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Molecular identification of hymenopteran parasitoids and their endosymbionts from agromyzids

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Abstract

Three polyphagous pest *Liriomyza* spp. (Diptera: Agromyzidae) have recently invaded Australia and are damaging horticultural crops. Parasitic wasps are recognized as effective natural enemies of leafmining species globally and are expected to become important biocontrol agents in Australia. However, the hymenopteran parasitoid complex of agromyzids in Australia is poorly known and its use hindered due to taxonomic challenges when based on morphological characters. Here, we identified 14 parasitoid species of leafminers based on molecular and morphological data. We linked DNA barcodes (5′ end *cytochrome c oxidase subunit I* (COI) sequences) to five adventive eulophid wasp species (*Chrysocharis pubicornis* (Zetterstedt), *Diglyphus isaea* (Walker), *Hemiptarsenus varicornis* (Girault), *Neochrysocharis formosa* (Westwood), and *Neochrysocharis okazakii* Kamijo) and two braconid species (*Dacnusa areolaris* (Nees) and *Opius cinerariae* Fischer). We also provide the first DNA barcodes (5′ end COI sequences) with linked morphological characters for seven wasp species, with three identified to species level (*Closterocerus mirabilis* Edwards & La Salle, *Trigonogastrella parasitica* (Girault), and *Zagrammosoma latilineatum* Ubaidillah) and four identified to genus (*Aprostocetus* sp., *Asecodes* sp., *Opius* sp. 1, and *Opius* sp. 2). Phylogenetic analyses suggest *C. pubicornis*, *D. isaea*, *H. varicornis,* and *O. cinerariae* are likely cryptic species complexes. *Neochrysocharis formosa* and *Aprostocetus* sp. specimens were infected with *Rickettsia*. Five other species (*Cl. mirabilis*, *D. isaea, H. varicornis, Opius* sp. 1, and *Opius* sp. 2) were infected with *Wolbachia*, while two endosymbionts (*Rickettsia* and *Wolbachia*) co-infected *N. okazakii.* These findings provide background information about the parasitoid fauna expected to help control the leafminers.

Introduction

The Agromyzidae (Diptera) is a family of small flies which has a significant economic impact on agricultural and horticultural crops around the world. The larvae of many species mine leaves, but some species mine stems or form galls (Spencer, 1973). The most well-known genus of this family is *Liriomyza,* which contains over 400 species; however, only a few species are considered destructive pests globally (Parrella, 1987). Most plant damage is caused by the larvae tunneling within the mesophyll of leaves, leaving serpentine or blotch mines (Parrella *et al*., 1985). Female flies can also damage plants by using their ovipositors to penetrate the epidermis of leaves, creating numerous punctures for feeding and ovipositing (Bethke and Parrella, 1985; Ge *et al*., 2019). Infested plants usually have reduced photosynthetic rates and young seedlings can die when very heavily mined (Johnson *et al*., 1983; Bueno *et al*., 2007).

Three polyphagous Neotropical *Liriomyza* species (*Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard, and *Liriomyza trifolii* (Burgess)) have become established around the world (Murphy and La Salle, 1999; Scheffer and Lewis, 2001, 2005, 2006; Weintraub *et al*., 2017), largely through the movement of infested plant material along trade routes (Minkenberg, 1988). Despite strict surveillance and quarantine programs, these three species are now established in mainland Australia (Xu *et al*., 2021a). *Liriomyza sativae* was detected for the first time in the islands of the Torres Strait in 2008 (Blacket *et al*., 2015), and then on the Australian mainland at Seisia in 2015 (IPPC 2017). This species has not been detected outside of Cape York for the last 7 years. *Liriomyza huidobrensis* was first confirmed at several sites in western Sydney and then in southern Queensland in 2020 (IPPC 2021a; Mulholland *et al*., 2022). *Liriomyza trifolii* was first detected in 2021 in Kununurra (northern Western Australia), Bamaga (Far North Queensland), and the Torres Strait (IPPC 2021b).

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Worldwide, farmers have routinely relied on synthetic chemical pesticides to control leafmining flies. However, the extensive use of pesticides has dramatically reduced their effectiveness due to the rapid evolution of resistance within some species and the negative impacts of these chemicals on natural enemies (Reitz *et al.*, 2013). Natural enemies, in particular parasitoid wasps, have now been recognized as effective bio-control agents that can suppress outbreaks of leafmining pests; indigenous parasitoid wasps can quickly suppress recent invasions of *Liriomyza* flies in pesticide-free areas (Murphy and La Salle, 1999; Liu *et al.*, 2009). In addition, some countries have introduced exotic parasitoid wasps to control *Liriomyza* spp. with encouraging results. For example, *Chrysocharis oscinidis* Ashmead and *Banacuniculus utilis* (Beardsley) (Hymenoptera: Figitidae: Eucoilinae) were successfully introduced to Hawaii, Guam, and Tonga for the management of *L. sativae and L. trifolii* (Johnson, 1993), while Japan imported *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) from Europe for augmentative biological control of *L. trifolii* in greenhouses (Mitsunaga and Yano, 2004).

Australia already has a suite of endemic and introduced parasitoids that parasitize a range of adventive and endemic agromyzid species, which would likely contribute to the regulation of exotic *Liriomyza* pests (Ridland *et al.*, 2020). It has been demonstrated that agromyzid species colonizing weeds and non-crop plants serve as useful reservoirs to support parasitoid wasps, potentially providing parasitoids for the biological control of invasive *Liriomyza* spp. (Lardner, 1991; Bjorksten *et al.*, 2005; Lambkin *et al.*, 2008; Wood *et al.*, 2010; Ridland *et al.*, 2020). However, numerous challenges impede the uptake of augmentative or conservation biological control programs including indiscriminate use of pesticides, lack of parasitoid reservoirs in intensive cropping systems, and the high cost of mass-rearing for augmentative biological control.

Accurate species identification of parasitoids underpins all these issues. Traditional morphological identification of parasitoids relies on taxonomic keys, which can be difficult to use by non-specialist researchers. Most published studies surveying parasitoids of agromyzids (e.g. Asadi *et al.*, 2006; Lambkin *et al.*, 2008; Mujica and Kroschel, 2011) have relied on morphological identification. However, the dearth of taxonomic expertize worldwide remains a major limiting factor for the authoritative morphological identification of specimens. High-resolution morphological information can now be collected more easily by non-specialists using techniques such as scanning electron microscopy (SEM) and multi-focus imaging, but still require validation from experts.

DNA barcodes are increasingly being used to supplement morphological studies in the identification of parasitoid wasps (Powell *et al.*, 2019). Some taxonomic studies now combine molecular data, morphological data and high-quality imaging in assessing parasitoids of leafminers such as eulophids attacking agromyzids (e.g. Perry and Heraty, 2019, 2021). However, morphological misidentifications and incorrect DNA barcodes on public databases hinder research assessing the impact of parasitoids (Lue *et al.*, 2021, 2022). This is an important issue, because validated DNA barcodes can be used by researchers with little taxonomic experience (Darling and Blum, 2007) and can also be applied to immature life stages and cryptic species (Waugh, 2007). Nevertheless, accurate DNA barcodes depend on the use of specimens that have been authoritatively identified (Lue *et al.*, 2021, 2022).

As well as assisting in identifying parasitoid species, DNA technology can be used to screen for the presence of bacterial endosymbionts within parasitoid specimens. Endosymbionts like *Wolbachia* are intracellular bacteria that are widespread in arthropod species, including parasitoid wasps (Floate *et al.*, 2006; Klopfstein *et al.*, 2018). *Wolbachia* is often associated with host reproductive effects that include male-killing, feminization, parthenogenesis, and cytoplasmic incompatibility (CI) (Sinkins *et al.*, 1997). Other endosymbionts such as *Cardinium* and *Rickettsia* are also common in insects and can affect traits such as reproduction (Montenegro *et al.*, 2005; Hagimori *et al.*, 2006). To date, endosymbiont surveys of parasitoids of agromyzids are limited (Tagami *et al.*, 2006), despite these endosymbionts having the potential to be used to generate strains of parasitoids with useful characteristics for future release. Moreover, endosymbionts can affect patterns of mtDNA variation (e.g. *Wolbachia* induced CI decreases mtDNA polymorphism as the *Wolbachia* and its associated mtDNA variant spreads in a population) and influence within-species variation in clades identified from mtDNA markers (Hale and Hoffmann, 1990).

Prior studies have found 27 genera of parasitoids of agromyzids in Australia, and some are likely to be important in controlling the invasive polyphagous *Liriomyza* species (Ridland *et al.*, 2020). Therefore, based on this background, our study mainly focuses on parasitoid wasp species reared from common agromyzids in Australia, aiming to determine which species are present and potentially helpful in suppressing the exotic *Liriomyza* pests. We compared DNA barcodes with sequences on public databases and verified morphological identifications where possible with SEM images. For species without DNA barcodes, we provide DNA information alongside morphological descriptions. Furthermore, we assessed endosymbiont infections in parasitoid wasps that might potentially be exploited for future augmentative biocontrol.

Materials and methods

Insects

We reared parasitoid wasps from three adventive agromyzid species: *Liriomyza brassicae* (Riley), *Phytomyza plantaginis* Goureau and *Phytomyza syngenesiae* (Hardy), and two native agromyzid species: *Liriomyza chenopodii* (Watt) and *Phytoliriomyza praecellens* Spencer. These agromyzids are likely to be reservoirs for parasitoids to attack invasive *Liriomyza* pests (Ridland *et al.*, 2020).

Mined plant leaves were primarily collected from locations in Melbourne (Victoria, Australia) and Bangalow (New South Wales, Australia). Samples reared from *Liriomyza huidobrensis* were received from Wyreema (Queensland, Australia). Table 1 gives detailed information on sampling. We also received samples of *Diglyphus isaea* (ex *L. bryoniae*) from a laboratory colony (Koppert BV) in The Netherlands and *Hemiptarsenus varicornis* (Girault) (ex *L. trifolii*) from Fiji. This enabled us to compare overseas accessions of these two widely distributed parasitoid species with Australian specimens, both in terms of COI haplotype and endosymbiont status. Leaf samples were first cleared of other insects and residues, then covered with paper towels and placed into individual Ziploc® bags (SC Johnson, Australia). Paper toweling was changed frequently to reduce the moisture content within the Ziploc® bags. We checked the bags regularly, and when adult flies and parasitoid wasps emerged within each bag, we removed and separated them (avoiding teneral insects, which can be difficult to identify). Flies and parasitoids were

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Table 1. The COI haplotypes and endosymbionts detected in parasitoid wasps characterized in this study

Parasitoid species	Collection site	Collection date	GPS	Host leafminer	Host plant	Individuals	COI haplotypes (*N*)	Endosymbionts
Aprostocetus sp.	Flemington Bridge, VIC, Australia	2022.03	−37.787, 144.939	*Phytoliriomyza praecellens*	*Rhagodia parabolica*	6♀	Ap.01(3), Ap.02(3)	*Rickettsia* (6♀)
Asecodes sp.	Flemington Bridge, VIC, Australia	2018.12	−37.787, 144.939	*Liriomyza brassicae*	*Brassica fruticulosa*	9♀ + 9♂	As.01(5), As.02(5), As.03 (1), As.04(2), As.05(1), As.06(4)	No
Chrysocharis pubicornis	Flemington Bridge, VIC, Australia	2019.08	−37.787, 144.939	*Phytomyza plantaginis*	*Plantago lanceolata*	55♀ + 55♂	Cp.01(14), Cp.02(67), Cp.03(17), Cp.04(3), Cp.05 (5), Cp.06(4)	No
Chrysocharis pubicornis	Flemington Bridge, VIC, Australia	2019.08	−37.787, 144.939	*Phytomyza syngenesiae*	*Sonchus oleraceus*	12♀ + 15♂	Cp.02(11), Cp.03(1), Cp.04 (15)	No
Closterocerus mirabilis	Flemington Bridge, VIC, Australia	2018.12	−37.787, 144.939	*Liriomyza brassicae*	*Brassica fruticulosa*	4♀ + 4♂	Cm.01 (8)	*Wolbachia* (4♀ + 4♂)
Diglyphus isaea	Glen Waverley, VIC, Australia	2019.09	−37.871, 145.145	*Phytomyza syngenesiae*	*Sonchus oleraceus*	4♀ + 3♂	D.01 (7)	*Wolbachia* (1♀ + 1♂)
Diglyphus isaea	Glen Waverley, VIC, Australia	2019.12	−37.871, 145.145	*Phytomyza plantaginis*	*Plantago lanceolata*	1♀	D.01(1)	No
Diglyphus isaea	Glen Waverley, VIC, Australia	2019.12	−37.871, 145.145	*Liriomyza brassicae*	*Brassica fruticulosa*	1♀	D.01(1)	No
Diglyphus isaea	Diggers Rd, Werribee, VIC, Australia	2019.03	−37.966, 144.685	*Liriomyza brassicae*	*Brassica fruticulosa*	1♂	D.01(1)	No
Diglyphus isaea	Federation Trail, Werribee, VIC, Australia	2018.11	−37.915, 144.668	*Phytomyza plantaginis*	*Plantago lanceolata*	1♂	D.01(1)	No
Diglyphus isaea	Federation Trail, Werribee, VIC, Australia	2019.01	−37.915, 144.668	*Phytomyza syngenesiae*	*Sonchus oleraceus*	1♂	D.01(1)	No
Diglyphus isaea	Flemington Bridge, VIC, Australia	2019.03	−37.787, 144.939	*Liriomyza brassicae*	*Brassica fruticulosa*	1♀	D.01(1)	No
Diglyphus isaea	Flemington Bridge, VIC, Australia	2019.06	−37.787, 144.939	*Phytomyza plantaginis*	*Plantago lanceolata*	1♀	D.01(1)	No
Diglyphus isaea	Flemington Bridge, VIC, Australia	2018.11	−37.787, 144.939	*Phytomyza syngenesiae*	*Sonchus oleraceus*	1♂	D.01(1)	No
Diglyphus isaea	The Netherlands	2020.07	51.991, 4.473	Laboratory colony reared on *Liriomyza bryoniae*	N.A.	4♀ + 5♂	D.02(7), D.03 (2)	No
Hemiptarsenus varicornis	Flemington Bridge, VIC, Australia	2018.12	−37.787, 144.939	*Liriomyza brassicae*	*Brassica fruticulosa*	18♀ + 16♂	H.01 (3), H.02 (2), H.03 (2), H.04 (2), H.05 (8), H.06 (1), H.10 (1), H.11 (2), H.12 (4),	*Wolbachia* (1♀)

