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Descriptions of three species of *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) parasitising *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in Australia based on morphological and molecular data

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Abstract Morphological and molecular systematic investigations have confirmed the existence in Australia of three species of *Eretmocerus* Haldeman that parasitise either the silverleaf whitefly, *Bemisia tabaci* (Gennadius), or the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). Two of the species *Eretmocerus warrae* sp. n. and *Eretmocerus queenslandensis* sp. n. are described. A third species is very similar to *Eretmocerus mundus* Mercet at both the morphological and molecular levels. However, Australian populations of *E. mundus* are distinct from those found elsewhere in being thelytokous, suggesting that the Australian populations are a distinct biotype; we refer to these Australian populations as *E. mundus* (Australian parthenogenetic form; APF) to reflect this distinction. The four gene regions investigated, mitochondrial cytochrome oxidase II, the nuclear ribosomal *ITS* region and the domain 2 and 3 expansion segments of the 28S ribosomal RNA gene gave separation of the species consistent with our morphological data. Studies of *COII*, *ITS1*, *ITS2* and *D3* indicate that these three species do not vary geographically within Australia. Field collections and laboratory studies confirm that *E. queenslandensis* and *E. mundus* (APF) parasitise only *B. tabaci*, while *E. warrae* parasitises only *T. vaporariorum*. *Eretmocerus warrae* was found across the southern half of Australia, *E. mundus* (APF) from northern New South Wales to northern Queensland, and *E. queenslandensis* in northern Queensland. The molecular data indicate that *E. queenslandensis* is conspecific with an undescribed *Eretmocerus* species from Hong Kong.

Key words aphelinid taxonomy, biological control, mitochondrial DNA, ribosomal DNA.

INTRODUCTION

In October 1994, the silverleaf whitefly (SLW), *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae), was first detected in Darwin, Northern Territory (Gunning *et al*. 1995). Subsequent surveys found that the whitefly was established in nurseries from northern New South Wales to far northern Queensland (De Barro 1995). The SLW colonises a wide range of plant species, including cotton and ornamental, vegetable and weed species. Damage is caused by: (i) direct feeding, which may induce irreversible physiological disorders and yield decline; (ii) contamination with honeydew and sooty mould; and (iii) SLW acting as a vector of geminiviruses (see De Barro 1995 for review). Through combinations of the above, SLW has caused serious damage to crops in virtually every country in which it occurs and hence its detection in Australia caused serious concern.

One of the key management challenges posed by SLW is its ability to develop resistance against insecticides. This is further compounded by the lack in Australia of effective insecticides and difficulties in obtaining minor use registration for new effective products. For this reason, reliance on insecticides as the sole means of managing infestations was considered at best a short-term solution. It is generally considered that long-term, sustainable management of SLW requires an integrated approach in which a range of management strategies are combined to control the pest. One of the key components in achieving this elsewhere has been the use of natural enemies, in particular parasitic Hymenoptera of the family Aphelinidae. Reductions in numbers of whiteflies through the use of natural enemies delays or decreases the need for applications of insecticides, thereby reducing selection pressure on insecticide resistance.

Australia has never before had a serious whitefly pest of outdoor crops and, consequently, has very little research experience of these organisms. It was therefore concluded that research into the management of SLW should commence before the inevitable economic effects of the pest were felt. One of the key areas targeted was biological control. Research overseas has indicated that parasitoids offer the best potential for control (Gerling 1986; Osborne

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et al. 1990; De Barro 1995; Goolsby *et al*. 1996, 1998; Kirk & Lacey 1996; Lacey *et al*. 1996; Legaspi *et al*. 1996; Nordlund & Legaspi 1996; for a review). However, our knowledge of the Australian whitefly parasitoid fauna was virtually nil. It was known that there was at least one indigenous biotype of *B. tabaci* that was widespread across the northern half of Australia (De Barro & Driver 1997; De Barro *et al*. 1998), as well as several other closely related indigenous species of *Bemisia* (Martin 1999). It was concluded that agents capable of contributing significantly to the biological control of this pest might already be in Australia.

To determine the distribution and diversity of parasitoids attacking *B. tabaci* in Australia, a series of surveys was undertaken. Because many parasitoids attack both *B. tabaci* and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), both were collected. It was clear that while surveys might find parasitoids, it was equally likely that their identification would not be possible, as keys to whitefly parasitoids in Australia did not exist and keys available for other regions were either unreliable or did not include Australian taxa.

There are approximately 45 species of *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) described worldwide and four have been recorded previously from Australia. Published descriptions are inadequate to enable recognition of species. However, recent work in North America has greatly improved understanding of the morphological criteria for characterising species of *Eretmocerus* (Rose & Zolnerowich 1997; Zolnerowich & Rose 1998). *Eretmocerus* was considered to offer great potential as an effective agent because *Eretmocerus* species had elsewhere proved to be consistently among the best-performing parasitoids both in the laboratory and the field (Goolsby *et al*. 1998).

The present paper describes morphological and molecular taxonomic criteria that are sufficiently discriminatory to enable separation of the different species of *Eretmocerus* attacking *B. tabaci* and *T. vaporariorum* in Australia.

MATERIALS AND METHODS

Surveys

Parasitised *B. tabaci* and *T. vaporariorum* from throughout Australia were collected in a series of surveys undertaken from mid-1995 to mid-1997. Surveys were carried out in: Perth, Pemberton and Kununurra (Western Australia); Darwin (Northern Territory); Mareeba, Cairns, Townsville, Ayr, Bowen, Bundaberg, Emerald, Biloela, Roma and the Darling Downs (Queensland); Adelaide and the Riverland (South Australia); Riverina District (Victoria); Canberra (Australian Capital Territory); and Lake Tandou, the Griffith area, Narrabri and Moree (New South Wales). All parasitoids were reared from whiteflies collected in the field. Additional samples were provided from several Queensland localities by the Queensland Department of Primary Industries.

Parasitised nymphs were placed in emergence chambers and parasitoids were removed after emergence. They were stored either as air-dried specimens in gelatine capsules or in vials in 95% ethanol at room temperature. Whiteflies were identified to species by using the fourth-instar pupal case from which the parasitoid had emerged (Martin 1987). *Bemisia tabaci* biotypes were identified by using adults collected with the parasitised nymphs using the method described in De Barro and Driver (1997). Each accession was given a unique code number to identify the location and date of collection, the host plant, the host whitefly species and the collector.

