

Invasive gall-forming wasps that threaten non-native plantation-grown *Eucalyptus*: diversity and invasion patterns

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Abstract

1. Gall-forming hymenopterans of *Eucalyptus* species are highly successful invaders causing significant damage in non-native plantation forests. To date, at least sixteen of these species have been recorded as invasive eucalypt gall formers, of which less than half are known from Australia where they are thought to be native. About 80% of the species have become invasive only in the last two decades, <10% of which were known from Australia beforehand.
2. Two species, *Leptocybe invasa* and *Ophelimus maskelli*, are global invaders that have become established in 43 and 23 countries, respectively, since 2000. They belong to a large number of wasps that cause similar damage and that could become invasive in the future.
3. The problem is exacerbated by the fact that many species lack taxonomic descriptions; over 80% of the invasive eucalypt gall wasps were first described from their invasive range. The small number of taxonomists able to identify these insects slows accurate diagnoses. Even when initial identifications have been made, these may be confused with morphologically similar but distinct cryptic species, which may differ in their host range and natural enemy interactions.
4. Furthermore, detailed information regarding their biology and native distribution is typically sparse or unknown. This lack of information delays initiation of management actions because breeding for resistance and biological control require accurate identification of the target pest.

5. The gall-forming hymenopterans associated with *Eucalyptus* represent an important group on which to focus the development of pre-emptive quarantine, monitoring and potential management options. Given the global nature of invasions by these insects an international and collaborative research approach is required, where knowledge and tools for study can be shared in a more effective manner.

Keywords: invasive species, plant galls, plantation forestry, gall-forming Hymenoptera

Introduction

Eucalyptus is native to Australasia, but is used in plantation forestry worldwide due to its fast growth and many commercial applications (Ciesla, 2015; Paine *et al.*, 2011). *Eucalyptus* species have become one of the most commonly planted hardwood trees worldwide. They are grown in more than 100 countries, on all continents with the exception of Antarctica (Rejmánek & Richardson, 2011), covering over 20 million hectares (Wingfield *et al.*, 2015). The popularity of *Eucalyptus* species is in part due to their adaptability to a range of environmental conditions, allowing them to replace native tree species previously utilised to meet, amongst other things, energy requirements (Bauhus *et al.*, 2010).

The productivity of *Eucalyptus* plantations has been challenged by the recent increase in invasive pests. It is well documented that global trade, travel and especially the movement of live plant material have contributed to the exponential increase in invasive insect pests in the last decade (Hurley *et al.*, 2016). This abundance of invasive pests and frequent records of new pests is especially threatening to plantation forestry, where uniform genetic material is planted (Wingfield *et al.*, 2015). Furthermore, plantation forestry is expanding globally, rapidly utilizing small numbers of *Eucalyptus* species, and thus providing increasing areas to which forestry pests can spread (Garnas *et al.*, 2013).

Gall-forming wasps are of particular interest to forestry because they include some of the most threatening insect pests on non-native species of *Eucalyptus* utilised in plantations globally (Paine *et al.*, 2011; Hurley *et al.*, 2016). The number of invasive Hymenoptera infesting non-native *Eucalyptus* have increased dramatically during the past 15 years, becoming the third-most recorded group of insect pests on these trees (Hurley *et al.*, 2016). The global distribution of *Eucalyptus* fulfils the first condition (host plant availability)

favouring successful invasion by gall-formers given their relatively narrow host ranges, which may also include an ability to circumvent host defences (Csóka *et al.*, 2017). Other factors favouring the invasiveness of gall-formers include concealment from detection and generalist natural enemies, absence of recorded parasitoids (enemy release), prevention of desiccation due to development within the plant tissue, parthenogenetic reproduction, aerial dispersal of adult stages and human-mediated movement of plant material to areas with suitable climates (Csóka *et al.*, 2017).

Although many gall-formers have been observed on *Eucalyptus* species in their native range in Australia (Kim, 2008), most are insufficiently important pests in Australian plantations (Nahrung & Swain, 2015) to have yet been described (e.g. Carnegie *et al.*, 2008) or warranted intervention. It is also likely that only a fraction of Australia's endemic hymenopteran eucalypt gall-formers have been described (Csóka *et al.*, 2017). An estimate of total numbers of insect species, based on collections examined (Austin *et al.*, 2004; Nielsen & West, 1994), indicated that Australia has between 84 000 and 140 000 insect species, and for most of which their biology is not well known (Austin *et al.*, 2004). This is with the exception of those that have become invasive and of subsequent economic importance. The thousands of undescribed hymenopteran species, including gall formers, are of particular concern as a potential for more invasions in other parts of the world.