(*Continued*)

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Table 1. (*Continued.*)

Parasitoid species	Collection site	Collection date	GPS	Host leafminer	Host plant	Individuals	COI haplotypes (*N*)	Endosymbionts
							H.13 (1), H.14 (1), H.15 (4), H.16 (1), H.17 (1), H.18 (1)	
Hemiptarsenus varicornis	Flemington Bridge, VIC, Australia	2018.12	−37.787, 144.940	*Liriomyza chenopodii*	*Stellaria media*	4♂	H.07 (1), H.08 (2), H.09 (1)	No
Hemiptarsenus varicornis	Koronivia, Nausori, Fiji	2020.02	−18.049, 178.541	*Liriomyza trifolii*	*Vigna unguiculata* ssp. *sesquipedalis*	4♀ + 4♂	H.19 (8)	*Wolbachia* (4♀ + 4♂)
Hemiptarsenus varicornis	Wainibokasi, Nausori, Fiji	2020.02	−18.060, 178.572	*Liriomyza trifolii*	*Vigna unguiculata* ssp. *sesquipedalis*	2♀ + 1♂	H.19 (3)	*Wolbachia* (2♀ + 1♂)
Neochrysocharis formosa	Wyreema, QLD, Australia	2021.05	−27.640, 151.866	*Liriomyza huidobrensis*	*Apium graveolens*	6♀	NF_5COI.03(6)	*Rickettsia* (6♀)
Neochrysocharis okazakii	Wyreema, QLD, Australia	2021.05	−27.640, 151.866	*Liriomyza huidobrensis*	*Apium graveolens*	3♀ + 3♂	Nok.01(1), Nok.02(3), Nok.03(1), Nok.04(1),	*Wolbachia* (3♀ + 3♂) *Rickettsia* (3♀ + 3♂)
Zagrammosoma latilineatum	Diggers Rd, Werribee, VIC, Australia	2019.03	−37.966, 144.685	*Liriomyza brassicae*	*Brassica fruticulosa*	2♀ + 2♂	Z.01 (1), Z.02 (1), Z.03 (1), Z.04 (1)	No
Dacnusa areolaris	Flemington Bridge, VIC, Australia	2020.06	−37.787, 144.939	*Phytomyza syngenesiae*	*Sonchus oleraceus*	2♀ + 2♂	Dac.01(4)	No
Opius sp. 1	Flemington Bridge, VIC, Australia	2018.12	−37.787, 144.939	*Liriomyza brassicae*	*Brassica fruticulosa*	7♀ + 6♂	Op1.01(12), Op1.02(1)	*Wolbachia* (1♀)
Opius sp. 1	Flemington Bridge, VIC, Australia	2018.12	−37.787, 144.939	*Phytomyza syngenesiae*	*Sonchus oleraceus*	2♀ + 2♂	Op1.01(3), Op1.02(1)	No
Opius sp. 2	Royal Park, VIC, Australia	2018.12	−37.795, 144.949	*Phytoliriomyza praecellens*	*Rhagodia parabolica*	7♀ + 8♂	Op2.01(3), Op2.02(11), Op2.03(1)	*Wolbachia* (4♀ + 3♂)
Opius cinerariae	Flemington Bridge, VIC, Australia	2018.12	−37.787, 144.939	*Liriomyza brassicae*	*Brassica fruticulosa*	3♀ + 3♂	Op3.01(1), Op3.02(4), Op3.03(1)	No
Opius cinerariae	Flemington Bridge, VIC, Australia	2020.1	−37.787, 144.939	*Liriomyza chenopodii*	*Stellaria media*	3♀	Op3.02(1), Op3.04(1), Op3.05(1)	No
Opius cinerariae	Bangalow, NSW, Australia	2018.09	−28.688, 153.520	*Phytomyza plantaginis*	*Plantago lanceolata*	1♂	Op3.01(1)	No
Trigonogastrella parasitica	Flemington Bridge, VIC, Australia	2019.07	−37.787, 144.939	*Phytomyza syngenesiae*	*Sonchus oleraceus*	1♀ + 2♂	T.01(1), T.02(1), T.03(1)	No

VIC, Victoria; QLD, Queensland; NSW, New South Wales.

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preserved in absolute ethanol and stored at −20°C for DNA extractions, while some parasitoid wasps were placed in 70% ethanol and stored at 4°C for SEM imaging. Different individuals of the same wasp species were processed in two ways. Approach 1, where whole bodies were used to extract DNA and detect symbiotic bacteria. Approach 2, where body parts (e.g. legs) were used for DNA extraction and SEM was undertaken on key body components to assist in species identification. The identifications of the agromyzid fly species were confirmed with the DNA barcodes we published previously (Coquilleau *et al.*, 2021; Xu *et al.*, 2021b). Voucher specimens were deposited in the Victorian Agricultural Insect Collection, AgriBio.

DNA extraction, amplification, and sequencing

We extracted genomic DNA using a Chelex (Bio-Rad Laboratories) DNA extraction method. For approach 1, DNA was extracted from whole parasitoid bodies, which involved crushing the body with two glass beads (3 mm) in 100 μl of 5% Chelex solution. For approach 2, we extracted DNA from the legs of wasps in order to preserve the remaining body parts for SEM imaging. For particularly tiny wasps (<1.5 mm in length), such as *Asecode*s sp. (Hymenoptera: Eulophidae) and *Closterocerus mirabilis* Edwards & La Salle (Hymenoptera: Eulophidae), we failed to successfully extract sufficient DNA from legs, so in these instances, we used the head & legs or the gaster and legs to extract DNA (in a total volume of 70 μl of 5% Chelex solution). In all cases, the Chelex mixture was incubated with 3 μl proteinase K (20 mg ml^{-1}, Roche Diagnostics) at 65°C for 1 h. The homogenates were then boiled at 95°C for 10 min to inactivate the proteinase K and these samples were used as templates for PCR.

The DNA barcodes we used in this study focused on the 5′ region of the *cytochrome c oxidase subunit I* (COI) gene. Additional sequences were obtained by sequencing the genes for some species (e.g., nuclear ribosomal internal transcribed spacer 1 (ITS1) sequences for *D. isaea*, 28S sequences for *Neochrysocharis okazakii* Kamijo (Hymenoptera: Eulophidae), and 3′ region COI sequences for *Asecodes* sp., *N. okazakii* and *Zagrammosoma latilineatum* Ubaidillah (Hymenoptera: Eulophidae)). For detailed information on primers used for DNA barcoding see table S1. It is noteworthy that primers LepF1/LepR1 used for *Cl. mirabilis* failed to sequence the COI gene because the endosymbiont *Wolbachia* was sequenced instead of the target. In this case, we undertook further DNA extractions following the methods described above, except we only used the head and legs due to the low *Wolbachia* densities in these organs (Narita *et al.*, 2007; Frentiu *et al.*, 2014; Amuzu and McGraw, 2016).

PCRs for DNA barcoding involved 2 μl DNA template, 3 μl 10 × ThermoPol® Reaction Buffer (New England BioLabs: B9004S), 2.4 μl dNTPs (2.5 mM), 1.5 μl of both forward and reverse primers (10 μM), and 0.3 μl BSA (New England BioLabs, B9000S), 0.2 μl Taq polymerase (New England BioLabs: M0267X) and ddH_2O to create a final 30 μl reaction volume. All PCRs included a sterile water sample (without genomic DNA) to confirm there was no DNA contamination. PCR products were directly sequenced in both directions using the primers detailed in table S1 at Macrogen (Seoul, Korea).

We determined the infection status of parasitoid wasps with three common endosymbionts (*Wolbachia*, *Cardinium*, and *Rickettsia*) using PCR reactions (and primers) described in Tagami *et al.* (2006). In brief, *wsp* sequence was selected to identify *Wolbachia* status and specific 16S rDNA sequences were selected to assess the presence of *Cardinium/Rickettsia*.

Sequence analysis

DNA sequences of parasitoid wasps were aligned and manually edited using Geneious 9.1.8 (Kearse *et al.*, 2012). Sequence similarities were searched first through BLAST (Altschul *et al.*, 1990; Ratnasingham and Hebert, 2007) and when matches were identified, we checked whether specimens had been identified. All available sequences were then downloaded from the BOLD System (Ratnasingham and Hebert, 2007) and the NCBI GenBank database (Benson *et al.*, 2018) and combined for further analyses. Polymorphism levels (haplotype and nucleotide diversity) were calculated using DnaSP version 6 (Rozas *et al.*, 2017). In this study, haplotype divergence within a specific species includes any sequence difference detected even if it involves a difference of only one nucleotide between the sequences. We excluded the duplicate haplotypes in the public dataset and the genetic cluster analysis. All other different haplotypes are included. A Neighbor-Joining tree (Kimura-2 parameter model) was generated with 1000 bootstrap replications using MEGA X (Kumar *et al.*, 2018). Pairwise genetic distances were calculated to assess the genetic similarity of sequences.

In those instances where the endosymbiont *Wolbachia* was detected, we allocated sequences to *Wolbachia* supergroups. To do this, *wsp* sequences from previously confirmed data (Baldo *et al.*, 2006) were obtained to construct a phylogenetic tree and allocate *Wolbachia* supergroups.

Morphological identification and scanning electron micrographs

Individual wasps were identified using Lucid keys (Reina and La Salle, 2003; Fisher *et al.*, 2005) and multiple published papers specific to each genus or species: *Aprostocetus* sp. (Bouček, 1988; Yang *et al.*, 2014), *Asecodes* sp. (Bouček, 1988; Hansson, 1994, 1996), *Chrysocharis pubicornis* (Hansson, 1985, 1987; Bouček, 1988; Ikeda, 1995, 1996), *Closterocerus mirabilis* (Edwards and La Salle, 2004), *D. isaea* (Hansson and Navone, 2017); *Hemiptarsenus* (Bouček, 1988; Fisher *et al.*, 2005), *Neochrysocharis formosa* (Westwood) (Hansson, 1990; Fisher *et al.*, 2005), *N. okazakii* (Kamijo, 1978), *Z. latilineatum* (Ubaidillah *et al.*, 2000; Perry and Heraty, 2021); *Trigonogastrella parasitica* Girault (Bouček, 1988), *Dacnusa areolaris* (Nees) (Wharton and Austin, 1991), *Opius cinerariae* Fischer, *Opius atricornis* Fischer and *Opius oleracei* Fischer (Belokobylskij *et al.*, 2004). For detailed morphological information, see Supplementary Information (figs S1–S10).

