a description of *C. orchamoplati* males from the type locality see Viggiani & Carver (1988: 45).



FIGURES 1–4. *Cales orchamoplati*, female. 1, body; 2, antenna; 3, head, front view; 4, fore wing.

Distribution. Australia: Queensland, South Australia.

Material examined. 3 ♀, AUSTRALIA: **South Australia:** Loxton, 34.4512°S, 140.5698°E, 50 m, December 2009 (J. Altman), ex *O. citri* (ZSM).

Genbank accession. HQ660516, JF750733.

Comments. *Cales orchamoplati* has previously been known only from its type locality in Adelaide, South Australia (Viggiani & Carver 1988) and from Mt. Glorious, Queensland (Mottern *et al.* 2011). It is distinguished from the only other Australian *Cales* species, *C. spenceri* (Girault), by its shorter marginal fringe (0.5X compared to 0.8X as long as the maximum width of disc in *C. spenceri*) (Mottern *et al.* 2011).

The 28S-D2 sequence data did not show any intraspecific variation. Amplification of the COI barcoding region consistently yielded sequences of endosymbiotic *Wolbachia*.

***Encarsia iris* (Girault)**

(Figs 5–8)

Coccophagus iris Girault 1930: 4. Holotype ♀, Australia, Queensland, Brisbane, Indooroopilly, 24 November 1929 (QMBA, type no. 4012, examined).

Coccophagus iris Girault: Dahms 1984: 730.

Encarsia iris (Girault): Viggiani 1985: 241–242. Change of combination.

Encarsia iris (Girault): Schmidt & Polaszek, 2007: 2173–2174 (redescription).

Diagnosis. FEMALE. Colour: head brown. Mesosoma brown except following parts yellow: mesoscutum posteriorly, posteromesal corner of axilla, scutellum, and metanotum. Gaster predominantly brown or basal tergites paler. Antenna yellow. Fore wing with dark band behind marginal vein (Fig. 8). Legs yellow except mid and hind coxae and occasionally hind femur brown.

Morphology. Stemmaticum with rugosely strigose surface sculpture (Fig. 7). Antennal formula 1,1,3,3 (Fig. 6). Pedicel longer than (1.1–1.3X) F1. F1 1.3–1.5X as long as its maximum width, shorter than F2 (0.7–0.9X) and F3 (0.7–0.8X). F2 slightly shorter than or subequal in length to F3 (0.9–1.0X). Flagellomeres with the following numbers of sensilla: F1: 0 or 1, F2: 2, F3–F6: 3 or 4. Midlobe of mesoscutum with 8 setae, arranged symmetrically, side lobes with 3 setae each. Scutellar sensilla widely separated (approximately 5–6X maximum width of a sensillum) (Fig. 5). Distance between anterior pair of scutellar setae slightly less than or subequal to distance between posterior pair (Fig. 5). Fore wing 2.3–2.4X as long as width of disc (Fig. 8). Marginal fringe 0.2X as long as width of disc (Fig. 8). Basal cell with 3–7 setae. Submarginal vein with 2 setae, marginal vein anteriorly with 6 or 7 setae. Tarsal formula 5,5,5. Apical spur of midtibia distinctly longer than half length of corresponding basitarsus (0.8–0.9X), the latter distally with 4 or 5 pegs. Tergites laterally with the following number of setae: T1: 0, T2: 1, T3: 1, T4: 1, T5: 2, T6: 2, T7: 4. Ovipositor longer than midtibia (1.7–2.2X) and 2.9–3.5X as long as clava. Third valvula 0.5–0.7X as long as second valvifer.

MALE. Body dark brown except midlobe of mesoscutum posteriorly and scutellum lighter. Legs pale, all coxae and hind femur brown. Antenna light brown (radicle, scape, and pedicel slightly darker brown). Flagellum 6-segmented with apical two segments partially fused and sensilla overlapping.

Species group placement. *E. opulenta* group.

Distribution. Australia: New South Wales, Queensland, Western Australia.