Given the importance of gall-forming hymenopterans as current and future pests of *Eucalyptus*, knowledge on the distribution and diversity of current invasive gall wasps as well as an understanding of the challenges associated with new introductions will be of value to inform responses to these threats. In this review we (i) provide a broad overview of the diversity and distributions of invasive gall-formers that have infested *Eucalyptus* established as non-natives for plantation forestry, and (ii) discuss the challenges that time lag preceding species description, identification of cryptic species and population level variation creates, in the context of increasing numbers of gall-formers appearing in *Eucalyptus* plantations.

Gall-forming Hymenoptera of *Eucalyptus*

Of the native Australian insects, the Hymenoptera is the most diverse order (Naumann, 1991) and is represented by gall-formers, seed eaters, predators, pollinators, parasitoids and social insects (Austin *et al.*, 2004). The Australian Hymenoptera are estimated to comprise

44 000 species, of which about 8 000 have been described, and where the Chalcidoidea is a major, biologically diverse but little studied component (Austin *et al.*, 2004; La Salle, 2005).

Many Chalcidoidea are associated with galls, either as gall-formers, inquilines or parasitoids. Chalcidoidea on *Eucalyptus* represent a notable Australian plant-associated radiation (Cranston, 2017), although there are a few *Eucalyptus* species endemic to New Guinea and Indonesia (Hill & Johnson, 2000). In contrast, in the northern hemisphere hymenopteran gall-formers largely belong to the Cynipidae, while in Australia most are in the Eulophidae and Pteromalidae (Austin *et al.*, 2004).

Within the Eulophidae, gall-formation has evolved twice, once in the Opheliminae and once in the Tetrastichinae (La Salle, 2005). Both subfamilies include Australian gall-formers (Austin *et al.*, 2004). The Tetrastichinae is a large subfamily with > 100 genera and approximately 1 600 species (Noyes, 2003), of which 20 described species in 12 genera are either gall-inducers, parasitoids or inquilines (Noyes, 2003). The gall-forming species have many different hosts, but a large proportion infest *Eucalyptus* species (Austin *et al.*, 2004; La Salle, 2005). Approximately 50 species of *Ophelimus* have been described and the hosts of these gall-formers are exclusively *Eucalyptus* species (Withers *et al.*, 2000; Austin *et al.*, 2004).

Identification and classification of gall-forming Hymenoptera is complex, resulting in numerous instances of misidentification, misunderstanding of trophic levels, and reclassification. Girault (1913) and later Bouček (1988) described gall-forming Hymenoptera in the Tetrastichinae associated with *Eucalyptus*, but also highlighted the need for further revision of the group. More recently the Tetrastichinae were placed in two groups, the Australian gall-formers associated with Myrtaceae, and the Neotropical species associated with galls as parasitoids, inquilines or gall-inducers (La Salle, 2005). The Australian gall-formers can be further divided into three groups based on the host parts galled, namely leaf gall-formers (e.g. *Epichrysocharis*), seed gall-formers (e.g. *Quadrastichodella*) and leaf and stem gall-formers (e.g. *Oncastichus* and *Leptocybe*) (La Salle, 2005).

Taxonomists have suggested that relatively few of the speciose Australian gall-forming Hymenoptera have been described, including those from *Eucalyptus* (Bouček, 1988; La Salle, 2005; Kim, 2008). In fact, many were only described after they became invasive elsewhere in the world (Figure 1).