Scanning electron micrograph (SEM) images were produced on an FEI Teneo Volumescope instrument (ThermoFisher Scientific, USA) at an operating voltage of 10 kV. Wasp specimens were dissected and fixed on the SEM specimen mount by double-sided carbon tape, followed by air drying for four hours. A 5 nm gold coating was applied to the samples using an Emitech K575x sputter coater (Quorum Technologies, Canada) before taking SEM images.

Results

Wasp identifications

Overall, we characterized 307 parasitoid individuals from 10 geographic locations (seven from Australia, two from Fiji, and a

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laboratory colony from The Netherlands). We found 14 species based on morphological identifications and DNA barcoding. Among these, eight species could be linked to DNA barcodes in public databases: *C. pubicornis*, *D. isaea*, *Dac. areolaris*, *H. varicornis*, *N. formosa*, *N. okazakii, O. cinerariae,* and *Z. latilineatum.* We compared our COI barcodes with sequences from databases to examine levels of genetic variation within taxa. For species without DNA barcodes in either BOLD or GenBank or where there was substantial sequence divergence (>5%), we provide additional morphological analyses (figs S1–S10). DNA sequences generated in this study were deposited in GenBank under accession numbers (table S2).

Chrysocharis pubicornis

All specimens were collected from the Flemington Bridge location, in Melbourne. Among these, 110 individuals were collected from mined leaves of *Plantago lanceolata* (host flies were *P. plantaginis*) and 27 individuals were collected from mined leaves of *Sonchus oleraceus* (host flies were *P. syngenesiae*). The 5′ region COI sequences of *C. pubicornis* revealed six haplotypes (Cp.01–Cp.06) were present in our study and the dominant haplotype was Cp.02 (representing 56.9% of sequences). Genetic divergence of *C. pubicornis* haplotypes in this study varied from 0.2 to 6.3% based on 409 bp COI sequences (table S3). However, we were unable to detect morphological differences between these haplotypes using taxonomic keys and SEM imaging.

A phylogenetic tree was generated that included specimens from our study and sequences of *C. pubicornis* from public databases (fig. 1). The genetically related species *Chrysocharis pallipes* (Nees) (Hymenoptera: Eulophidae) was used as an outgroup based on recent studies of the phylogenetic relationship of Hymenoptera (Derocles *et al.*, 2015). Phylogenetic analyses suggested at least five major COI clades in *C. pubicornis*: *C. pubicornis*-A–E. This and the presence of substantial sequence divergence suggests the possibility of cryptic species within this taxon. *Chrysocharis pubicornis* collected in Australia are separated into two clades. Haplotypes Cp.01 and Cp.02 are within clade *C. pubicornis*-A, together with samples from Canada, the UK, and Norway. Genetic variation in clade *C. pubicornis*-A ranges from 0.2 to 1.4%. *C. pubicornis*-B is nearby to clade *C. pubicornis*-A and includes samples from Germany and Canada, with a genetic variation of 1.4%. Australian haplotypes Cp.03 to Cp.06 are within clade *C. pubicornis*-C alone, with genetic variation ranging from 0.2 to 0.4%. *C. pubicornis*-D includes samples from Belarus, Germany, and Japan, with genetic variation ranging from 0.2 to 5.6%. *C. pubicornis*-E includes samples from the UK, Canada and Norway, with genetic variation ranging from 0.2 to 2.2%. The genetic distances across all *C. pubicornis* samples ranged from 0.2 to 9.0% (table 2). The smallest genetic distances between *C. pubicornis* samples and outgroup species *Chrysocharis pallipes* was 9.5% (table S3)

Diglyphus isaea

In total, 15 *D. isaea* individuals were collected from three host species (*L. brassicae*, *P. plantaginis,* and *P. syngenesiae*) (table 1) in Melbourne sites, and only one COI haplotype (D.01) was

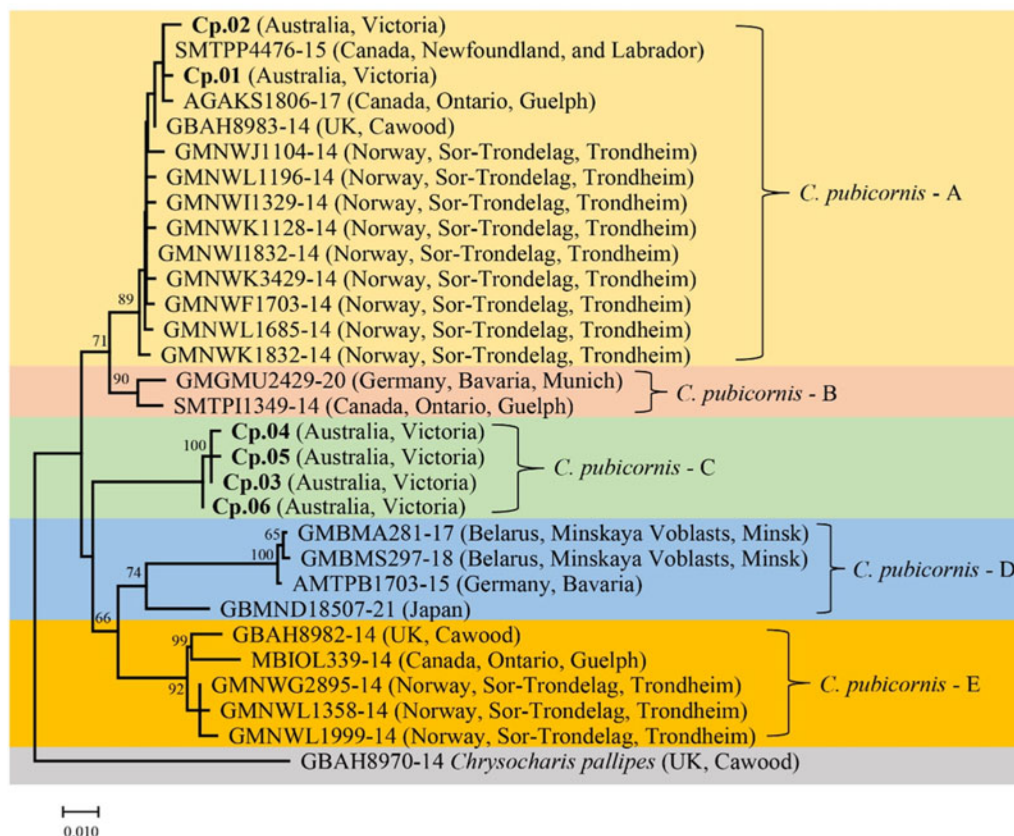

Figure 1. COI phylogenetic tree of *Chrysocharis pubicornis* was generated using the Neighbor-Joining method (1000 bootstrap replications, Kimura-2 parameter model) based on 409 bp sequence data. The scale bar indicates nucleotide substitutions per site. Haplotypes in this study are highlighted in bold, and the remainder of the sequences are from the BOLD database. *Chrysocharis pallipes* is the outgroup species.

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Table 2. Uncorrected pairwise distances among *Chrysocharis pubicornis* clades based on 409 bp COI sequence data

Clade	*C. pubicornis*-A	*C. pubicornis*-B	*C. pubicornis*-C	*C. pubicornis*-D	*C. pubicornis*-E
C. pubicornis-A	0.2–1.4%				
C. pubicornis-B	2.4–3.1%	1.4%			
C. pubicornis-C	4.8–6.3%	4.6–5.3%	0.2–0.4%		
C. pubicornis-D	4.8–8.0%	5.8–9.0%	6.3–8.3%	0.2–5.6%	
C. pubicornis-E	4.8–6.6%	5.6–7.3%	6.1–7.0%	4.8–8.0%	0.2–2.2%

found. We detected two COI haplotypes (D.02 and D.03) in nine *D. isaea* individuals obtained from the Netherlands. Sha *et al.* (2006) suggested that *D. isaea* in China is very likely a complex of cryptic species because five main clades were identified based on COI sequences and this was further supported by ITS1 sequences (Sha *et al.*, 2007). In this study, we reconstructed the COI phylogenetic tree including our sequences of *D. isaea* and those from China (fig. 2). We found *D. isaea* from Australia and the Netherlands clustered together in Group I, the largest clade, which also included *D. isaea* from northern and southern populations in China.

Hemiptarsenus varicornis

Forty-nine *H. varicornis* individuals were collected from three host species (*L. brassicae*, *L. chenopodii* and *L. trifolii*) (table 1) with 18 COI haplotypes (H.01–H.18) identified in Victoria and one COI haplotype (H.19) found from Fiji. There are 77 *H. varicornis* COI sequences in the BOLD database. Alignment yielded a 375

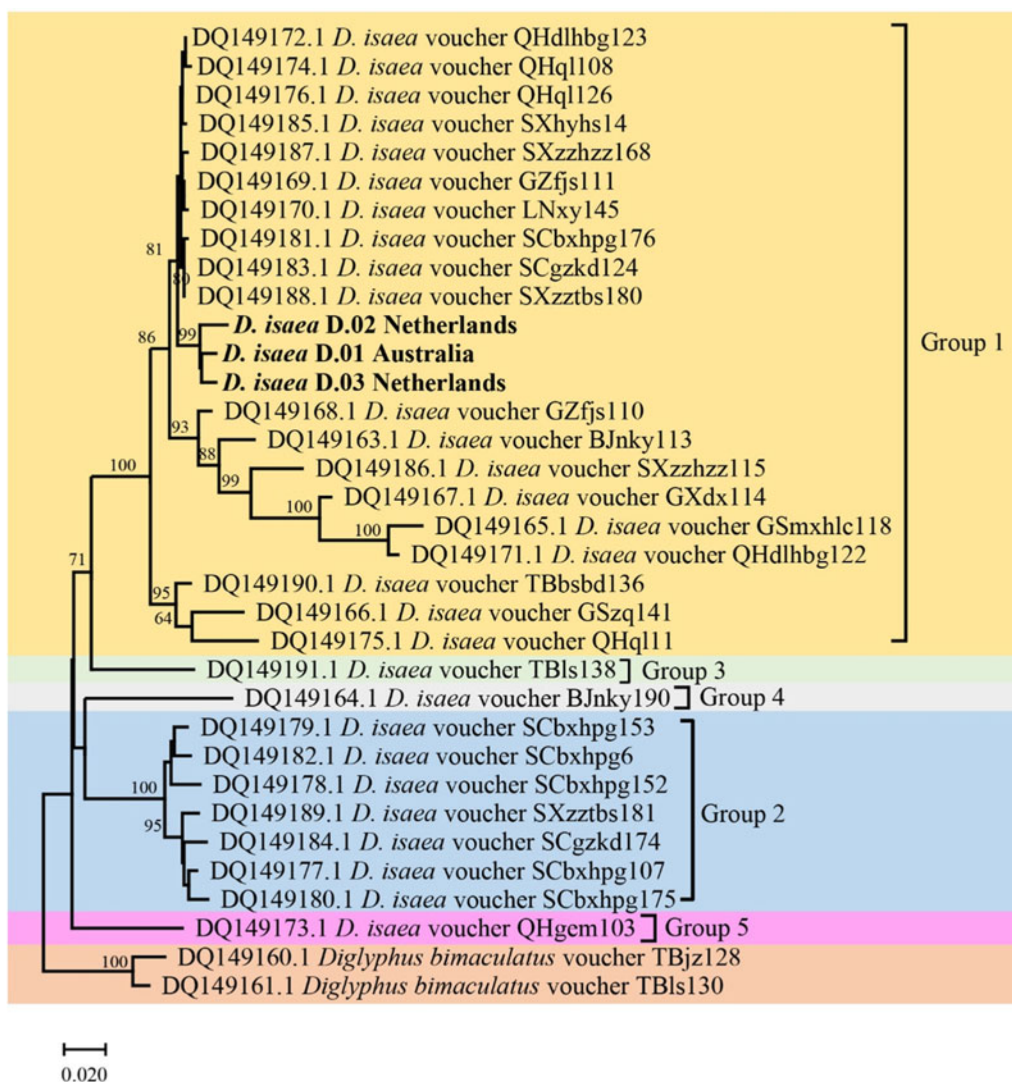

Figure 2. COI phylogenetic tree of *Diglyphus isaea* was generated using the Neighbor-Joining method (1000 bootstrap replications, Kimura-2 parameter model) based on 745 bp sequence data. The scale bar indicates nucleotide substitutions per site. Haplotypes in this study are highlighted in bold. The remainder of the sequences of *D. isaea* and the outgroup *D. bimaculatus* are from China and taken from Sha *et al.* (2006).