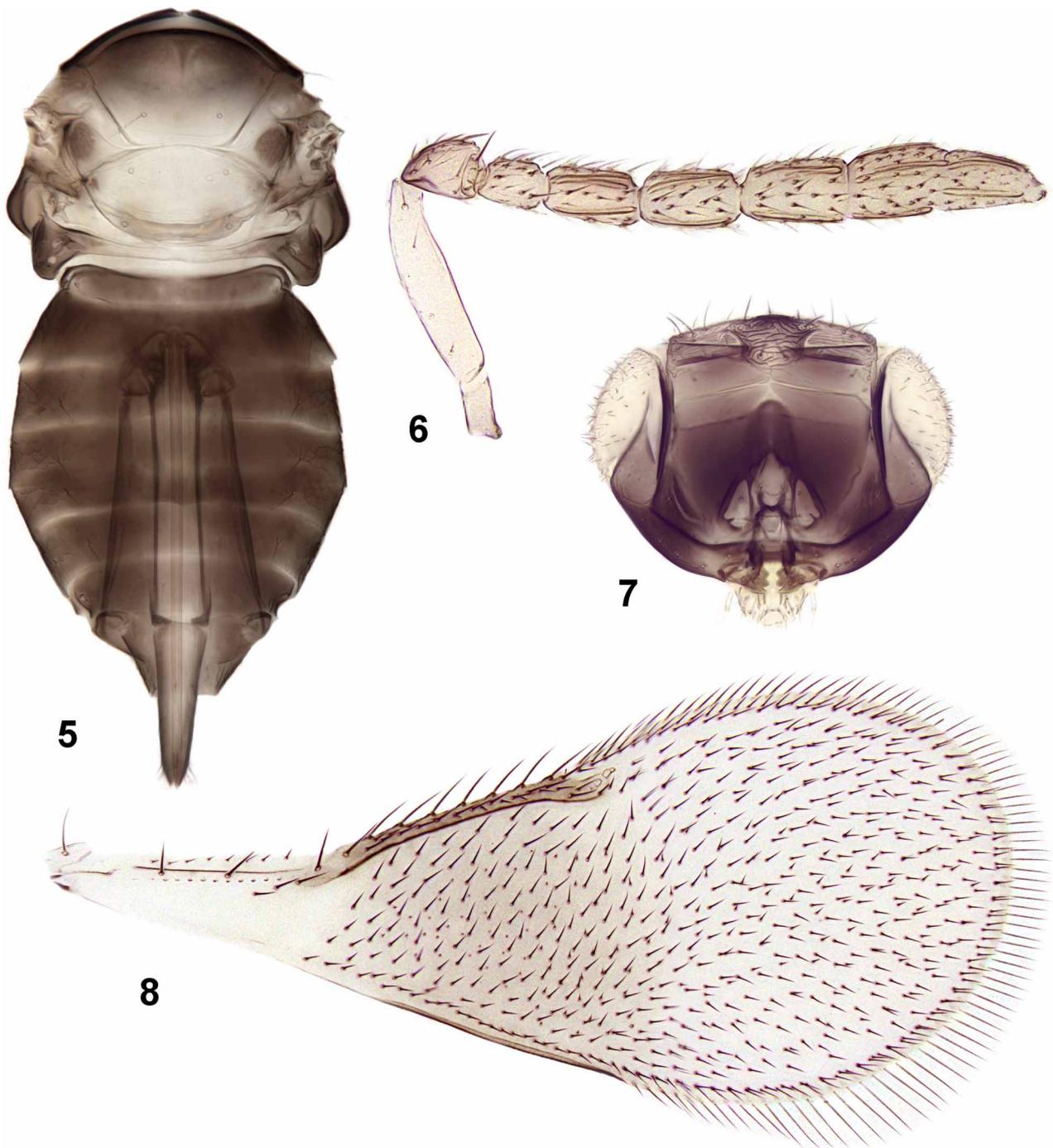
Host. *Orchamoplatus citri* (Takahashi).