DNA extraction, amplification and sequencing

DNA was extracted from single, whole specimens in the manner described by De Barro and Driver (1997). The crude lysate was boiled for 5 min and samples were stored at – 20°C until required.

The domain 3 gene region (*D3*) of the 28S rRNA gene, the ribosomal *ITS1* and *ITS2* gene regions and a central portion of the mitochondrial cytochrome oxidase II (*COII*) gene were used to compare three species of parasitoids across their known range in Australia (Table 1). To compare the Australian fauna with species of *Eretmocerus* found to be effective agents from other regions (Goolsby *et al*. 1998; Goolsby *et al*. 1999), the *COII* region from *Eretmocerus* sp. 1 (M96076, Ethiopia) was compared with the three Australian species, as was *ITS1* from *Eretmocerus mundus* Mercet (M92014, Spain), *Eretmocerus hayati* Rose and Zolnerowich (M95012, Pakistan) and *Eretmocerus* sp. 1 (Table 2).

In addition, the domain 2 gene region (*D2*) of the 28S rRNA gene was compared across a smaller subset of the three species from Australia. These sequences were, in turn, compared with the same region from *Eretmocerus* sp. 2, a thelytokous species from Hong Kong, the biology of which is described by McAuslane and Nguyen (1996). *Eretmocerus* sp. 2 is the only other thelytokous species known from the Asia–Australia region (Table 1).

The polymerase chain reaction (PCR) was used to amplify the gene regions for each specimen. All reaction volumes were 50 µL, containing 20 pmol of each primer, 200 µmol of each of dGTP, dATP, dCTP and dTTP, 1.5–2.5 mmol $MgCl_2$, 2 µL DNA lysate, 1× supplied buffer and 2.5 U Taq polymerase (Geneworks, Adelaide, Australia). The PCR amplifications were carried out in a Hybaid thermocycler (Hybaid, Teddington, UK) and included a precycle denaturation step for 5 min at 94°C, followed by the addition of the Taq polymerase, then a final postcycle extension step at 72°C for 5 min (Table 1).

The *D2*, *D3* and *COII* amplicons were purified and prepared for sequencing by electrophoresis in 0.8% Tris acetate EDTA (TAE) agarose gels containing 10 µg mL^{-1} ethidium bromide (Sambrook *et al*. 1989). Fragments were excised and transferred to a microfuge tube. The agarose slices were mashed in 30 µL sterile distilled water using a toothpick, then incubated at 50°C for 1 h. Samples were left at room temperature overnight to allow the DNA to elute from the gel. The samples were stored at – 20°C until required. The *ITS1* and *ITS2* amplicons were selectively precipitated and ligated into the pPCR-Script Amp SK(+) cloning vector from

Table 1 Primer sequences and cycling conditions

	Primer	Cycling conditions			
		Denaturation	Annealing	Extension	Cycles
28S-*D3**					
D3A	5′ GACCCGTCTTGAAACACACGGA 3′	94°C	55°C	72°C	30
D3B	5′ TCGGAAGGAACCAGCTACTA 3′	(1 min)	(1 min)	(1.5 min)	
ITS1					
TW81	5′ GTTTCCGTAGGTGAACCTGC 3′	94°C	55°C	72°C	30
Aed5.8R	5′ GAGAACAGCAGGAACACAGAAC 3′	(1 min)	(1 min)	(1.5 min)	
ITS2†					
Aed5.8F	5′ GTGAACTGCAGGACACATGAAC 3′	94°C	55°C	72°C	30
AedAB28	5′ ATATGCTTAAATTTAGGGGGT 3′	(1 min)	(1 min)	(1.5 min)	
COII‡					
C2-J-3400	5′ ATTGGACATCAATGATATTGA 3′	94°C	52°C	72°C	32
C2-N-3661	5′ CCACAAATTTCTGAACATTGACCA 3′	(1 min)	(1 min)	(1.5 min)	
28S-*D2*§					
*D2*F	5′ CGT GTT GCT TGA TAG TGC AGC 3′	94°C	55°C	72°C	35
*D2*R	5′ TTG GTC CGT GTT TCA AGA CGG 3′	(1 min)	(1 min)	(1.5 min)	

*Nunn *et al*. (1996); †Brust *et al*. (1998); ‡Simon *et al*. (1994); §Dr B. Campbell, United States Department of Agriculture–Agricultural Research Service, Western Regional Research Centre, USA.

Stratagene (La Jolla, California, USA), according to the manufacturer's protocol.

We used 5 µL of the eluted PCR-amplicons or approximately 250 ng cloned plasmid DNA and the appropriate PCR primers for sequencing according to the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit Manual (PE-Applied BioSystems, Forest City, CA, USA). Both strands of each fragment were sequenced and reactions were loaded onto an ABI Model 373 A Sequencer (PE-Applied BioSystems, Forest City, CA, USA).

Analysis of DNA sequences

The *D2*, *D3*, *COII*, *ITS1* and *ITS2* gene sequences were aligned using Clustal W (Thompson *et al*. 1994). The alignments were checked visually and minor adjustments made manually. Secondary structural models, such as those described by Michot *et al*. (1990), for the *D3* gene region, were not employed for the alignment because there were no size polymorphisms in the sequences from this gene region. No further analyses of the data were deemed necessary, except for the *ITS1* region, which was subjected to phylogenetic analysis to highlight the relationships between sequences from different *E. mundus* populations.

Phylogenetic analysis using maximum parsimony of the 17 unique *ITS* sequences was undertaken using PAUP* 4.0b4a (Swofford 1998). We conducted 100 full heuristic search bootstrap replicates with the following parameters. Optimisation was accelerated transformation (ACCTRAN), starting trees were generated by simple stepwise addition and branch swapping was by tree-bisection and reconnection (TBR). All 754 characters were included and were assigned equal weight. Gaps were treated as missing characters.

Slide preparation and media

Specimens in ethanol were dried before mounting. All were glued onto a card mount with the wings accessible, the wings were then removed and mounted (under coverslip 1) on a slide. The dried specimen was then removed by soaking and cleared with KOH. After drying, specimens in ethanol were left in KOH for 72 h at room temperature; other specimens were heated in KOH for 5 min at just below boiling point. All specimens were then treated (10–20 min each bath) with glacial acetic acid, distilled water, an ethanol gradient (35, 70, 82.5 and 95%) and clove oil before being transferred to the slide already bearing the wings. Separate mounts were made of antennae (coverslip 2), head (coverslip 3) and the remainder of the body, dorsal side up and with legs displayed at the sides (coverslip 4). Canada balsam was the mounting medium, and histolene was used as a thinning agent.