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bp fragment, allowing us to incorporate these BOLD sequences (table S4) into our analysis, which revealed a further 20 haplotypes. The phylogenetic tree demonstrated three major clades within this species: *H. varicornis*-A, *H. varicornis*-B, and *H. varicornis*-C (fig. 3). Clade *H. varicornis*-A represents all *H. varicornis* from Australia; clade *H. varicornis*-B is a single specimen from Fiji and this clade is nearby to Clade *H. varicornis*-C, which includes specimens from Pakistan, Malaysia, and Vietnam. Notably, the COI sequence of *H. varicornis* (AB721362) in GenBank is nearly identical to *N. okazakii* (AB721363) (with only three base pair differences) (Nakamura *et al.*, 2013). We suspect that these two sequences are both incorrect (neither *H. varicornis* nor *N. okazakii*). The uncorrected pairwise distances within clade *H. varicornis*-A + B (haplotype H.01–H.34) was 0.2–3.2% while the genetic distances within clade *H. varicornis*-C (haplotype H.35–H.38) were 0.2–0.8% (table S5). There is a clear genetic divergence between Australian *H. varicornis* and other locations with the largest divergence being 3.5% (haplotype H.35-Vietnam and haplotype H.24-WA, Australia) (table S5).

Opius *spp.*

We detected three *Opius* species with one species identified to species level as *O. cinerariae* and the other two putative species based on morphology and sequence divergence identified to genus level (*Opius* sp. 1 and *Opius* sp. 2). We found two COI haplotypes (Op1.01 and Op1.02) in *Opius* sp. 1, which were reared from two host flies (*L. brassicae* and *P. syngenesiae*) (table 1). The dominant haplotype was Op1.01, which accounted for 88% of the samples. For *Opius* sp. 2, we found three haplotypes (Op2.01, Op2.02, and Op2.03), all from *P. praecellens*. Op2.02 was the dominant haplotype, accounting for 73% of *Opius* sp. 2 samples. For *O. cinerariae*, five COI haplotypes (Op3.01–Op3.05) were detected from three host flies (*L. brassicae*, *L. chenopodii,* and *P. plantaginis*) (table 1). Among these, Op3.01–Op3.03 were reared from *L. brassicae* and *P. plantaginis*, while Op3.04–Op3.05 were only reared from *L. chenopodii*. A phylogenetic tree based on 577 bp COI sequence data was constructed and showed clear species boundaries among the three *Opius* species (fig. 4). *Opius* sp. 1 is genetically closer to *Opius* sp. 2 (uncorrected pairwise distances ranging from 6.6–8.3%) than *O. cinerariae*, (fig. 4, table 3, table S6). Furthermore, genetic divergence is evident within the *O. cinerariae* clade. The uncorrected pairwise distances of *O. cinerariae* from Australia (in this study) and New Zealand (NZHYM868–11 and NZHYM870–11) range from 6.9 to 8.0%, pointing to the possibility of cryptic species.

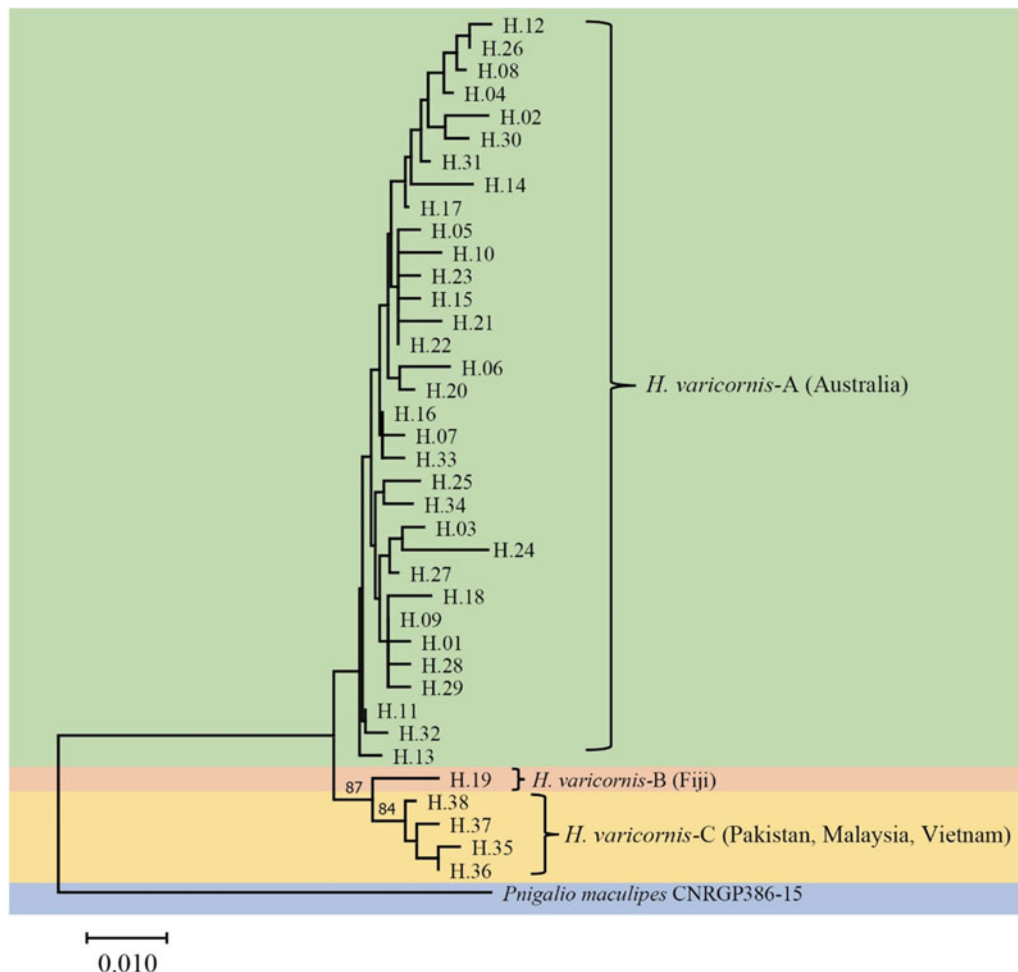

Figure 3. COI phylogenetic tree of *Hemiptarsenus varicornis* was generated using the Neighbor-Joining method (1000 bootstrap replications, Kimura-2 parameter model) based on 375 bp sequence data. The scale bar indicates nucleotide substitutions per site. Information about the haplotypes used in this study can be found in table S4. *Pnigalio maculipes* is the outgroup species.

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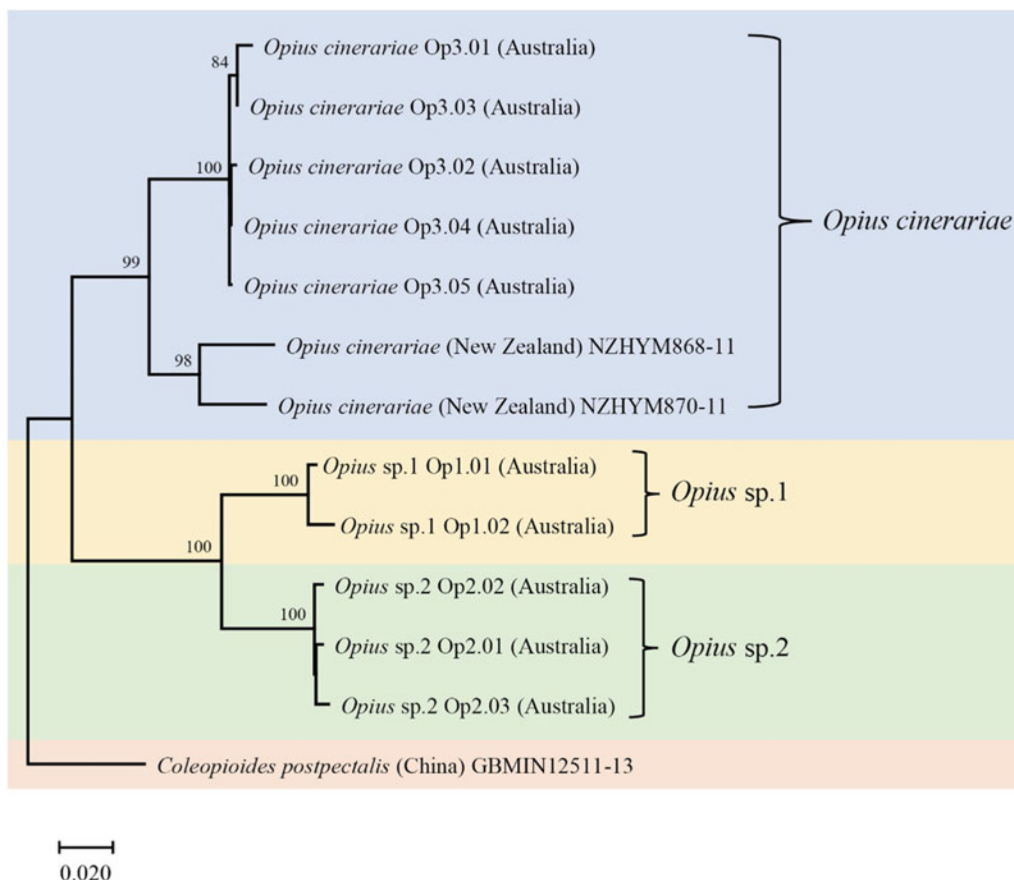

Figure 4. The COI phylogenetic tree of *Opius* spp. was generated using the Neighbor-Joining method (1000 bootstrap replications, Kimura-2 parameter model) based on 577 bp sequences. The scale bar indicates nucleotide substitutions per site. *Coleopioides postpectalis* was the outgroup species (Li *et al*., 2013).

Other parasitoid species

There are two species (*Dac. areolaris, N. okazakii*) with DNA barcodes on BOLD and four species (*Aprostocetus* sp., *Asecodes* sp., *Cl. mirabilis,* and *T. parasitica*) without DNA barcodes. In this study, we provide both morphological and COI information for these six species (table 1, figs S1–S3, S5–S7). For *Dac. areolaris*, we only found a single COI haplotype (Dac.01) and sequences were 99.8% similar to specimens from Germany (BOLD: GBMIX500–14). For *N. okazakii*, 28S sequences were also obtained and indicated our samples from Queensland are 100% identical to *N. okazakii* (NCBI: AB526861) from Japan (Adachi-Hagimori *et al*., 2011). However, the 3′ COI gene region results suggest our *N. okazakii* from Queensland are not the same species as *N. okazakii* (NCBI: AB721363) based on sequences provided by Nakamura *et al*. (2013). The pairwise distances between our *N. okazakii* sequences and these *N. okazakii* (NCBI: AB721363) were in the range of 15.4–16.1%. However, we consider the *N. okazakii* sequences provided by Nakamura *et al.* (2013) problematic given that their COI sequences for *N. okazakii* (NCBI: AB721363.1) are identical to *H. varicornis* (NCBI: AB721362). Our morphological identification also supported our conclusion that our specimen is *N. okazakii.* (fig. S5). We provide the first COI barcodes for *Aprostocetus* sp., *Asecodes* sp., *Cl. mirabilis,* and *T. parasitica.* Among these, we found two haplotypes for *Aprostocetus* sp., six haplotypes for *Asecodes* sp., one haplotype for *Cl. mirabilis,* and three haplotypes for *T. parasitica* (table 1).

Prior studies already provide detailed molecular and morphological data on *N. formosa* and *Z. latilineatum* (Perry and Heraty, 2021; Xu *et al*., 2022), so we compared our COI data with these sequences. For *N. formosa*, Xu *et al.* (2022) found two 5′ COI haplotypes (NCBI: OK076720, NF_5COI.01 and OK076721, NF_5COI.02) from Victorian collections. In this study, we only found one 5′ COI haplotype (NF_5COI.03) for *N. formosa* from Queensland, which is a single base pair different from NF_5COI.02. For *Z. latilineatum*, Perry and Heraty (2021) only provided 3′ COI sequences (NCBI: MK753233, Australia), which were 0.5–2.0% similar to the *Z. latilineatum* sequences we generated in this study.