Material examined. AUSTRALIA: **New South Wales:** 2 ♀, Burringbar, Howards Rd, 28.4220°S, 153.4786°E, 87 m, 8.ii.2010 (L. Howie, A. Marcora), ex *O. citri* on orange (ZSM); 3 ♀, 625 Myocum Rd, 28.8180°S, 153.4922°E, 34 m, 8.ii.2010 (L. Howie, A. Marcora), ex *O. citri* on lemon (ZSM); 1 ♂, Newybar, 19 Johnston Rd, 28.7244°S, 153.5189°E, 114 m, 8.ii.2010 (L. Howie, A. Marcora), ex *O. citri* on Honey Murcart Mandarins (ZSM). **Queensland:** 1 ♀, Biggenden, Cherelley Rd, 4621, Longatong Orchard (Cooranga Citrus P/L), 25.2862°S, 151.9570°E, 120 m, 1.iv.2010 (E. Carlton, D. Papacek, A. Guanilo) (ZSM); 2 ♀, Coochin Creek Farms, 1755 Roys Rd Beerwah, 26.892°S, 153.086°E, 13.v.2009 (L. Howie, A. Marcora), ex *O. citri* (ZSM); 1 ♀, Frank Van Der Meer Cnr Steve Irvin Way & Kings Rd, Glasshouse Mts, 26.891°S, 152.962°E, 13.v.2009 (L. Howie, A. Marcora), ex *O. citri* (ZSM); 1 ♀, Mundubbera, Durong Rd 4626, Benhams Orchard (Joey Citrus P/L), 25.6333°S, 151.2833°E, 140 m, 15.iii.2010 (E. Carlton, D. Papacek, A. Guanilo) (ZSM).

Genbank accession. HQ660515, HQ660517, JF750716-JF750728.

Comments. *Encarsia iris* was the most common parasitoid species reared from *O. citri*. It was collected at coastal locations from central Queensland south to Victoria, sometimes in large numbers. *Orchamoplatus citri* is the first host record of this species.

The 28S-D2 sequence data did not show any intraspecific variation between populations from locations in Queensland (Brisbane, Glasshouse Mts) and New South Wales (Burringbar, Myocum, Newrybar). The COI sequences exhibited an overall mean distance of 0.8%, with a maximum of 1.4% between populations from Burringbar (NSW) and Brisbane (QLD).



FIGURES 5–8. *Encarsia iris*, female. 5, body; 6, antenna; 7, head, front view; 8, fore wing.

***Eretmocerus orchamoplati* Schmidt, sp. nov.**

(Figs 9–12)

Description. FEMALE. Colour: yellow.

Morphology [measurements of holotype in square brackets]: Stemmaticum with rugose-reticulate surface structure. Antenna (Fig. 10) with radicle 3.6–3.8 [3.6]X as long as wide. Scape 3.9–4.7 [4.4]X as long as wide, 1.7–2.1 [1.8]X length of pedicel, 0.5–0.6 [0.6]X length of clava. Pedicel 2.1–2.7 [2.5]X as long as wide, 0.5–0.6 [0.6]X length of scape, 0.3–0.4 [0.3]X length of clava. First funicular segment transverse, about half as long as its maximum width; second funicular segment slightly broader than long. Clava 3.6–5.0 [4.7]X as long as wide. Apex of clava obliquely truncate (Fig. 10). Mesoscutum with 2 pairs of setae arranged symmetrically (Fig. 9), side lobes with 2 setae each. Distance between anterior pair of scutellar setae slightly less than, or subequal to, distance between posterior pair (Fig. 9). Scutellar sensilla lateral to and closer to posterior than to anterior setae. Fore wing 2.2X as long as width of disc (Fig. 12). Marginal fringe 0.2–0.3 [0.2]X as long as width of disc (Fig. 12). Basal cell without setae. Submarginal vein with 2 setae, marginal vein anteriorly with 4 setae. Marginal vein separated from linea calva by irregular row of 8–10 setae (Fig. 12). Approximately 3–6 tubercles present on ventral surface of wing near posterior end of linea calva. Wing disc posteriorly with an area of less dense setation (Fig. 12). Tergites laterally with the following number of setae: T1: 1, T2: 1 or 2, T3: 2, T4: 2, T5: 2 or 3, T6: 2, T7: 4. Ovipositor longer than midtibia (1.3–1.4 [1.4]X) and 1.4–1.5 [1.5]X as long as clava. Third valvula 0.2–0.3X as long as second valvifer.