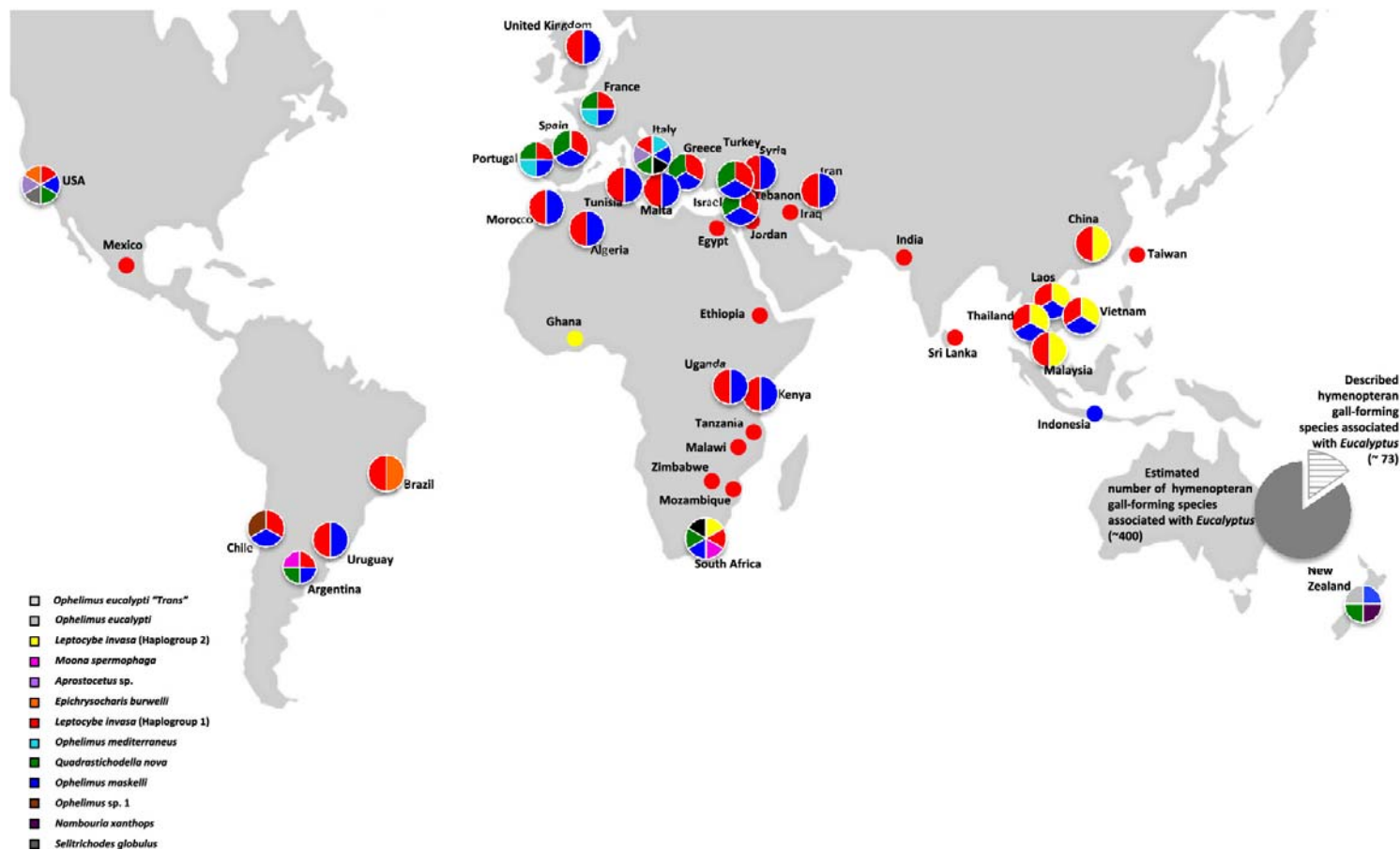


Figure 1. The current global distribution of invasive (non-native to Australia) true gall-forming wasps associated with *Eucalyptus*. The colours of the dots or segments of the pie charts relate to colours assigned to each hymenopteran species used in this Figure. *Megastigmus eucalypti* is not indicated as it has been recorded from a region and not a specific country. The dark grey pie chart in Australia indicates the estimated number of Australian hymenopteran gall-forming species associated with *Eucalyptus*; the shaded piece indicates the number of described hymenopteran gall-forming species associated with *Eucalyptus*.