Table 3. Uncorrected pairwise distances among Opius species based on 577 bp COI sequence data

Species	*Opius cinerariae*	*Opius* sp. 1	*Opius* sp. 2
Opius cinerariae	0.1–8.0%		
Opius sp. 1	13.3–15.8%	1.2%	
Opius sp. 2	13.7–16.5%	6.6–8.3%	0.5–4.1%

Endosymbiont detections

In the Australian samples, we found *H. varicornis* (1 positive/38 total), *D. isaea* (2/24), *Cl. mirabilis* (8/8), *Opius* sp. 1 (1/17),

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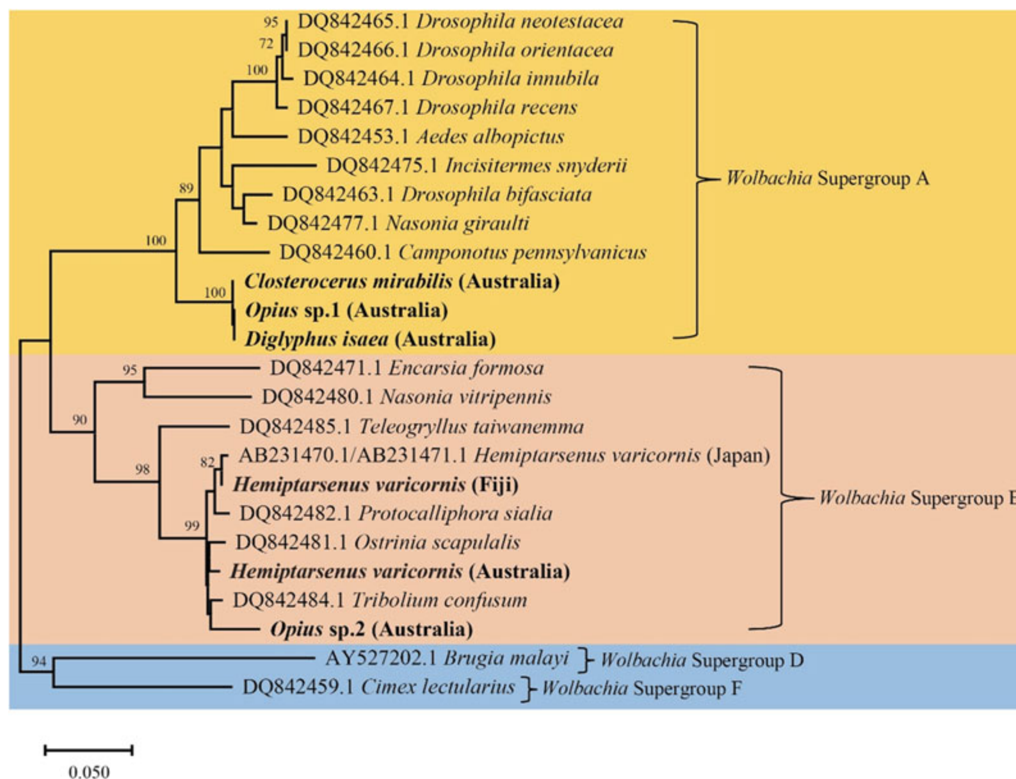

Figure 5. Phylogenetic tree of *Wolbachia wsp* sequences in different insect hosts generated using the Neighbor-Joining method (1000 bootstrap replications, Kimura-2 parameter model) based on 382 bp sequence data. The scale bar indicates nucleotide substitutions per site. Species in this study are highlighted in bold and the remainder of the sequences are from Baldo *et al*. (2006).

and *Opius* sp. 2 (7/14) infected with *Wolbachia,* but often at low frequency. In contrast, *Aprostocetus* sp. and *N. formosa* samples were uniformly infected with *Rickettsia* sp. (table 1). Additionally, we found *N. okazakii* infected with two endosymbionts (*Wolbachia* and *Rickettsia* sp.) simultaneously. Based on these findings, we constructed a phylogenetic tree using *Wolbachia wsp* sequences (fig. 5). The *Wolbachia wsp* sequences of *Cl. mirabilis, Opius* sp. 1, and *D. isaea* were identical and belong to *Wolbachia* Supergroup A. This *wsp* sequence is identical to the *wsp* sequence of *L. sativae* (*w*LsatC) from Vietnam (Xu *et al*., 2021a). The *Wolbachia wsp* sequences of *H. varicornis* and *Opius* sp. 2 belong to *Wolbachia* Supergroup B. There is only a single base pair difference between the *Wolbachia wsp* sequences of *H. varicornis* from Fiji (this study where all 11 specimens were infected) and Japan (NCBI: AB231470.1/AB231471.1) (Tagami *et al*., 2006). Additionally, the *Wolbachia wsp* sequence of *H. varicornis* from Fiji is identical to the *wsp* sequence of *L. bryoniae* from Japan (*w*LbryB) (Tagami *et al*., 2006; Xu *et al*., 2021a). The *Wolbachia wsp* sequence of *H. varicornis* from Australia (this study) is three base pairs different to the *wsp* sequence of *L. bryoniae* (*w*LbryA) from the Netherlands (Xu *et al*., 2021a). The *Wolbachia wsp* sequence of *Opius* sp. 2 is previously undescribed and we did not find similar sequences in any other host.

Both *N. formosa* and *N. okazakii* were infected with the same *Rickettsia* sp. based on 253 bp COI sequence data, which is identical to *N. formosa* previously screened from Australia, Japan, and China (Xu *et al*., 2022). The *Rickettsia* sp. sequence of *Aprostocetus* sp. is only two base pairs different to *N. formosa* and *N. okazakii* sequences generated in this study. Moreover, *N. okazakii is* infected with *Wolbachia,* with the *wsp* sequence identical to *L. brassicae* (NCBI: MW047082.1) (Xu *et al*., 2021a).

Discussion

Biological control has become important in managing *L. huidobrensis*, *L. sativae* and *L. trifolii*, largely because chemical controls have become ineffective due to resistance to pesticides, and because biological control helps circumvent unwanted side effects of chemical applications, including the destruction of natural enemy communities and environmental pollution (Murphy and La Salle, 1999; Reitz *et al*., 2013; Ridland *et al*., 2020). Within Australia, it is important to improve the understanding of the indigenous and adventive parasitoid wasps given the quarantine challenges of deliberately introducing exotic species into the country. In this study, we deployed DNA barcoding and morphological diagnosis to identify 14 parasitoid species which we reared from agromyzids in Australia. Based on our phylogenetic analyses, we found clear genetic divergence within *C. pubicornis, D. isaea, H. varicornis,* and *Opius* spp., highlighting the importance of further taxonomic studies on these taxa. In addition, we provide new barcodes with convincing morphological characterization for other species. We also checked for endosymbiont infection status and found both *Rickettsia* and *Wolbachia* infections and evidence for a superinfection in one species.

Chrysocharis pubicornis is a koinobiont endoparasitoid of agromyzid leafminers (Lardner, 1991; Baeza Larios, 2007) and is an adventive species in Australia, found in New South Wales, South Australia, Tasmania, and Victoria (Bouček, 1988). It is primarily a pupal parasitoid but occasionally acts as a larval–pupal

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parasitoid (Hansson, 1985; Lardner, 1991; Baeza Larios, 2007). This species is an important parasitoid wasp of *Phytomyza* spp. which pupate in the leaf mine (e.g., *P. horticola*) but may only have a minor impact on *Liriomyza* pests which generally pupate in the soil (Baeza Larios, 2007; Coquilleau, 2020; Ridland *et al*., 2020). In this study, we found 0.2–6.3% COI divergence in *C. pubicornis* specimens from the same population with haplotypes potentially associated with different host leafmining species. This situation is also found in the UK, where individuals found on *P. horticola* (BOLD: GBAH8983–14) varied 6.1% from individuals found on *Scaptomyza flava* (Fallen) (BOLD: GBAH8982–14) (Derocles *et al*., 2015) (table S3). Additionally, the sympatric genetic divergence of *C. pubicornis* has been recorded in Norway (where an individual (BOLD: GMNWK1832–14) varied 6.1% from another individual (BOLD: GMNWL1999–14) although the host flies remain unknown. These high and variable levels of sequence divergence in *C. pubicornis* may indicate cryptic species that require further analysis.

Diglyphus isaea is a synovigenic idiobiont ectoparasitoid of many leafmining Diptera (Zhang *et al*., 2011) and is released extensively in glasshouses for augmentative biological control of *L. sativae, L. trifolii, L. huidobrensis* and *L. bryoniae* (Van Lenteren, 2012). This species has become cosmopolitan after inoculative introductions into other regions (e.g., Japan, Hawaii, Canada, and New Zealand) (Minkenberg, 1989; Abe 2017). Based on the analyses of COI and ITS1 sequence data, Sha *et al*. (2006, 2007) indicated a probable complex of cryptic species present in Chinese *D. isaea*.

Derocles *et al*. (2015) also suggested *D. isaea* found in the UK is a species complex due to high intraspecific variability, and our results support this notion. In Australia, *D. isaea* is an adventive species and possibly introduced from New Zealand, where introductions from Pakistan were released in an attempt to suppress leafminers infesting forage brassicas in the 1970s (McGregor, 1989). In this study, we compared specimens with the samples collected by Sha *et al*. (2006, 2007) and found *D. isaea* samples from Australia were genetically similar to those from the Netherlands and also clustered with Chinese *D. isaea* in the largest clade (Group I). Our phylogenetic analyses indicated that Chinese and Australian *D. isaea* strains are probably the result of the movement of European *D. isaea* across the world although more populations and individuals are needed to support this hypothesis.

Hemiptarsenus varicornis is a synovigenic idiobiont ectoparasitoid, primarily attacking third instar agromyzid larvae (Bordat *et al*., 1995). Host-killing behaviors of this wasp include parasitism, host feeding, and host stinging, which account for 26, 58, and 16% of mortality, respectively (Cheng *et al*., 2017). *Hemiptarsenus varicornis* is widely distributed throughout Australia (Bouček, 1988) and its biology is well studied overseas (Bordat *et al*., 1995; Thu and Ueno, 2002; Cheng *et al*., 2017). In this study, we found all Australian *H. varicornis* individuals were clustered together, separated from samples from Fiji, Pakistan, Malaysia and Vietnam but nevertheless with relatively low sequence divergence. Prijono *et al*. (2004) found *H. varicornis* in Australia to be more susceptible to abamectin compared with Indonesian *H. varicornis*. It is possible that insecticide tolerance differs across regions reflecting past histories of chemical selection and/or genetic differences between haplotypes.

Opius spp. are koinobiont larval–pupal endoparasitoids and form one of the largest genera in the family Braconidae (Wharton, 1988). Many *Opius* species play important roles in the control of leafmining Agromyzidae (Belokobylskij *et al*., 2004). For example, in Florida, *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) was the most abundant parasitoid of *L. trifolii* on *Phaseolus vulgaris* L., and a direct density-dependent relationship was detected between *O. dissitus* parasitism and *L. trifolii* (Li *et al*., 2012). A handful of field surveys in Australia demonstrated *Opius* spp. commonly parasitize agromyzids and might potentially be used to suppress exotic leafmining pests (Lardner, 1991; Bjorksten *et al*., 2005; Lambkin *et al*., 2008).

However, knowledge of *Opius* spp. attacking agromyzids in Australia remains poor since relatively few species are known from rearing records, and many species have no host records (Belokobylskij *et al*., 2004). In this study, we found three *Opius* species with one identified as *O. cinerariae* and the other two (*Opius* sp. 1 and *Opius* sp. 2) identified to genus level. The morphological diagnosis of *O. cinerariae* is based on the key and re-description in Belokobylskij *et al*. (2004), who examined wasps from *L. chenopodii* in Australia. In other work, Bjorksten *et al*. (2005) found *O. cinerariae* from *L. chenopodii* on *Beta vulgaris* and Lardner (1991) found *O. cinerariae* attacking *L. brassicae* on *Brassica napus*, *Raphanus raphanistrum*, *Raphanus rugosum,* and *Sisymbrium officinale*. Belokobylskij *et al*. (2004) examined many Australian specimens, including the holotype from Queensland, and noted that *O. cinerariae* is more variable than the original description by Fischer (1963). In this study, the genetic divergence of *O. cinerariae* specimens between Australia and New Zealand ranged from 6.9–8.0%. Further collections of this species in other regions are needed to determine if there is a cryptic species complex. *Opius* sp. 1 and *Opius* sp. 2 are more genetically similar than *O. cinerariae* but the divergence between the two is still substantial. Apart from *O. cinerariae,* Lardner (1991) collected *Opius atricornis* from *L. brassicae* in South Australia. Further studies are needed to check if our specimens of *Opius* sp. 1 and *Opius* sp. 2 are in fact *Opius atricornis* (Belokobylskij *et al*., 2004).

The Victorian *Aprostocetus* (*Aprostocetus*) sp. belongs to the subfamily of Tetrastichinae (Graham, 1987; LaSalle, 1994). In Australia, 207 *Aprostocetus* species have been described but no doubt many more species remain undescribed (Bouček, 1988). Three *Aprostocetus* species (spp. 1, 2 and 3) were reared from *P. praecellens* on *R. candolleana* and one species (sp. 2) from *R. parabolica* in South Australia (Wood *et al*., 2010), but it is not known whether the Victorian *Aprostocetus* (*Aprostocetus*) sp. reared from *P. praecellens* on *R. parabolica* is the same as *Aprostocetus* (sp. 2). Further molecular and morphological work will be essential to unravel the identity of the *Aprostocetus* species attacking Australian agromyzids given this is a very large and taxonomically diverse genus, with a large number of undescribed species.