RESULTS AND DISCUSSION

Sequences for each of the parasitoids examined are lodged with GenBank, *ITS1* (AF273621–AF273649), *ITS2* (AF273650–AF273662), *D2* (AF273666–AF273669), *D3* (AF273663–AF273665) and *COII* (AF275275–AF275306).

D3 expansion segment of the 28S rRNA

Sequences for all three species identified vary strictly in accordance with their placement into one of three morphologically defined taxonomic groups (Fig. 1); *Eretmocerus warrae*, *E. mundus* (Australian parthenogenetic form) or *Eretmocerus queenslandensis*. Only five positions vary (positions 39, 40, 68, 85 and 106) from 312 positions in the alignment, with no size polymorphisms between the three taxa. For each taxonomic group, no sequence divergence was found between specimens collected in the same locality or between sites across Australia and Nauru. This gene region is the most conserved of the three gene regions sequenced, and the secondary structural organisation of the sequences is consistent with models described by Michot *et al*. (1990).

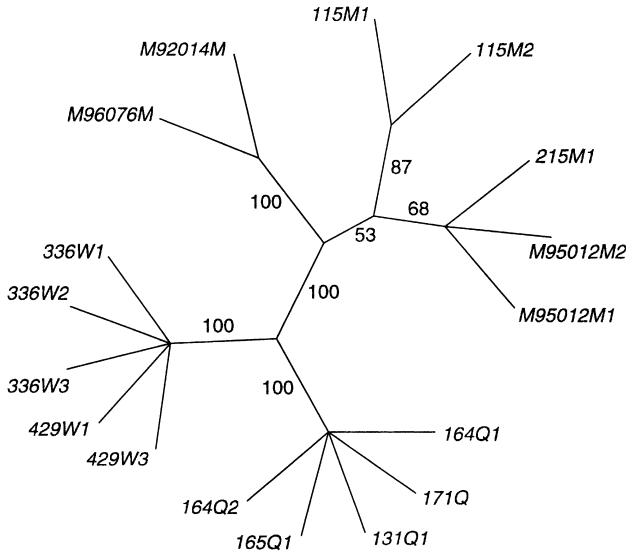


Fig. 1. Strict consensus most parsimonious unrooted network tree of the *ITS1* data set of *Eretmocerus* spp. Numbers above or below each branch represent bootstrap support values for 100 iterations.

Mitochondrial cytochrome oxidase II

DNA was extracted from single individuals, therefore any heteroplasmy or polymorphisms observed are specific to those individuals. A degree of heteroplasmy was evident in the sequences of almost all individuals tested, this ranged from almost 0 to approximately 5% of bases in the codons. Generally, this redundancy was in third-position sites of the codons that would not result in amino acid substitutions in the putative translation product. However, a low level of alternate codon usage for serine and first and second position changes that would result in amino acid substitutions were observed.

Mitochondrial DNA has been used to demonstrate intraspecific variation, which may also be localised geographically. Such variation at the level of amino acid substitutions occurs in all three *Eretmocerus* species. There is only scant evidence that such intraspecific variation is geographically localised in *E. warrae* and *E. queenslandensis*, but specimens of *E. mundus* from two separate collections from Nauru (nos 215, 216, Table 2), as well as two individuals from the United States Department of Agriculture collected from Ethiopia (*Eretmocerus* sp. 1, M96076) show amino acid substitutions at positions 64 and 79, and 40 and 79, respectively, which is more indicative of geographically localised variation. The sequence data and amino acid translation products place the Ethiopian specimens in the *E. mundus* cluster.

Nucleotide base composition and amino acid sequences also vary in accordance with the morphological designations for these taxa. There are 22 (24.7%) variable sites among the 89 positions in the amino acid alignment, which unambiguously delineate the taxa. The morphologically defined species vary from each other by very similar amounts; *E. mundus* (Australian parthenogenetic form; APF) and *E. warrae* by 17 (19%) sites in the alignment, *E. mundus* (APF) and *E. queenslandensis* by 15 sites (16.9%) and *E. warrae* from *E. queenslandensis* by 19 (21.3%) sites in the alignment.

Ribosomal *ITS*

A total of 29 *ITS1* and 17 *ITS2* clones were sequenced for all three species of Australian *Eretmocerus* spp. Clones for each population were derived from a single individual. As would be expected for this gene region, low levels of polymorphism can be seen between gene copies from an individual. This level of polymorphism is similar to the level of intraspecific variation shown by individuals from different geographical sites.

The 17 unique *ITS1* sequences contained 161 parsimony informative sites. An initial heuristic search yielded 32 equally most parsimonious unrooted trees of 213 steps in length, with a consistency index of 0.945 and a retention index of 0.985. The strict consensus and 50% majority rule consensus trees are identical (Fig. 1). Bootstrap support values (based on 100 bootstrap replicates) are shown for each branch. An identical topology was recovered by neighbour joining methods. The unrooted network clearly shows three well-supported monophyletic clades corresponding to the three species of *Eretmocerus*. There is some additional hierarchical structure within the *E. mundus* clade, indicating specimens from Spain (M92014), *Eretmocerus hayati* from Pakistan (M95012) and *Eretmocerus* sp. 1 from Ethiopia (M96076) unequivocally fall into the *E. mundus* cluster, but exhibit some geographically localised variation compared with Australian populations. Such variation in *ITS* sequences that correlated with geographical distribution was observed in biotypes of tiger beetles in the New England area of the USA (Vogler & De Salle 1994). In the absence of any good distinguishing morphological characters, it is difficult to conclude that the variation between *E. mundus*, *E. mundus* (APF), *E. hayati* and *Eretmocerus* sp. 1 is more than intraspecific population variation.

These sequences also vary in a species-specific manner, and sequence divergence is such that there is scope for identifications based on size polymorphisms of the PCR amplicons, RFLP (restriction fragment length polymorphism) patterns of the gene fragments or by the design of species-specific primers.