No hymenopteran gall-formers are significant pests of *Eucalyptus* grown commercially in Australia (see Nahrung & Swain, 2015), with only three undescribed species (*Ditripinotella* sp. and two *Ophelimus* spp.) mentioned in forest industry guides (Elliott *et al.*, 1998; Carnegie *et al.*, 2008). Interestingly, none of these species are among the invasive species outside Australia. Indeed, only two of sixteen hymenopteran species recorded as invasive *Eucalyptus* gallers were known from Australia before they were encountered outside that country (Girault, 1915; Timberlake, 1957). These were *Quadrastichodella nova* and *Megastigmus eucalypti*. Only five species (*Leptocybe invasa* Haplogroup 2, *Ophelimus maskelli*, *O. mediterraneus*, *Megastigmus zebrinus* and *Moona spermophaga*) have been found in Australia since they became invasive elsewhere in the world. Two of these (*M. eucalypti* and *M. zebrinus*) have subsequently been reclassified: *M. eucalypti* was likely misidentified and *M. zebrinus* was reclassified as a parasitoid (Le *et al.*, 2018).

In this review, the Australian Plant Pest Database (APPD), which collates vouchered specimens from herbaria and insect collections across Australia, was searched for records of phytophagous Hymenoptera associated with *Eucalyptus* (*Eucalyptus* and *Corymbia*). While there were > 16 450 records of insects identified to family level associated with *Eucalyptus*, only 45 (< 0.3 %) were Eulophidae and Torymidae. Of the ~ 1 030 species of insects recorded in the APPD associated with *Eucalyptus*, only two reside in these families. One of them (*Chrysonotomyia mira*) is a likely parasitoid and the other, *Megastigmus eucalypti*, is of uncertain galling/parasitoid/invasive status (see Le *et al.*, 2018). Such a paucity of records reflects the lack of economic importance of this group in Australia and that knowledge on the identity, diversity and biology of the endemic *Eucalyptus* gall-forming Hymenoptera is sparse.

Kim (2008) lists 23 species of Tetrastichinae thought to be gall-formers of *Eucalyptus*. Predominant genera in this list include species of *Aprostocetus*, *Epichrysocharis* and *Quadrastichodella* (Kim, 2008), all of which are represented among the invasive species. Most *Quadrastichodella* spp. are thought to be seed-gallers whereas it is not known which host organs are galled by *Aprostocetus* spp. (Kim, 2008).

Invasion history and biology

Sixteen Hymenoptera species have been recorded as invasive alien *Eucalyptus* gall formers of likely Australian origin (Table 1). Two of these (*L. invasa* Haplogroup 2, and *O. eucalypti* 'Trans') were discovered as cryptic species within the first invasive populations recorded of that species. Three (*Megastigmus eucalypti*, *M. zebrinus*, *Leprosa milga*) were later considered more likely to be parasitoid species or misidentifications of parasitoid species. Currently, there are thirteen invasive Australian hymenopteran gall formers that have become established across >40 countries. Of these, 77% became invasive in the last two decades, none of which were known from their native range before they were encountered as alien invasives.

A comparison of the number of recorded invasive species associated with *Eucalyptus* per continent indicates that Europe, North America and Africa have the greatest diversity. Ten of the 13 species (in differing combinations of 5-6 per region) have been recorded from these continents (Figure 1). *Leptocybe invasa* Haplogroup A, *Quadrastichodella nova* and *Ophelimus maskelli* are the most widespread species, and have been recorded as invasive on four continents. Most countries recorded three or less (overall average of 2.3 ± 0.3 across 44 eucalypt-growing countries) invasive gall-forming species on *Eucalyptus*, with the exception of Argentina, France, Italy, New Zealand, Portugal, South Africa and the USA (Figure 1, 2), which averaged 4.9 ± 0.3 species per country. However, of these, New Zealand and the USA had three and two unique species, respectively. Just over half of the invasive species have spread to more than one country. Only *Aprostocetus* sp., *Nambouria xanthops*, *Ophelimus* sp. 1 and *Selitrichodes globulus* are currently present adventively only in a single country, and the distribution of the two cryptic species of *O. eucalypti* is unclear (Figure 1, 2). Of the seven species that have spread to more than one continent, two were first detected in Europe and two in the USA.