Asecodes is a small genus with a cosmopolitan distribution (Noyes, 2019). To date, all *Asecodes* species reared from agromyzids were originally considered to be *Teleopterus*. Boucˇek (1988) noted that there were 3–4 species of *Teleopterus* in Australia, with only *T. atripes* (Girault, 1915a) described from Queensland, but without host data. Subsequently, *Teleopterus* was synonymized with *Asecodes* (Hansson, 1996). Gumovsky (2001) then synonymized *Asecodes*, *Neochrysocharis*, *Hispinocharis,* and *Mangocharis* with *Closterocerus*. However, molecular analysis led Burks *et al*. (2011) to remove *Neochrysocharis* and *Asecodes* from synonymy. *Asecodes delucchii* and *A. erxias* are two common parasitoids of agromyzid wasps and may play an important role in suppressing *Liriomyza* pests and *P. horticola* (Arakaki and Kinjo, 1998; Tran *et al*., 2005; Amano *et al*., 2008; Tran 2009). In this study, we

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only identify *Asecodes* to genus level and DNA sequences demonstrate our *Asecodes* sp. is not *A. erxias* (Genbank: MG836471.1/MG836472.1) or other *Asecodes* species present in BOLD and GenBank. Coquilleau (2020) showed the *Asecodes* sp. sequenced in this study is commonly found parasitizing *L. brassicae*, *P. plantaginis,* and *P. syngenesiae* in Melbourne and we have also found this *Asecodes* sp. regularly reared from *L. huidobrensis* in Queensland (P. Ridland, unpub. data).

Closterocerus mirabilis is an idiobiont ectoparasitoid but little is known about its biology (Lardner, 1991; Ridland *et al*., 2020). It has been found in ACT, South Australia, Victoria and Queensland (Edwards and La Salle, 2004; Bjorksten *et al*., 2005; Lambkin *et al*., 2008; Coquilleau, 2020) and is suspected to be present in Indonesia (Edwards and La Salle, 2004). *Closterocerus mirabilis* is morphologically close to *Closterocerus cruy* (Girault), but the former has only been recorded from agromyzid leafminers whereas the latter has only been recorded from lepidopteran hosts (Berry, 2007a). Both these species are morphologically very similar to *Closterocerus separatus* Li & Li (Li and Li 2021). *Closterocerus mirabilis* has been reared from *P. plantaginis*, *P. syngenesiae, L. chenopodii*, *L. brassicae,* and *L. sativae* (Edwards and La Salle, 2004; Bjorksten *et al*., 2005; Lambkin *et al*., 2008; Coquilleau, 2020; Ridland *et al*., 2020) and is an abundant indigenous parasitoid of agromyzid leafminers in eastern Australia. We found only a single haplotype in this study.

Dacnusa areolaris is a koinobiont endoparasitoid, which oviposits in the early larval stages and emerges from the puparia of agromyzid hosts (Haviland, 1922). This species is thought to have been accidentally introduced into Australia and New Zealand (Wharton and Austin, 1991; Berry, 2007b) and no native *Dacnusa* species have been found in Australia to date (Wharton and Austin, 1991). In Australia, *Dac. areolaris* have been recorded from South Australia, Victoria, Tasmania, the Australian Capital Territory, and New South Wales; the earliest collection records are from 1927 (Wharton and Austin, 1991). Griffiths (1966) recorded *Dac. areolaris* reared from three hosts including *P. syngenesiae*, *Phytomyza asteris* (Hendel), and *Phytomyza nigra* (Meigen). Similar to *Cl. mirabilis,* we only found a single haplotype of *Dac. areolaris* in this study.

Neochrysocharis formosa and *N. okazakii* are synovigenic idiobiont endoparasitoids (Chien and Chang, 2009a, 2009b). Both species are widely distributed in Asian countries and parasitize *Liriomyza* (Tran *et al*., 2007; Sunari *et al*., 2016). In Australia, Bjorksten *et al*. (2005) recorded one specimen of *N. okazakii* on *L. brassicae* and one specimen of an unidentified *Neochrysocharis* sp. collected from *P. syngenesiae*. Lambkin *et al*. (2008) found unidentified *Neochrysocharis* sp. from *P. plantaginis* and *P. syngenesiae*, and field collections in Victorian have found *N. formosa* reared from *L. brassicae, L. chenopodii*, *P. plantaginis* and *P. syngenesiae* (Coquilleau, 2020; Xu *et al*., 2022). Currently, only female wasps have been found in Australia and thelytokous strains of *N. formosa* have been recorded in Japan and China which are associated with *Rickettsia* infection (Hagimori *et al*., 2006; Zhang *et al*., 2014; Yang *et al*., 2017; Xu *et al*., 2022). For *N. okazakii*, only limited DNA barcodes are publicly available. Our 28S sequence of *N. okazakii* is identical to *N. okazakii* (NCBI: AB526861) from Japan (Adachi-Hagimori *et al*., 2011), but there is still 6.3% genetic distance from Chinese *N. okazakii* based on 430 bp COI sequence data (Personal Communication W-X Liu). We suspect there are cryptic species within this taxon despite not finding any morphological differences through SEM imaging.

Trigonogastrella parasitica is a larval–pupal koinobiont endoparasitoid of agromyzids and has been found in Queensland, New South Wales, ACT, Victoria, and Tasmania (Bouc˘ek, 1988). The species was described by Girault (1915b) from Victorian specimens together with another species, *Cryptoprymnoides rabiosus* from Queensland, which was subsequently re-classified as *Trigonogastrella rabiosa* (Girault) (Bouček, 1988). *Trigonogastrella rabiosa* has been recorded in Queensland, New South Wales, South Australia, and Western Australia without detailed host information (Bouc˘ek, 1988). The known agromyzid hosts of *T. parasitica* include *P. syngenesiae*, *Liriomyza* spp., *Ophiomyia* spp., and *P. plantaginis* (Ridland *et al*., 2020). Limited field surveys illustrated that *T. parasitica* is a common parasitoid species reared from *P. syngenesiae* and *P. plantaginis* (Lambkin *et al*., 2008; Coquilleau, 2020) and we found at least three COI haplotypes exist in Australia.

Zagrammosoma latilineatum was first described in Indonesia and Australia and has been found parasitizing *L. huidobrensis* in southeast Asia (Ubaidillah *et al*., 2000). This idiobiont ectoparasitoid species is widely distributed in Australia, recorded in every state except for Tasmania (Lardner, 1991; Perry and Heraty, 2021). Bjorksten *et al*. (2005) reared *Z. latilineatum* from *L. brassicae* and *L. chenopodii* in Victoria. Wood *et al*. (2010) found that *Z. latilineatum* was commonly reared from *Phytoliriomyza praecellens* in South Australia. It was also the most frequently reared species from *L. sativae* on Thursday Island, Horn Island and Seisia between 2018 and 2019 (Ridland *et al*., 2020). Given *Z. latilineatum* is widely distributed and can inflict high rates of parasitism on exotic *Liriomyza* pests (Ridland *et al*., 2020), this species is considered a potentially important biological control agent for the management of *Liriomyza* pests in Australia.

Endosymbiont assays in the present study found *H. varicornis* was infected with *Wolbachia* at a low frequency in Victoria, whereas all individuals from Fiji were infected with *Wolbachia*. Both *H. varicornis* and its host *L. brassicae* at the Victorian collection site (Flemington Bridge) were infected by *Wolbachia* with identical *wsp* sequences. Likewise, Tagami *et al*. (2006) found nearly identical *wsp* sequences in *H. varicornis* and *L. bryoniae*. In Japan, *Wolbachia* infection did not induce cytoplasmic incompatibility (CI) or parthenogenesis in *H. varicornis*. We also found *Opius* sp. 1 and *Opius* sp. 2 infected with *Wolbachia* at low frequencies. The *wsp* sequences of *Opius* sp. 1 and *D. isaea* are identical to the *wsp* sequences of *L. sativae* (*w*LsatC: MW310402), *P. syngenesiae* (MW047083) and *P. praecellens* (MW310408) (Xu *et al*., 2021a). This suggests horizontal transmission of *Wolbachia* may occur through host-parasitoid interactions. Moreover, we found all *Cl. mirabilis* individuals in this study are infected with *Wolbachia* but it is unknown if there are any phenotypes associated with this *Wolbachia* strain.

We found three wasp species infected with *Rickettsia* including one species with *Wolbachia*/*Rickettsia* co-infections. In *N. formosa*, only one COI haplotype (NF_5COI.03) was found in Queensland and this is a single base pair different to *N. formosa* collected in Victoria (NCBI: OK076721, NF_5COI.02). Interestingly, NF_5COI.02 and another *N. formosa* haplotype collected at the same location, NF_5COI.01 (NCBI: OK076720), were found to be thelytokous (Xu *et al*., 2022). Given no males of *N. formosa* were collected from Queensland in this study and all specimens were infected with *Rickettsia*, it is possible that NF_5COI.03 is also thelytokous. In *N. okazakii*, we found all individuals (three females and three males) are not only infected with *Rickettsia* (same as found in *N. formosa*, NCBI: OK086364.1) but also

infected with *Wolbachia* (same as found in *L. brassicae*, NCBI: MW047082.1). The *Rickettsia* found in *N. okazakii* was not associated with thelytoky but it has been suggested to cause thelytoky in *N. formosa* (Hagimori *et al*., 2006). The *Wolbachia* strain in *N. okazakii* has been suggested to cause cytoplasmic incompatibility in *L. brassicae* (Xu *et al*., 2021a). It would be worthwhile exploring how these two endosymbionts interact in *N. okazakii* and if there are any phenotypic effects detected. For *Aprostocetus* sp., only females were collected in this study and all individuals tested were infected with *Rickettsia* with two base pairs different from *Rickettsia* detected in *N. formosa* and *N. okazakii*.

Although the total number of wasps tested was somewhat limited, endosymbiont infection status appears to be associated with the mtDNA haplotypes. Species with diverse COI haplotypes were often infected with a low frequency of *Wolbachia* or were devoid of *Wolbachia,* whereas species infected with *Wolbachia* at a high frequency (e.g., *Cl. mirabilis* in Australia, *H. varicornis* in Fiji) often possess fewer haplotypes. A decrease of mitochondrial variation is thought to relate to *Wolbachia*-induced cytoplasmic incompatibility, which results in the infected individuals spreading in a population and eventually throughout the whole range of a species (Hale and Hoffmann, 1990).

Conclusion

Overall, our study provides important DNA barcodes and morphological information of 14 parasitoid wasp species that are potentially important in agromyzid control in Australia. Given the taxonomic challenges and limited DNA barcoding information available on public databases, our study provides a solid foundation that facilitates future research into agromyzid wasps. Furthermore, our study provides information on endosymbiont infections across parasitoid species, with the potential of manipulating endosymbionts to alter the mode of reproduction in populations of the parasitoids in the future.

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References

Abe Y (2017) Invasion of Japan by exotic leafminers *Liriomyza* spp. (Diptera: Agromyzidae) and its consequences. *Applied Entomology and Zoology* **52**, 175–182.

Adachi-Hagimori T, Miura K and Abe Y (2011) Gene flow between sexual and asexual strains of parasitic wasps: a possible case of sympatric speciation caused by a parthenogenesis-inducing bacterium. *Journal of Evolutionary Biology* **24**, 1254–1262.

Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403–410.

Amano K, Suzuki A, Hiromori H and Saito T (2008) Relative abundance of parasitoids reared during field exposure of sentinel larvae of the leafminers *Liriomyza trifolii* (Burgess), *L. sativae* Blanchard, and *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae). *Applied Entomology and Zoology* **43**, 625–630.

Amuzu HE and McGraw EA (2016) *Wolbachia*-based dengue virus inhibition is not tissue-specific in *Aedes aegypti*. *PLoS Neglected Tropical Diseases* **10**, e0005145.

Arakaki N and Kinjo K (1998) Notes on the parasitoid fauna of the serpentine leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) in Okinawa, southern Japan. *Applied Entomology and Zoology* **33**, 577–581.

Asadi R, Talebi AA, Fathipour Y, Moharramipour S and Rakhshani E (2006) Identification of parasitoids and seasonal parasitism of the agromyzid leafminers genus *Liriomyza* (Dip.: Agromyzidae) in Varamin, Iran. *Journal of Agricultural Science and Technology* **8**, 293–303.