D2 expansion segment of the 28S rRNA

Sequences for all three Australian species identified vary strictly in accordance with their placement into one of the three morphologically defined taxonomic groups, and provide the same level of species separation as the *D3*, *ITS* and *COII* gene regions. There are 29 positions that vary of the 590 positions in the alignment of the sequences. In addition, there is a nine-base deletion in *E. mundus* (APF). While the sample compared was small for each taxonomic

Table 2 Collection details and number of individuals sequenced for the three *Eretmocerus* species

Species	Code	Whitefly host‡	State/country	Locality	Host plant	*D3*	*COII*	*ITS1/2*	*D2*
E. warrae	155	*T. vaporariorum*	New South Wales	Narrabri	*Xanthium occidentale*	W	W	W/W	–
	336	*T. vaporariorum*	New South Wales	Lake Tandou	*Xanthium occidentale*	W	W	W/W	–
	370	*T. vaporariorum*	South Australia	McLaren Vale	*Salvia* sp.	2W	W	–/–	–
	429	*T. vaporariorum*	Western Australia	Pemberton	*Cyphomandra fragrans*	W	2W	W/W	–
	142	*T. vaporariorum*	Queensland	Highfields	*Verbena bonariensis*	2W	W	–/–	–
	518	*T. vaporariorum*	Queensland	Jondaryan	*Xanthium occidentale*	2W	2W	–/–	–
	537	*T. vaporariorum*	Queensland	Goondiwindi	*Helianthus annus*	2W	W	–/–	–
	531	*T. vaporariorum*	Queensland	Oakey	*Lactuca serriola*	2W	W	–/–	–
	T-6735	*T. vaporariorum*	Queensland	Bowenville	*Helianthus annus*	2W	2W	–/–	–
	ERW1	*T. vaporariorum*	New South Wales	Bellata	*Xanthium occidentale*	–	–	–/–	W
	ERW2	*T. vaporariorum*	New South Wales	Bellata	*Xanthium occidentale*	–	–	–/–	W
E. mundus (APF)	135	*B. tabaci*, EAN*	Queensland	Warra	*Gossypium hirsutum*	M	M	–/–	–
	709	*B. tabaci*, EAN*	Queensland	Warra	*Gossypium hirsutum*	M	M	–/–	–
	143	*B. tabaci*, EAN*	Queensland	Dalby	*Gossypium hirsutum*	2M	2M	–/–	–
	159	*B. tabaci*, B*	Queensland	Mt Isa	*Sonchus oleraceus*	2M	M	–/M	–
	520	*B. tabaci*, EAN*	Queensland	Toowoomba	*Gossypium hirsutum*	2M	2M	–/–	–
	115	*B. tabaci*, B*	New South Wales	Winmallee	*Euphorbia pulcherrima*	2M	2M	M/M	–
	ERM1	*B. tabaci*, B*	Queensland	Warra	*Sonchus oleraceus*	–	–	–/–	M
	ERM2	*B. tabaci*, B*	Queensland	Warra	*Sonchus oleraceus*	–	–	–/–	M
	ERM3	*B. tabaci*, B*	Queensland	Warra	*Sonchus oleraceus*	–	–	–/–	M
E. mundus (biparental form)	215	*B. tabaci*, Nauru*	Nauru		*Phyllanthus amarus*	M	M	M/M	–
	216	*B. tabaci*, Nauru*	Nauru		*Cleome viscosa*	M	M	–/–	–
	M92014	*B. tabaci*, non-B*	Spain	Murcia	*Gossypium hirsutum*, culture	–	–	M/–	–
E. hayati	M95012	*B. tabaci*, non-B*	Pakistan	Multan	*Gossypium hirsutum*, culture	–	–	M/–	–
Eretmocerus sp. 1	M96076	*B. tabaci*, non-B*	Ethiopia	Melka	*Gossypium hirsutum*, culture	–	2M	M/–	–
E. queenslandensis	164	*B. tabaci*, B*	Queensland	Townsville	*Hibiscus rosa-sinensis*	2Q	Q	Q/–	–
	165	*B. tabaci*, B*	Queensland	Townsville	*Hibiscus rosa-sinensis*	2Q	2Q	Q/Q	–
	131	*B. tabaci*, B*	Queensland	Townsville	*Hibiscus rosa-sinensis*	Q	Q	Q/Q	–
	171	*B. tabaci*, B*	Queensland	Townsville	*Hibiscus rosa-sinensis*	Q	Q	Q/–	–
	168	*B. tabaci*, B*	Queensland	Cairns	*Sonchus oleraceus*	Q	2Q	–/–	–
	130	*B. tabaci*, B*	Queensland	Ayr	*Hibiscus rosa-sinensis*	Q	Q	–/–	–
	262	*B. tabaci*, B*	Queensland	Ayr	*Sonchus oleraceus*	Q	Q	–/Q	–
	ERQ1	*B. tabaci*, B*	Queensland	Townsville	*Hibiscus rosa-sinensis*†	–	–	–/–	Q
	ERQ2	*B. tabaci*, B*	Queensland	Townsville	*Hibiscus rosa-sinensis*†	–	–	–/–	Q
Eretmocerus sp. 2	ERHK1	*B. tabaci*, unknown	Hong Kong		Culture, H. J. McAuslane	–	–	–/–	H
	ERHK2	*B. tabaci*, unknown	Hong Kong		Culture, H. J. McAuslane	–	–	–/–	H

*Biotype of *Bemisia tabaci*. †Specimens from the culture at CSIRO Entomology, Canberra. ‡*Trialeurodes vaporariorum* or *Bemisia tabaci*. APF, Australian parthenogenetic form.

group, there was no sequence divergence found between specimens from the same locality. The sequence of *Eretmocerus* sp. 2 from Hong Kong was identical to that of *E. queenslandensis*, with the exception of a single consistent point mutation at position 206. This suggests that the two are conspecific, with the point mutation being consistent with variation at the population level.

The comparison of various regions of DNA obtained from the *Eretmocerus* spp. from Australia with effective agents found elsewhere suggests that populations of *Eretmocerus* with sequences similar to *E. mundus* have the potential to be more effective than species that do not. Further, the recent phylogenetic analysis of *B. tabaci* (Frohlich *et al*. 1999; De Barro *et al*. 2000) suggests an interesting interpretation of the approach to biological control of SLW, in particular, the search for effective natural enemies. Not surprisingly, some of the most effective natural enemies of this biotype are from the north-eastern Africa/Middle-East region, yet nearly all countries in this region have current serious problems with the B biotype (Goolsby *et al*. 1998). This suggests that many of the problems associated with controlling SLW may not be the result of having ineffective natural enemies, but are, instead, issues of crop management. Effective agents are also found in countries containing *B. tabaci* biotypes that are quite distinct from SLW (Goolsby *et al*. 1998). This is contrary to one of the tenets of biological control because it suggests, in this case, that knowing the origin of SLW is not essential in terms of finding effective agents. Again, these findings support the notion that crop management is the key aspect to control. A similar situation was recently hypothesised to exist regarding the cane toad, *Bufo marinus*. Where a pest organism exists in its natural range as two or more distinct populations, control organisms may be potentially more effective if selected from population-clusters other than the one from which the pest has dispersed (Slade & Moritz 1998).