MALE. Colour: yellow with pronotum, propodeum, and apical margins of gastral tergites brownish.

Distribution. Australia: New South Wales, Queensland, Victoria.

Host. *Orchamoplatus citri* (Takahashi).

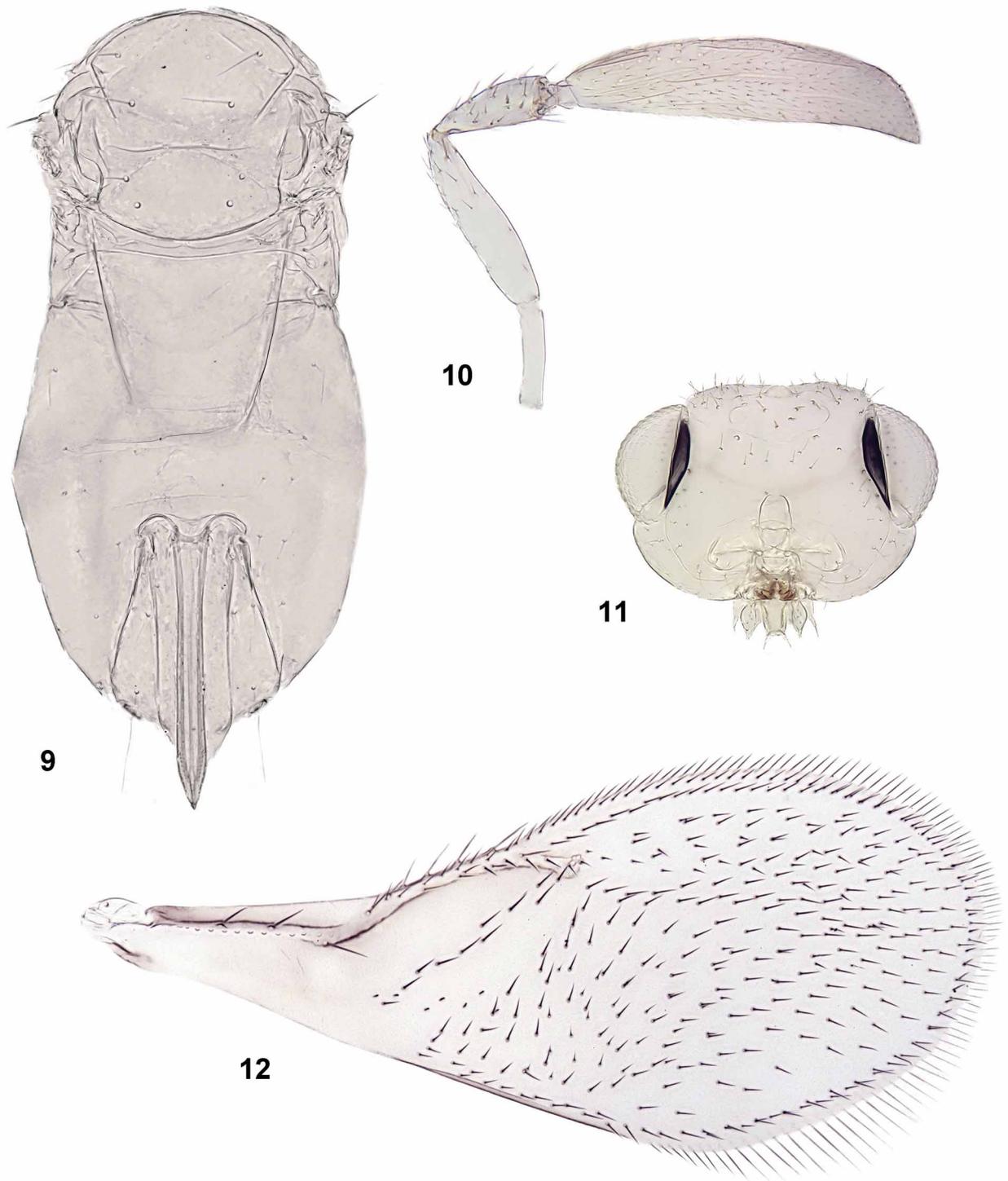
Material examined. Holotype female, labelled “AUSTRALIA, Queensland, Mundubbera, Durong Rd, 4626, Benhams Orchard (Joey Citrus P/L), 25.6333°S, 151.2833°E, 140 m, 15.iii.2010, E. Carlton, D. Papacek & A. Guanilo, DNA-Sample ZSM-HYM-AE415-04” (ANIC).