Baeza Larios GL (2007) Assessment of ecological features of Chrysocharis pubicornis (Hymenoptera: Eulophidae) as biological control agent of Liriomyza leafminers (Diptera: Agromyzidae) (PhD thesis). University of Miyazaki. Available at https://ci.nii.ac.jp/naid/500000495834/?l=en (Accessed 21 February 2018).

Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MC, Tettelin H and Werren JH (2006) Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Applied and Environmental Microbiology* **72**, 7098–7110.

Belokobylskij SA, Wharton RA and La Salle J (2004) Australian species of the genus *Opius* Wesmael (Hymenoptera: Braconidae) attacking leafmining Agromyzidae, with the description of a new species from South-east Asia. *Australian Journal of Entomology* **43**, 138–147.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt and KD and Sayers (2018) GenBank. *Nucleic Acids Research* **41**, D36–D42.

Berry JA (2007a) Redescription of *Closterocerus cruy* (Girault) (Hymenoptera: Eulophidae: Entedoninae), with new geographical and host records. *Australian Journal of Entomology* **46**, 294–299.

Berry JA (2007b) Alysiinae (Insecta: Hymenoptera: Braconidae). *Fauna of New Zealand* **58**, 1–95.

Bethke JA and Parrella MP (1985) Leaf puncturing, feeding and oviposition behavior of *Liriomyza trifolii*. *Entomologia Experimentalis et Applicata* **39**, 149–154.

Bjorksten TA, Robinson M and La Salle J (2005) Species composition and population dynamics of leafmining flies and their parasitoids in Victoria. *Australian Journal of Entomology* **44**, 186–191.

Blacket MJ, Rice AD, Semeraro L and Malipatil MB (2015) DNA-based identifications reveal multiple introductions of the vegetable leafminer *Liriomyza sativae* (Diptera: Agromyzidae) into the Torres Strait Islands and Papua New Guinea. *Bulletin of Entomological Research* **105**, 533–544.

Bordat D, Coly EV and Roux-Olivera C (1995) Morphometric, biological and behavioural differences between *Hemiptarsenus varicornis* (Hym., Eulophidae) and *Opius dissitus* (Hym., Braconidae) parasitoids of *Liriomyza trifolii* (Dipt., Agromyzidae). *Journal of Applied Entomology* **119**, 423–427.

Bouček Z (1988) Australasian Chalcidoidea (Hymenoptera). *A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species*. Wallingford, UK: CAB International.

Bueno AD, Zechmann B, Hoback WW, Bueno RC and Fernandes OA (2007) Serpentine leafminer (*Liriomyza trifolii*) on potato (*Solanum tuberosum*): field observations and plant photosynthetic responses to injury. *Ciência Rural* **37**, 1510–1517.

Burks RA, Heraty JM, Gebiola M and Hansson C (2011) Combined molecular and morphological phylogeny of Eulophidae (Hymenoptera: Chalcidoidea), with focus on the subfamily Entedoninae. *Cladistics* **27**, 581–605.

Cheng X-Q, Cao F-Q, Zhang Y-B, Guo J-Y, Wan F-H and Liu W-X (2017) Life history and life table of the host-feeding parasitoid *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae). *Applied Entomology and Zoology* **52**, 287–293.

Chien C-C and Chang S-C (2009a) Morphology and life history of *Closterocerus okazakii* (Kamijo) (Hymenoptera: Eulophidae). *Formosan Entomologist* **29**, 25–36.

Chien C-C and Chang S-C (2009b) Influence of temperature on the life table and host-killing capability of *Closterocerus okazakii* (Kamijo) (Hymenoptera: Eulophidae). *Formosan Entomologist* **29**, 37–50.

Coquilleau MP (2020) *Seasonality and community composition of parasitoid wasps of four agromyzid leafminer species (Diptera: Agromyzidae) in Victoria* (MPhil Thesis). The University of Melbourne. Available at https://minerva-access.unimelb.edu.au/handle/11343/252730 (Accessed 07 August 2021).

Coquilleau MP, Xu X, Ridland PM, Umina PA and Hoffmann AA (2021) Variation in sex ratio of the leafminer *Phytomyza plantaginis* Goureau (Diptera: Agromyzidae) from Australia. *Austral Entomology* **60**, 610–620.

Darling JA and Blum MJ (2007) DNA-based methods for monitoring invasive species: a review and prospectus. *Biological Invasions* **9**, 751–765.

Derocles SA, Evans DM, Nichols PC, Evans SA and Lunt DH (2015) Determining plant–leaf miner–parasitoid interactions: a DNA barcoding approach. *PloS One* **10**, e0117872.

Edwards CM and La Salle J (2004) A new species of *Closterocerus* Westwood (Hymenoptera: Eulophidae), a parasitoid of serpentine leafminers (Diptera: Agromyzidae) from Australia. *Australian Journal of Entomology* **43**, 129–132.

Fischer M (1963) Neue Zuchtergebnisse von Braconiden (Hymenoptera). *Zeitschrift für Angewandte Zoologie* **50**, 195–214.

Fisher N, Ubaidillah R, Reina P and La Salle J (2005) Liriomyza parasitoids in Southeast Asia. Available at https://keys.lucidcentral.org/keys/v3/Liriomyza/index.html (Accessed 15 February 2022).

Floate KD, Kyei-Poku GK and Coghlin PC (2006) Overview and relevance of *Wolbachia* bacteria in biocontrol research. *Biocontrol Science and Technology* **16**, 767–788.

Frentiu FD, Zakir T, Walker T, Popovici J, Pyke AT, van den Hurk A, McGraw EA and O'Neill SL (2014) Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. *PLoS Neglected Tropical Diseases* **8**, e2688.

Ge J, Wei J, Tao Y and Kang L (2019) Sexual cooperation relies on food controlled by females in agromyzid flies. *Animal Behaviour* **149**, 55–63.

Girault AA (1915a) Australian Hymenoptera Chalcidoidea – IV. Supplement. *Memoirs of the Queensland Museum* **3**, 180–299.

Girault AA (1915b) Australian Hymenoptera Chalcidoidea – VI. Supplement. *Memoirs of the Queensland Museum* **3**, 313–346.

Graham MD (1987) A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae), with a revision of certain genera. *Bulletin of the British Museum (Natural History), Entomology Series* **55**, 1–392.

Griffiths GCD (1966) The Alysiinae (Hym. Braconidae) parasites of the Agromyzidae (Diptera). III. The parasites of *Paraphytomyza* Enderlein, *Phytagromyza* Hendel and *Phytomyza* Fallén. *Beiträge zur Entomologie* **16**, 775–951.

Gumovsky AV (2001) The status of some genera allied to *Chrysonotomyia* and *Closterocerus* (Hymenoptera: Eulophidae, Entedoninae), with description of a new species from Dominican amber. *Phegea* **29**, 125–141.

Hagimori T, Abe Y, Date S and Miura K (2006) The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. *Current Microbiology* **52**, 97–101.

Hale LR and Hoffmann AA (1990) Mitochondrial DNA polymorphism and cytoplasmic incompatibility in natural populations of *Drosophila simulans*. *Evolution* **44**, 1383–1386.

Hansson C (1985) Taxonomy and biology of the Palearctic species of *Chrysocharis* Forster, 1856 (Hymenoptera: Eulophidae). *Entomologica Scandinavica Supplement* **26**, 1–130.

Hansson C (1987) Revision of the New World species of *Chrysocharis* Forster (Hymenoptera: Eullophidae). *Entomologica Scandinavica Supplement* **31**, 1–86.

Hansson C (1990) A taxonomic study on the Palearctic species of *Chrysonotomyia* Ashmead and *Neochrysocharis* Kurdjumov (Hymenoptera: Eulophidae). *Insect Systematics & Evolution* **21**, 29–52.

Hansson C (1994) Re-evaluation of the genus *Closterocerus* Westwood (Hymenoptera: Eulophidae), with a revision of the Nearctic species. *Insect Systematics & Evolution* **25**, 1–25.

Hansson C (1996) The status of the genera *Asecodes* Förster, *Ionympha* Graham and *Teleopterus* Silvestri (Hymenoptera: Eulophidae), with a review of Nearctic species. *Insect Systematics & Evolution* **27**, 159–167.

Hansson C and Navone P (2017) Review of the European species of *Diglyphus* Walker (Hymenoptera: Eulophidae) including the description of a new species. *Zootaxa* **4269**, 197–229.

Haviland MD (1922) On the larval development of *Dacnusa areolaris* Nees (Braconidae), a parasite of Phytomyzinae (Diptera), with a note on certain chalcid parasites on phytomyzids. *Parasitology* **14**, 167–173.

Ikeda E (1995) Revision of the Japanese species of *Chrysocharis* (Hymenoptera, Eulophidae), I. *Japanese Journal of Entomology* **63**, 261–274.

Ikeda E (1996) Revision of the Japanese species of *Chrysocharis* (Hymenoptera, Eulophidae), III. *Japanese Journal of Entomology* **64**, 275–287, 551–569.

International Plant Protection Convention (IPPC) (2017) Detection of *Liriomyza sativae* in Far North Queensland. IPPC Pest Report, AUS 80/1. Available at https://www.ippc.int/en/countries/Australia/pestreports/2017/04/detection-of-liriomyza-sativae-in-far-north-queensland/ (Accessed 04 August 2021).

International Plant Protection Convention (IPPC) (2021a) *Liriomyza huidobrensis* (serpentine leafminer) in New South Wales and Queensland. IPPC Pest Report AUS 103/3. Available at https://www.ippc.int/en/countries/australia/pestreports/2021/07/liriomyza-huidobrensis-in-new-south-wales-and-queensland/ (Accessed 04 August 2021).

International Plant Protection Convention (IPPC) (2021b) *Liriomyza trifolii* (American serpentine leafminer) in Queensland and Western Australia. IPPC Pest Report AUS 104/1. Available at https://www.ippc.int/en/countries/australia/pestreports/2021/07/liriomyza-trifolii-american-serpentine-leafminer-in-queensland-and-western-australia/ (Accessed 04 August 2021).

Johnson MW (1993) Biological control of *Liriomyza* leafminers in the Pacific Basin. Micronesica, *Supplement* **4**, 81–92.

Johnson MW, Welter SC, Toscano NC, Ting P and Trumble JT (1983) Reduction of tomato leaflet photosynthesis rates by mining activity of *Liriomyza sativae* (Diptera: Agromyzidae). *Journal of Economic Entomology* **76**, 1061–1063.

Kamijo K (1978) Chalcidoid parasites (Hymenoptera) of Agromyzidae in Japan, with description of a new species. *Japanese Journal of Entomology* **46**, 455–469.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C and Thierer T (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* **28**, 1647–1649.

Klopfstein S, van Der Schyff G, Tierney S and Austin AD (2018) *Wolbachia* infections in Australian ichneumonid parasitoid wasps (Hymenoptera: Ichneumonidae): evidence for adherence to the global equilibrium hypothesis. *Biological Journal of the Linnean Society* **123**, 518–534.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547.

Lambkin CL, Fayed SA, Manchester C, La Salle J, Scheffer SJ and Yeates DK (2008) Plant hosts and parasitoid associations of leaf mining flies (Diptera: Agromyzidae) in the Canberra region of Australia. *Australian Journal of Entomology* **47**, 13–19.

Lardner RM (1991) Comparative host stage utilization of two parasitoids of *Liriomyza brassicae* (Diptera: Agromyzidae) (PhD Thesis). University of Adelaide. Available at http://digital.library.adelaide.edu.au/dspace/handle/2440/21631 (Accessed 15 February 2019).

LaSalle J (1994) Taxonomic notes on African *Aprostocetus* Westwood (Hymenoptera: Eulophidae). *African Entomology* **2**, 107–109.

Li M-R and Li C-D (2021) Four new species of *Closterocerus* Westwood (Hymenoptera, Eulophidae) from China, with a key to Chinese species. *ZooKeys* **1017**, 21–36.

Li J, Seal DR, Leibee GL and Liburd OE (2012) Seasonal abundance and spatial distribution of the leafminer, *Liriomyza trifolii* (Diptera: Agromyzidae), and its parasitoid, *Opius dissitus* (Hymenoptera: Braconidae), on bean in southern Florida. *Florida Entomologist* **95**, 128–135.

Liu TX, Kang L, Heinz M and Trumble J (2009) Biological control of *Liriomyza* leafminers: progress and perspective. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **4**, No. 004.

Li X-X, van Achterberg K and Tan J (2013) Revision of the subfamily Opiinae (Hymenoptera, Braconidae) from Hunan (China), including thirty-six new species and two new genera. *ZooKeys* **268**, 1–186.