Terminology for descriptions and repositories

Morphological conventions and terms employed in the descriptions follow Rose and Zolnerowich (1997) and Zolnerowich and Rose (1998). All specimens examined have been deposited in the Australian National Insect Collection (ANIC), CSIRO Entomology, Canberra.

Key to species of *Eretmocerus* associated with *Bemisia tabaci* and *Trialeurodes vaporariorum* in Australia (females)

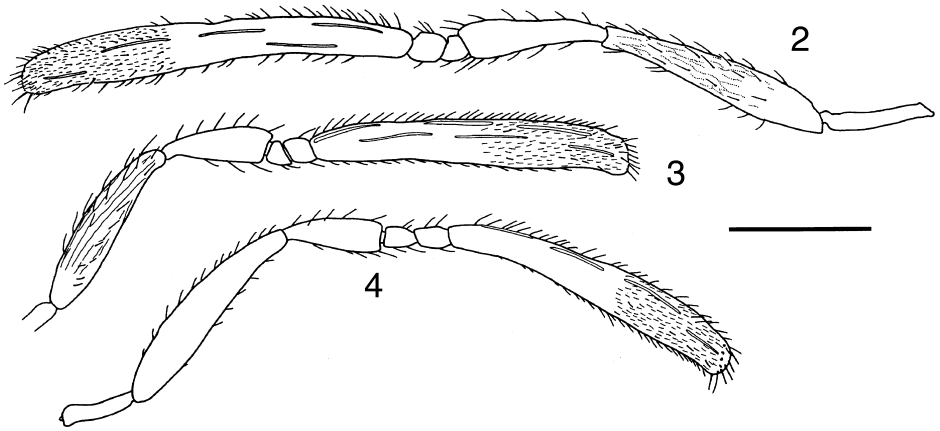
1. First funicular segment almost cylindrical in lateral view, dorsal surface slightly shorter than ventral surface (Fig. 4). Marginal vein straight proximally (Fig. 9). Mesoscutum with 2 pairs of setae (Fig. 5). Parasitoid of *Bemisia tabaci* .... *mundus* Mercet (Australian parthenogenetic form)
– First funicular segment subtriangular in lateral view, ventral surface at least twice as long as dorsal surface (Figs 2,3). Marginal vein curved proximally (Figs 8,10). Mesoscutum usually with 3 pairs of setae (Fig. 7), occasionally 1–2 setae of anteromedian pair missing (Fig. 6) 2
– Gastral tergites 2–4 with 2 seta, one on each side (Fig. 11), occasionally a tergite with 3 setae. Pedicel elongate, at least 3.7 times longer than wide (Fig. 2). Body yellow. Parasitoid of *Trialeurodes vaporariorum* ..... *warrae* sp. n.
– Gastral tergites 2–4 with 2 pairs of setae, one pair on each side, occasionally a tergite with 3 or 5 setae. Pedicel stouter, not more than 3.1 times longer than wide (Fig. 3). Body fuscous. Parasitoid of *Bemisia tabaci* ..... *queenslandensis* sp. n.

Eretmocerus mundus (Australian parthenogenetic form; Figs 4,5,9)

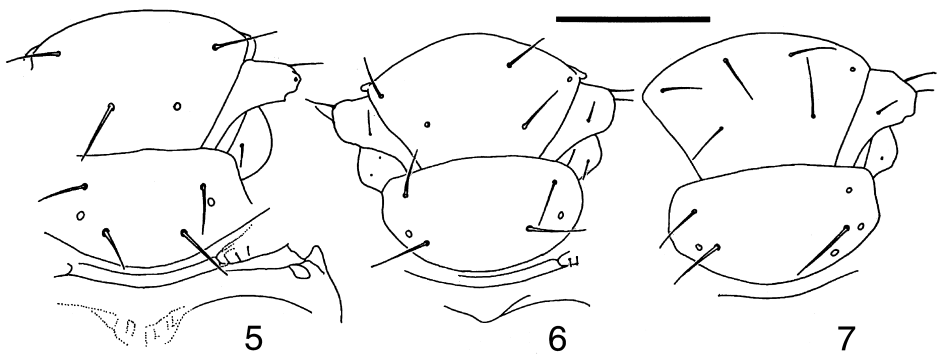
Eretmocerus mundus Mercet 1931: 395–398; Compere 1936: 277–321; Ferrière 1965: 171; Viggiani 1965: 257–262; Gerling 1972: 157; Hayat 1972: 99–106; Viggiani & Battaglia 1983: 97–101.

Material examined. ***Queensland.*** 12 females, all ex *B. tabaci*: 4 females, ex laboratory culture originating from Warra or Ayr, x.1997, P. De Barro, on *Hibiscus*; 3 females, Warra, 24.iii.1997, 29.v.1997, x.1997, B. A. Franzmann, D. R. Lea, on cotton or on *Sonchus oleraceus*; 4 females, Mount Isa, 3.x.1996, P. De Barro, on *S. oleraceus*; 1 female, Ayr, iii.1997, P. De Barro, on *S. oleraceus*; 3 females, from laboratory culture originating from Warra on *S. oleraceus*. All in ANIC.