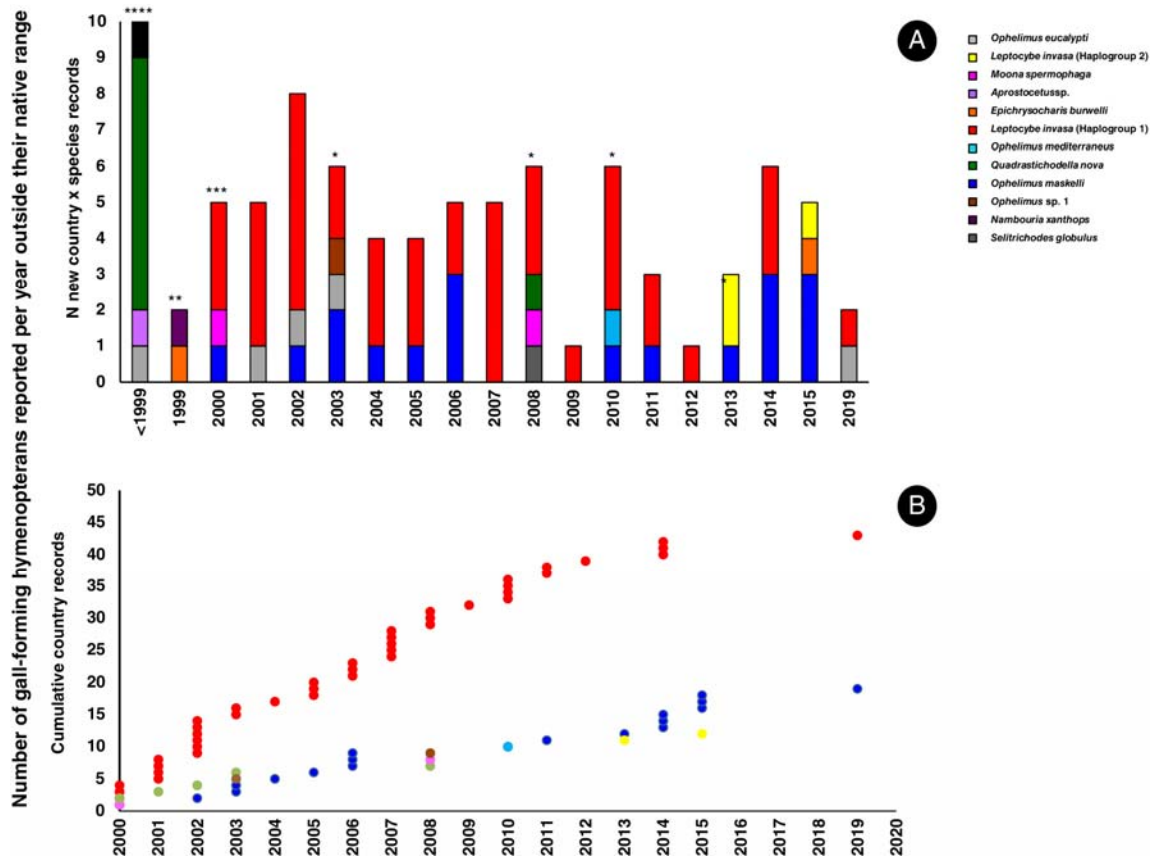


Figure 2. The number of gall-forming wasps recorded for a specific year outside their native range. (A) New species-country record prior to and since 1999 of the thirteen invasive primary gall-forming Hymenoptera on *Eucalyptus*. Years in which new species became invasive are marked with an asterix. (B) Cumulative records per country of gall-forming hymenopterans reported per year outside their native range.

There was no relationship between the time of detection of first invasive and number of countries invaded by a particular species (Spearman rank correlation, $\rho = -0.42$, $P = 0.46$). On average, there were 4.2 new species-country records per year between 1999 and 2015: 0.6 new invasive species per year (representing movement from Australia, or from undiscovered invasive populations elsewhere), and 3.6 new country records for all previously recorded invasive species (representing movement from bridgehead populations, or additional introductions from Australia) (Figure 2B). For example, following the simultaneous discovery of *L. invasa*, in Europe and the Middle East, 77% of subsequent first records were from countries neighbouring previously-infected countries within geographic regions (Appendix Table 1). The first three records in Asia (India 2001, Vietnam 2002, Thailand 2004) were non-neighbouring, but all eight new country records thereafter were in countries neighbouring at least one of these. Likewise, spread within South America