Lue CH, Buffington ML, Scheffer S, Lewis M, Elliott TA, Lindsey AR, Driskell A, Jandova A, Kimura MT, Carton Y and Kula RR (2021) DROP: molecular voucher database for identification of *Drosophila* parasitoids. *Molecular Ecology Resources* **21**, 2437–2454.

Lue CH, Abram PK, Hrcek J, Buffington ML and Staniczenko PPA (2022) Metabarcoding and applied ecology with hyper-diverse organisms: recommendations for biological control research. *Molecular Ecology* **00**, 1–13. https://doi.org/10.1111/mec.16677.

McGregor PG (1989) Agromyzidae, leaf miners (Diptera). In Cameron PJ, Hill RL, Bain J and Thomas WP (eds), *A Review of Biological Control of Invertebrate Pests and Weeds in New Zealand 1874 to 1987*. Wallingford: CAB International, pp. 45–49.

Minkenberg OPJM (1988) Dispersal of *Liriomyza trifolii*. *Bulletin OEPP/EPPO Bulletin* **18**, 173–182.

Minkenberg OP (1989) Temperature effects on the life history of the eulophid wasp *Diglyphus isaea*, an ectoparasitoid of leafminers (*Liriomyza* spp.), on tomatoes. *Annals of Applied Biology* **115**, 381–397.

Mitsunaga T and Yano E (2004) The effect of multiple parasitism by an endoparasitoid on several life history traits of leafminer ectoparasitoids. *Applied Entomology and Zoology* **39**, 315–320.

Montenegro H, Solferini VN, Klaczko LB and Hurst GDD (2005) Male-killing *Spiroplasma* naturally infecting *Drosophila melanogaster*. *Insect Molecular Biology* **14**, 281–287.

Mujica N and Kroschel J (2011) Leafminer fly (Diptera: Agromyzidae) occurrence, distribution and parasitoid associations in field and vegetable crops along the Peruvian coast. *Environmental Entomology* **40**, 217–230.

Mulholland S, Gopurenko D, Mirringto R, Löcker B, Gillespie P, Rossiter L and Anderson C (2022) First report of the serpentine leafminer *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) and its impacts in Australia. *Austral Entomology* **61**, 350–357.

Murphy ST and LaSalle J (1999) Balancing biological control strategies in the IPM of New World invasive *Liriomyza* leafminers in field vegetable crops. *Biocontrol News and Information* **20**, 91N–104N.

Nakamura S, Masuda T, Mochizuki A, Konishi K, Tokumaru S, Ueno K and Yamaguchi T (2013) Primer design for identifying economically important *Liriomyza* species (Diptera: Agromyzidae) by multiplex PCR. *Molecular Ecology Resources* **13**, 96–102.

Narita S, Nomura M and Kageyama D (2007) Naturally occurring single and double infection with *Wolbachia* strains in the butterfly *Eurema hecabe*: transmission efficiencies and population density dynamics of each *Wolbachia* strain. *FEMS Microbiology Ecology* **61**, 235–245.

Noyes JS (2019) *Universal Chalcidoidea Database*. The Natural History Museum. World Wide Web Electronic Publication. Available at https://www.nhm.ac.uk/our-science/data/chalcidoids/database/ (Accessed 8 June 2019).

Parrella MP (1987) Biology of *Liriomyza*. *Annual Review of Entomology* **32**, 201–224.

Parrella MP, Jones VP, Youngman RR and Lebeck LM (1985) Effect of leaf mining and leaf stippling of *Liriomyza* spp. on photosynthetic rates of chrysanthemum. *Annals of the Entomological Society of America* **78**, 90–93.

Perry RK and Heraty JM (2019) A tale of two setae: how morphology and ITS2 help delimit a cryptic species complex in Eulophidae (Hymenoptera: Chalcidoidea). *Insect Systematics and Diversity* **3**, 1–23.

Perry RK and Heraty JM (2021) Read between the lineata: a revision of the tattooed wasps, *Zagrammosoma* Ashmead (Hymenoptera: Eulophidae), with descriptions of eleven new species. *Zootaxa* **4916**, 001–108.

Powell C, Caleca V, Sinno M, van Staden M, van Noort S, Rhode C, Allsopp E and van Asch B (2019) Barcoding of parasitoid wasps (Braconidae and Chalcidoidea) associated with wild and cultivated olives in the Western Cape of South Africa. *Genome* **62**, 183–199.

Prijono D, Robinson M, Rauf A, Bjorksten T and Hoffmann AA (2004) Toxicity of chemicals commonly used in Indonesian vegetable crops to *Liriomyza huidobrensis* populations and the Indonesian parasitoids *Hemiptarsenus varicornis*, *Opius* sp., and *Gronotoma micromorpha*, as well as the Australian parasitoids *Hemiptarsenus varicornis* and *Diglyphus isaea*. *Journal of Economic Entomology* **97**, 1191–1197.

Ratnasingham S and Hebert PD (2007) BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular Ecology Notes* **7**, 355–364.

Reina P and La Salle J (2003) Key to the world genera of Eulophidae parasitoids (Hymenoptera) of leafmining Agromyzidae (Diptera). Available at https://keys.lucidcentral.org/keys/v3/eulophidae_parasitoids/index.html (Accessed 07 August 2021).

Reitz SR, Gao Y and Lei Z (2013) Insecticide use and the ecology of invasive liriomyza leafminer management. In Tradan S (ed.), *Insecticides–Development of Safer and More Effective Technologies*. Rijeka, Croatia: InTech, pp. 233–253.

Ridland PM, Umina PA, Pirtle EI and Hoffmann AA (2020) Potential for biological control of the vegetable leafminer, *Liriomyza sativae* (Diptera: Agromyzidae), in Australia with parasitoid wasps. *Austral Entomology* **59**, 16–36.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE and Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* **34**, 3299–3302.

Scheffer SJ and Lewis ML (2001) Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Annals of the Entomological Society of America* **94**, 648–653.

Scheffer SJ and Lewis ML (2005) Mitochondrial phylogeography of vegetable pest *Liriomyza sativae* (Diptera: Agromyzidae): divergent clades and invasive populations. *Annals of the Entomological Society of America* **98**, 181–186.

Scheffer SJ and Lewis ML (2006) Mitochondrial phylogeography of the vegetable pest *Liriomyza trifolii* (Diptera: Agromyzidae): diverged clades and invasive populations. *Annals of the Entomological Society of America* **99**, 991–998.

Sha ZL, Zhu CD, Murphy RW, La Salle J and Huang DW (2006) Mitochondrial phylogeography of a leafminer parasitoid, *Diglyphus isaea* (Hymenoptera: Eulophidae) in China. *Biological Control* **38**, 380–389.

Sha ZL, Zhu CD, Murphy RW and Huang DW (2007) Diglyphus isaea (Hymenoptera: Eulophidae): a probable complex of cryptic species that forms an important biological control agent of agromyzid leaf miners. *Journal of Zoological Systematics and Evolutionary Research* **45**, 128–135.

Sinkins S, Curtis C and O'Neill SL (1997) The potential application of inherited symbiont systems to pest control. In O'Neill SL, Hoffman AA and Werren JH (eds), *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, 1st Edn. Oxford, UK: Oxford University Press, pp. 155–175.

Spencer KA (1973) Agromyzidae (Diptera) of Economic Importance. Series Entomologica 9, DrW. Junk B.V. Publishers The Hague, Netherlands.

Sunari AAAAS, Supartha IW, Wijaya IN and Laba IW (2016) The abundance parasitoid populations of *Neochrysocharis formosa* and *Neochrysocharis okazakii* (Hymenoptera: Eulophidae) on *Liriomyza* spp. (Diptera: Agromyzidae) associated with vegetable crop in Bali. *Journal Biology, Agriculture and Healthcare* **6**, 51–56.

Tagami Y, Doi M, Sugiyama K, Tatara A and Saito T (2006) Survey of leafminers and their parasitoids to find endosymbionts for improvement of biological control. *Biological Control* **38**, 210–216.

Thu GHT and Ueno T (2002) Biology of *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae), a parasitoid wasp of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae). *Journal of the Faculty of Agriculture, Kyushu University* **47**, 45–54.

Tran DH (2009) Agromyzid leafminers and their parasitoids on vegetables in central Vietnam. *Journal of the International Society for Southeast Asian Agricultural Sciences* **15**, 21–33.

Tran TT, Tran DH, Konishi K and Takagi M (2005) The vegetable leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) and its parasitoids on cucumber in the Hochiminh Region of Vietnam. *Journal of the Faculty of Agriculture, Kyushu University* **50**, 119–125.

Tran DH, Ueno T and Takagi M (2007) Comparison of the suitability of *Liriomyza chinensis* and *L. trifolii* (Diptera: Agromyzidae) as hosts for

Neochrysocharis okazakii (Hymenoptera: Eulophidae). *Biological Control* **41**, 354–360.

Ubaidillah R, LaSalle J and Rauf A (2000) A new species of *Zagrammosoma* (Hymenoptera: Eulophidae) from the Indo-Australian region, a parasitoid of the invasive pest species *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Oriental Insects* **34**, 221–228.

Van Lenteren JC (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl* **57**, 1–20.

Waugh J (2007) DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays* **29**, 188–197.

Weintraub PG, Scheffer SJ, Visser D, Valladares G, Correa AS, Shepard BM, Rauf A, Murphy ST, Mujica N, MacVean C, Kroschel J, Kishinevsky M, Joshi RC, Johansen NS, Hallett RH, Çivelek HS, Chen B and Metzler HB (2017) The invasive *Liriomyza huidobrensis* (Diptera: Agromyzidae): understanding its pest status and management globally. *Journal of Insect Science* **17**, 1–27.

Wharton RA (1988) Classification of the braconid subfamily Opiinae (Hymenoptera). *The Canadian Entomologist* **120**, 333–360.

Wharton RA and Austin AD (1991) Revision of Australian Dacnusini (Hymenoptera: Braconidae: Alysiinae), parasitoids of cyclorrhaphous Diptera. *Australian Journal of Entomology* **30**, 193–206.

Wood G, Siekmann G, Stephens C, DeGraaf H, La Salle J and Glatz R (2010) Native saltbush (*Rhagodia* spp.; Chenopodiaceae) as a potential reservoir for agromyzid leafminer parasitoids on horticultural farms. *Australian Journal of Entomology* **49**, 82–90.

Xu X, Ridland PM, Umina PA, Gill A, Ross PA, Pirtle E and Hoffmann AA (2021a) High incidence of related *Wolbachia* across unrelated leaf-mining Diptera. *Insects* **12**, 788. doi: 10.3390/insects12090788

Xu X, Coquilleau MP, Ridland PM, Umina PA, Yang Q and Hoffmann AA (2021b) Molecular identification of leafmining flies from Australia including new *Liriomyza* outbreaks. *Journal of Economic Entomology* **114**, 1983–1990.

Xu X, Hoffmann AA, Umina PA, Coquilleau MP, Gill A and Ridland PM (2022) Identification of two leafminer parasitoids (Hymenoptera: Eulophidae), *Neochrysocharis formosa* and *Proacrias* sp. from Australia, with both showing thelytoky and infection by *Rickettsia*. *Austral Entomology* **61**, 358–369.

Yang MM, Lin YC, Wu YJ, Fisher N, Saimanee T, Sangtongpraow B, Zhu C, Chiu WCH and La Salle J (2014) Two new *Aprostocetus* species (Hymenoptera: Eulophidae: Tetrastichinae), fortuitous parasitoids of invasive eulophid gall inducers (Tetrastichinae) on *Eucalyptus* and *Erythrina*. *Zootaxa* **3846**, 261–272.

Yang YM, Xuan LL, Ye FY, Guo JY, Yang LY and Liu WX (2017) Molecular identification of the thelytokous strain of *Neochrysocharis formosa* (Hymenoptera: Eulophidae) newly found in China and detection of its endosymbiont *Rickettsia*. *Acta Entomologica Sinica* **60**, 582–593.

Zhang YB, Liu WX, Wang W, Wan FH and Li Q (2011) Lifetime gains and patterns of accumulation and mobilization of nutrients in females of the synovigenic parasitoid, *Diglyphus isaea* Walker (Hymenoptera: Eulophidae), as a function of diet. *Journal of Insect Physiology* **57**, 1045–1052.

Zhang YB, Lu SL, Liu WX, Wang WX, Wang W and Wan FH (2014) Comparing immature development and life history traits in two coexisting host-feeding parasitoids, *Diglyphus isaea* and *Neochrysocharis formosa* (Hymenoptera: Eulophidae). *Journal of Integrative Agriculture* **13**, 2690–2700.

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