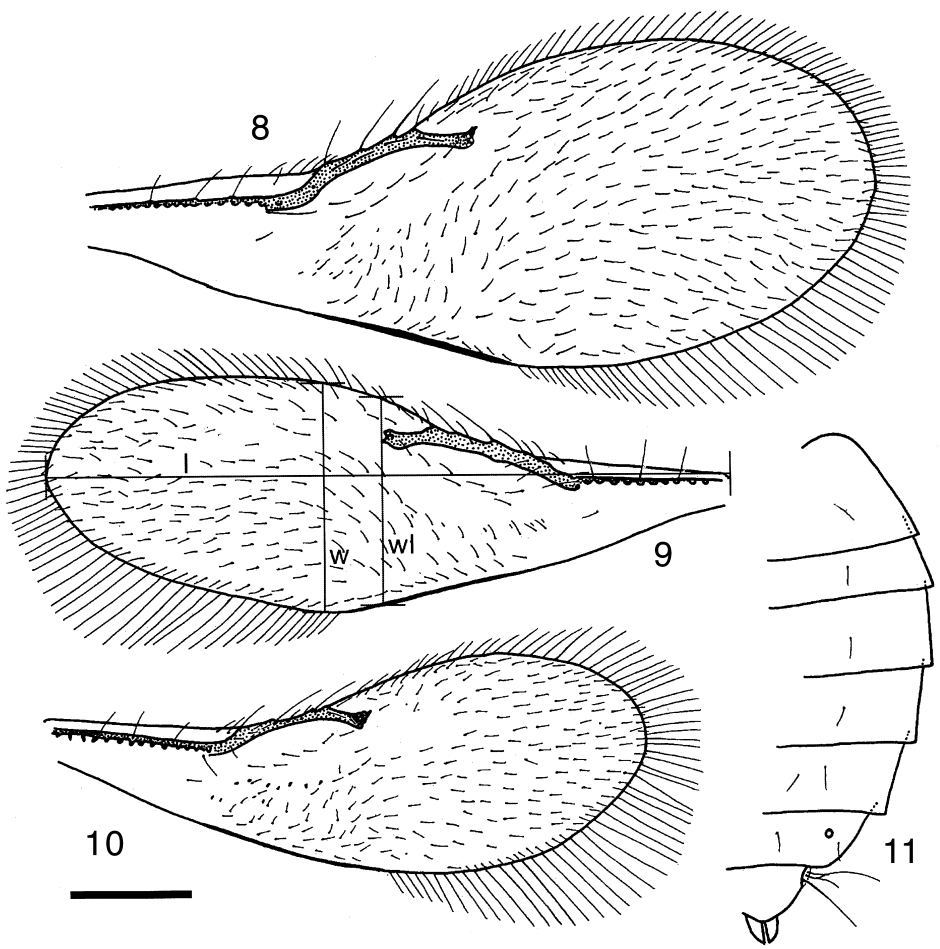
Female. ***Colouration.*** Dried, unmounted specimens pale yellow. Specimens in balsam: body pale yellow. Anterior margin of mesoscutum sometimes slightly fuscous. Antennae slightly fuscous, club darker. Legs pale except last tarsal segment slightly fuscous. Wings hyaline except fuscous veins and forewing slightly infuscate in basal half and anteriorly near frenal fold. ***Morphology.*** Frons and occiput with transverse, substrigulate microsculpture; lower face more vertically substrigulate. Antenna (Fig. 4) with radicle 3.6–3.8 times as long as wide. Scape 5.1–6.4 times as long as wide, 2.4–2.6 times length of radicle, 1.9–2.3 times length of pedicel, 0.7 times length of club. Pedicel 2.6–3.4 times as long as wide, 1.1–1.2 times length of radicle, 0.5 times length of scape, 0.34–0.37 times length of club. First funicular segment 1.0–1.5 times as long as wide; second funicular segment 1.1–1.7 times as long as wide. Club 5.8–6.8 times as long as wide, 1.4–1.5 times length of scape, 2.7–3.4 times length of pedicel. Apex of club truncate. Mesoscutum (Fig. 5) with 4 setae and with reticulate sculpture except posteriorly longitudinally reticulate and posteromedially longitudinally reticulate to substrigulate. Parapsis with 2 setae and reticulate to substrigulate sculpture; axilla with 1 seta and substrigulate sculpture. Scutellum with longitudinally reticulate sculpture laterally and substrigulate sculpture medially and with 4 setae. Placoid sensilla lateral to and equidistant or



Figs 2–4. *Eretmocerus* spp., antenna: **(2)** *E. warrae*; **(3)** *E. queenslandensis*; **(4)** *E. mundus* (Australian parthenogenetic form). Scale line = 0.1 mm.



Figs 5–7. *Eretmocerus* spp., mesosoma of females: **(5)** *E. mundus* (Australian parthenogenetic form); **(6,7)** *E. queenslandensis*. Scale line = 0.1 mm.



Figs 8–11. *Eretmocerus* spp. **(8–10)** Female forewings: **(8)** *E. warrae*; **(9)** *E. mundus* (Australian parthenogenetic form) (l, Length of forewing; w, greatest width of disc; wI, width I); **(10)** *E. queenslandensis*; **(11)** *E. warrae* female, right side of metasoma: Scale line = 0.1 mm.

closer to anterior than to posterior setae. Propodeum with substrigulate sculpture. Endophragma usually extending to posterior margin of gastral tergite 2. Forewing (Fig. 9) length 0.57–0.62 mm, 3.0–3.6 times as long as wide at width 1 (Fig. 9, wI), 2.7–3.3 times as long as maximum width of disc. Longest anterior alary fringe 0.20–0.31 times maximum width of disc, longest posterior alary fringe 0.36–0.45 times width of disc. Basal cell of wing with (0–) 1 seta. Costal cell distally with (1–)2–3 setae. Marginal vein with 3(–4) long setae, separated from linear calva by irregular row of 6–8(–9) setae. Linea calva closed posteriorly by setae. Approximately 5–9 tubercles present on ventral surface of wing near posterior end of linea calva. Hindwing 7.4–7.9 times as long as wide. Gastral tergite 1 anteriorly with transverse, reticulate microsculpture, becoming substrigulate laterally, tergites 2–6 weakly reticulate laterally. Gastral tergites 1–6 with paired setae as follows: 1, 1, 1, 1(–2), 2(–3), 2. Syntergum with 4–6 setae. Ovipositor slightly exserted, 1.0–1.1 times as long as antennal club, 1.3–1.6 times as long as scape, 1.0–1.1 times length of midtibia.

Male. ***Colouration.*** Three specimen in balsam from laboratory culture. Head pale. Antenna with radicle slightly fuscous, pedicel dark fuscous and club fuscous, pedicel darker than club. Mesoscutum, scutellum and metanotum slightly darker than rest of mesosoma. Pronotum and anterior mesoscutum fuscous. Metanotum fuscous. Propodeum fuscous, slightly more pale than metanotum. Legs and gaster pale.