Paratypes. New South Wales: 2♀, Burringbar, Howards Rd, 28.4220°S, 153.4786°E, 87 m, 8.ii.2010 (L. Howie, A. Marcora), ex *O. citri* on orange (ZSM); 3♀, 625 Myocum Rd, 28.8180°S, 153.4922°E, 34 m, 8.ii.2010 (L. Howie, A. Marcora), ex *O. citri* on lemon (ZSM); 1♂, Newybar, 19 Johnston Rd, 28.7244°S, 153.5189°E, 114 m, 8.ii.2010 (L. Howie, A. Marcora) ex *O. citri* on Honey Murcart Mandarins (ZSM); 1♀, Bateman's Bay, 4 Peninsula Drive, 35.7082°S, 150.1747°E, 5 m, 14.iv.2010 (A. Chhagan), ex *O. citri* (ZSM). **Queensland:** 1♂, 2♀, same data as holotype (ZSM); 3♀, Coochin Creek Farms, 1755 Roys Rd, Beerwah, 26.892°S, 153.086°E, 13.v.2009 (L. Howie, A. Marcora), ex *O. citri* (NZAC, ZSM); 2♀, Mundubbera, 25.6°S, 151.3°E, 12.ii.2009 (D. Papacek), collected in close association with *O. citri*. (ZSM); 1♀, Frank Van Der Meer Cnr Steve Irvin Way & Kings Rd, Glasshouse Mtn 26.891°S, 152.962°E, 13.v.2009 (L. Howie, A. Marcora), ex *O. citri* (NZAC); 2♀, Biggenden, Cherelley Rd, 4621, Longatong Orchard (Cooranga Citrus P/L), 25.2862°S, 151.9570°E, 120 m, 1.iv.2010 (E. Carlton, D. Papacek, A. Guanilo) (ZSM); 2♀, Indooroopilly, 27.5000°S, 152.9824°E, 21 m, September 2009 (P. De Barro) (ZSM). **Victoria:** 4♀, Mallacoota, Blue Wren Motel, 37.5587°S, 149.7541°E, 19 m (A. Chhagan), ex Aleyrodidae (ZSM); 1♀, Lakes Entrance, 9 Prince Hwy, 33.828°S, 144.817°E, 25 m (A. Chhagan), ex Aleyrodidae (ZSM).

Genbank accession. HQ660514, HQ660518, JF750711-JF750715, JF750729-JF750732.

Comments. *Eretmocerus orchamoplati* sp. nov. was collected at coastal locations from central Queensland south to Victoria. The species can be separated from the other Australian *Eretmocerus* species by a combination of characters, including the transverse first and slightly broader than long second funicular segments, the short marginal fringe of the fore wing, and the few tubercles on the ventral surface of the fore wing near the posterior end of the linea calva. In the key to Australian *Eretmocerus* species associated with *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (De Barro *et al.* 2000), *E. orchamoplati* would key out at the first couplet because of its transverse first funicular segment that is neither cylindrical as in *E. mundus* Mercet, nor subtriangular as in *E. warrae* (Naumann & Schmidt) or *E. queenslandensis* (Naumann & Schmidt).

As in the other two parasitoid species examined, the 28S-D2 sequence data did not exhibit any intraspecific variation between populations from several locations in Queensland (Beerwah, Brisbane, Mundubbera), New South Wales (Batemans Bay), and Victoria (Mallacoota). The COI sequences exhibited an overall mean distance of 1.0%, with a maximum of 1.7% between populations from Biggenden (Queensland) and Mallacoota (Victoria).



FIGURES 9–12. *Eretmocerus orchamoplatis* sp. nov., female. 9, body; 10, antenna; 11, head, front view; 12, fore wing.

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