Discussion. *Eretmocerus mundus* is widespread from Spain, through the Mediterranean region, to Sudan and India. It is here recorded from Australia for the first time. We have also examined specimens of *E. mundus* from the North Mariana Islands and Nauru (P. J. De Barro & I. D. Naumann, unpubl. data, 2000). *Eretmocerus mundus* appears to be specific to *B. tabaci* (Gerling 1972), and is immediately recognisable among the Australian species of *Eretmocerus* by the combination of the shape of the first funicular segment, straight marginal vein, two pairs of setae on the mesoscutum and one pair of setae on the anterior 3–4 gastral tergites. Unlike *E. mundus* from elsewhere in the world, the form of the species in Australia is thelytokous. To distinguish the Australian population from sexual populations of *E. mundus* found elsewhere, we have added the term Australian parthenogenetic form (APF) to the name. Males are rarely produced and are non-functional.

Eretmocerus queenslandensis Naumann and Schmidt sp. n. (Figs 3,6,7,10)

Types. ***Queensland.*** Holotype female, Townsville (Garden View Nursery), 4.x.1996, P. De Barro, ex *B. tabaci* on *Hibiscus* sp. Paratypes: 11 females, 3 males, all ex *B. tabaci*: 1 female, Cairns, 30.ix.1996. P. De Barro, on *S. oleraceus*; 1 female, Bowenville, 14.v.1996, B. A. Franzmann, on *Helianthus annus*; 4 females, 1 male, same data as holotype, or 28.x.1995; 5 females, Ayr, 26.x.1995, 13.xi.1996, P. De Barro, on *Sonchus oleraceus* or *Hibiscus* sp.; 2 males from laboratory culture originating Ayr, ex *B. tabaci* on *Hibiscus* sp. All in ANIC.

Female. ***Colouration.*** Dried, unmounted specimens fuscous. Specimens in balsam: head fuscous in ventral half, or uniformly pale to fuscous. Body and antenna uniformly fuscous to pale or pronotum, mesoscutum, axillae, scutellum, metanotum, propodeum, hind coxae and femora and area around ovipositor slightly darker than remaining body. Wings hyaline except fuscous veins and forewing more or less infuscate in basal half. ***Morphology.*** Frons and occiput with transverse, substrigulate microsculpture; lower face vertically reticulate to substrigulate. Antenna (Fig. 3) with radicle 3.4–3.8 times as long as wide. Scape 4.2–5.4 times as long as wide, 2.2–2.9 times length of radicle, 1.6–2.2 times length of pedicel, 0.6 times length of club. Pedicel 2.4–3.1 times as long as wide, 1.3–1.4 times length of radicle, 0.5–0.6 times length of scape, 0.32–0.39 times length of club. First funicular segment 0.9–1.3 times as long as wide; second funicular segment 1.2–1.5 times as long as wide. Club 4.8–6.4 times as long as wide, 1.6–1.7 times length of scape, 2.6–3.1 times length of pedicel. Apex of club obliquely truncate. Mesoscutum usually with 6 setae (anteromedian pair occasionally missing or only 1 seta of that pair present) and anteriorly with reticulate sculpture, posteriorly longitudinally reticulate, posteromedially substrigulate. Parapsis with 2(–3) setae and reticulate sculpture. Axilla with 1 seta and substrigulate sculpture. Scutellum with longitudinally reticulate sculpture and with 4 setae. Placoid sensilla usually closer to posterior setae. Occasionally 1–2 additional placoid sensilla present in posterior half of scutellum. Metanotum more or less smooth. Propodeum with substrigulate sculpture. Endophragma almost reaching posterior margin of gastral tergite 2. Forewing (Fig. 10) length 0.53–0.62 mm, 3.0–3.6 times as long as wide at width I (Fig. 9, wI), 2.7–3.0 times as long as maximum width of disc. Longest anterior alary fringe 0.20–0.27 times maximum width of disc, longest posterior alary fringe 0.34–0.47 times width of disc. Basal cell of wing usually with 1 seta. Costal cell distally with 2–4 setae. Marginal vein with 3 long setae, separated from linear calva by irregular row of 5–9 setae. Linea calva closed posteriorly by setae. Approximately 11–13 tubercles present on ventral surface of wing near posterior end of linea calva. Hind wing 7.7–9.0 times as long as wide. Gastral tergite 1 with transverse reticulate microsculpture, this becoming more longitudinally reticulate to substrigulate laterally. Tergites 2–6 weakly reticulate laterally. Gastral tergites 1–6 with paired setae as follows: 1(–2) (1–)2, 2, 2(–3), 2(–3), 3(–5). Syntergum with 5–6 setae. Ovipositor slightly exserted, 1.0–1.1 times as long as antennal club, 1.3–1.6 times as long as scape, 1.0–1.1 times length of midtibia.

Male. ***Colouration.*** Three specimens in balsam: head and body slightly fuscous except the following parts darker: head ventrally, pronotum, mesoscutum, scutellum slightly, metanotum, propodeum and hind coxa. Antenna light fuscous with radicle, scape and pedicel slightly darker than club. Wings hyaline except fuscous veins and forewing more or less infuscate basally.

Discussion. *Eretmocerus queenslandensis* differs from *Eretmocerus australis* Girault in the subtriangular shape of funicle I, the stouter funicle II, the more truncate apex of the antennal club, the larger number of tubercles on the ventral surface of the forewing posterior to the linea calva, and the larger number of setae separating the linea calva from the marginal vein (approximately 4 in *australis*). *Eretmocerus queenslandensis* is similar to *Eretmocerus lativentris* Girault in the shape of the funicle segments, but the club of *E. lativentris* is much stouter and apically very rounded. *Eretmocerus lativentris* also has a small number of tubercles (approximately 6) on the ventral surface of the forewing. The row of setae behind the marginal vein of *E. lativentris* has a similar number of setae to that present in *E. queenslandensis* but the setae are more irregularly inserted. The forewing of *E. queenslandensis* is similar to that of *Eretmocerus nativus* Girault. The two species can be distinguished by the development of the posterior alary fringe: it is at most 0.26 times as long as the maximum width of the disc in *nativus* compared to 0.34–0.47 times in *queenslandensis*. *Eretmocerus queenslandensis* is thelytokous. Males are rarely produced and are non-functional.

Eretmocerus warrae Naumann and Schmidt sp. n. (Figs 2,8,11)

Types. ***Queensland.*** Holotype female, Warra, 7.v.1997, D. R. Lea, ex *T. vaporariorum* on *Sonchus oleraceus*. Paratypes: 37 females, 1 male, all ex *T. vaporariorum*. ***Queensland.*** 14 females, Oakey, 1.v.1997, 13.v.1997, 29.v.1997, 25.vi.1997, D. R. Lea, on *Verbena bonariensis*, *Urtica* sp., *Xanthium occidentale*, *Anoda cristata*, *Conyza albida*, *Malvastrum coromandelium*, sunflower, wild cotton; 1 female, Oakey, 20.xi.1996, B. A. Franzmann, on *Verbena bonariensis*; 1 female, 1 male, Oakey, 13.v.1997, D. R. Lea, on wild cotton; 1 female, Oakey, 20.iii.1997, B. A. Franzmann, on *Anoda cristata*; 4 females, same locality and collector as holotype, 7.v.1997, 29.v.1997, 25.vi.1997, on *Cucumis melo*, *Verbena bonariensis*, prickly malvastrum, *Anoda cristata*. ***New South Wales.*** 6 females, Lake Tandou, 6.i.1997, P. De Barro, on *Xanthium occidentale*; 1 female, Griffith, 8.i.1997, P. De Barro on *Sonchus oleraceus*. ***Victoria.*** 2 females, Red Cliffs, 2.i.1997, P. De Barro, on *Helianthus annus*, *Euphorbia peplus*. ***South Australia.*** 3 females, McLaren Vale, 2.i.1997, P. De Barro, on hollyhock, *Salvia* sp. ***Western Australia.*** 1 female, Wanneroo, 1.xi.1996, P. De Barro, on *Hibiscus* sp.; 3 females, Pemberton, 22.i.1997, P. De Barro, on *Cyphomandra fragrans*. All in ANIC.

Female. ***Colouration.*** Dried, unmounted specimens pale yellow. Head and body of specimen mounted in Canada balsam pale. Antennae slightly fuscous, club darker towards apex. Legs pale except tarsi apically sometimes slightly fuscous. Wings hyaline except fuscous veins and forewing slightly infuscate in basal third. ***Morphology.*** Frons and occiput with transverse, substrigulate microsculpture; lower face more vertically substrigulate. Antenna (Fig. 2) with radicle 4.1–5.5 times as long as wide. Scape 4.7–5.7 times as long as wide, 2.0–2.4 times length of radicle, 1.5–1.6 times length of pedicel, 0.6–0.7 times length of club. Pedicel 3.7–4.4 times as long as wide, 1.3–1.5 times length of radicle, 0.6–0.7 times length of scape, 0.39–0.42 times length of club. First funicular segment 1.0–1.3 times as long as wide; second funicular segment 1.2–1.4 times as long as wide. Club 6.3–8.2 times as long as wide, 1.5–1.7 times length of scape, 2.4–2.6 times length of pedicel. Apex of club truncate. Mesoscutum with 6(–7) setae, anterior half with reticulate sculpture, posteriorly longitudinally reticulate, posteromedially longitudinally reticulate to substrigulate. Parapsis with 1 seta and substrigulate sculpture. Axilla with 1 seta and reticulate to substrigulate sculpture. Scutellum with longitudinally reticulate sculpture laterally, substrigulate medially and with 4 setae. Placoid sensilla lateral to and closer to posterior than to anterior setae, or equidistant. Propodeum with substrigulate sculpture. Endophragma usually almost extending to posterior margin of gastral tergite 2. Forewing (Fig. 8) length 0.54–0.73 mm, 2.8–3.1 times as long as wide at width I (Fig. 9, wI), 2.5–2.8 times as long as maximum width of disc. Longest anterior alary fringe 0.11–0.21 times maximum width of disc, longest posterior alary fringe 0.29–0.40 times width of disc. Basal cell usually with 1 seta. Costal cell distally with 4–6 setae. Marginal vein with 3 long setae, separated from linear calva by irregular row of about 8 setae. Linea calva closed posteriorly by setae. Approximately 14–16 tubercles present on ventral surface of wing near posterior end of linea calva. Hindwing 6.4–7.4 times long as wide. Gastral tergite 1 with weak, transverse reticulate microsculpture, becoming more substrigulate laterally, tergites 2–6 reticulate laterally; gastral tergites 1–6 with paired setae as follows: 1, 1, 1, 1(–2), 2, 2 (Fig. 11). Syntergum with 6 setae. Ovipositor slightly exserted, 0.7–0.9 times as long as antennal club, 1.2–1.4 times as long as scape, 0.8–0.9 times length of midtibia.

Male. ***Colouration.*** One specimen in balsam: head pale, antenna slightly fuscous. Mesoscutum fuscous, darkest along anterior margin and midline. Scutellum fuscous, becoming lighter posteriorly and posterolaterally. Metanotum fuscous. Propodeum fuscous, slightly more pale than metanotum. Legs slightly fuscous. Gaster pale.

Discussion. With respect to the elongate scape and mesoscutal setation *E. warrae* is similar to the North American species *Eretmocerus haldemani* Howard and *Eretmocerus tejanus* Rose and Zolnerowich (Rose & Zolnerowich 1997). However, *E. warrae* differs from both of these species in the proportions of the scape. The female of *E. haldemani* is known only from one poorly preserved specimen. The female of *E. tejanus* differs from that of *E. warrae* in having paired setae on gastral tergites 2–4. The mesoscutal infuscation patterns are quite different in the males of *E. tejanus* and *E. warrae*. In *E. tejanus*, the mesoscutum has a pair of longitudinal, fuscous bands separated by a pale band. The mesoscutum of the male of *E. warrae* is fuscous medially. *Eretmocerus warrae* can be distinguished readily from three species of *Eretmocerus* previously described from Australia, that is, *E. nativus* Girault (1930), *E. lativentris* Girault (1932)

and *E. australis* Girault (1921). *Eretmocerus warrae* differs from *E. nativus* in having a longer posterior alary fringe (longest fringe setae less than 0.25 times width of forewing in *E. nativus*) and more tubercles posterior to the linea calva of the forewing (14–16 versus 11 in the holotype of *E. nativus*). The antenna of *E. nativus* was not described and has been lost from the type. The female club of *E. lativentris* Girault (1932) is much stouter than that of *E. warrae* (approximately 4.8 times longer than wide in the holotype). The club of *E. australis* is relatively elongate (7.3 times longer than wide in the holotype). However, the forewing of *E. australis* is more slender (slightly more than 3.0 times longer than maximum width in holotype) and has only four or five tubercles posterior to the linea calva. *Eretmocerus warrae* is thelytokous. Males are rare.

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