	Australia	Algeria	Argentina	Brazil	Canada	China	China	Egypt	Ethiopia	France	Ghana	Greece	India	Indonesia	Iran	Iraq	Israel	Italy	Jordan	Korea	Laos	Lebanon	Malawi	Malaysia	Mali	Mexico	Morocco	Mozambique	Netherlands	Panama	Poland	South Africa	Spain	Sri Lanka	Syria	Taiwan	Tanzania	Thailand	Tunisia	Turkey	United Kingdom	Uruguay	USA	Vietnam	Zimbabwe	No. countries/ species		
<i>Ophiurus muskandi</i>	A	2010	2013			2014				2005	2002		2015	2015	**		2003	2000			**						2011				2006	2015	2003	2014				2006	2004	**	2006		2014	2015	24			
<i>Ophiurus eucalypti</i>													2019	2015														1987	1991															2				
<i>Ophiurus eucalypti</i> "Travis"																																													1			
<i>Ophiurus rigidiorum</i>						2003																																							1			
<i>Ophiurus mediterraneus</i>	A									2010																																				3		
<i>Selichochius globulix</i>																																													2008	1		
<i>Miona spinosiplaga</i> ^b	A		2000																																										2			
<i>Apariticulus</i> sp.																																														1998	1	
<i>Epichrysichne burnsi</i>																																															1999	3
<i>Quadrantic hoodellana</i>	B		1975										1979																																	1977	11	
<i>Leprona mita</i> ^b																																															2004	2
<i>Leptocybe invasa</i> (haplogroup 1)		2002	2003	2007	2007	2010	2007	2001	2002	2005			2004	2001	2010	2005	2000	2005	2001	2002	2002	2002	2002	2002	2002	2002	2002	2011		2014	2003	2007	2003	2010	2001	2010	2005	2004	2004	2002	2006	2011	2002	2007	2007	4		
<i>Leptocybe invasa</i> (haplogroup 2)	A										2013										2013																										7	
<i>Nembotria xanthope</i>																													1990																		1	
<i>Megastigma eucalypti</i> ^c	B																																														2000	1
<i>Megastigma zebrina</i>	A																																														2008	1
Number of recorded species per country ^d	2	4	2	1	3	2	1	1	4	1	3	1	1	2	1	3	5	1	2	2	2	1	1	2	2	1	2	5	1	5	5	3	1	2	1	1	4	2	3	2	2	2	6	3	1			

^aNative range – recorded in Australia before (B) or after (A) discovery overseas.

^bGallers associated with *Eucalyptus* seed capsules.

^cUnreliable record.

^dNumber of recorded species per country excludes non-phytophagous species.

References: Anonymous, 2006 (loc.cit. Branco *et al.*, 2016); Flock, 1957; Bain, 1977; Beardsley & Perreira, 2000; Schauff & Garrison, 2000; Berry & Withers, 2002; Kim *et al.*, 2005; Döganlar & Mendel, 2007; Kim & La Salle, 2008; Wingfield *et al.*, 2008; La Salle *et al.*, 2009; Branco *et al.*, 2016; Mansfield, 2016; Dittrich-Schröder *et al.*, 2018; <https://bicep.net.au> (accessed January 2020); <https://www.cabi.org/isc/search/> (accessed January 2020) <https://app.oxfordabstracts.com/events/691/program-app/submission/92664> (accessed May 2020).

Table 1. The invasive *Eucalyptus* gall-forming wasp species and their current global distribution. Where available, dates of the first report for each species and country are indicated. The relatedness of the gall-formers is indicated by a phylogenetic tree. Species within the same subfamily are indicated in the same colour. The subfamilies Euderinae and Tetrastichinae are members of the Eulophidae, the subfamily Ormocerinae belongs within the Pteromalidae and the subfamily Megastigminae belongs within the Torymidae. Grouping within the Tetrastichinae at the genus level is arbitrary. Grey stippling is used to indicate non-phytophagous species. Colours used in this table correspond to the colours used in Figs 1 and 2

occurred contiguously following its discovery in Brazil (2007), with a separate discovery in 2008 in USA. Thus, apart from inter-regional ‘jumps’, spread seems to have mainly occurred from previously-invaded countries, a notion supported by scenario modelling in Dittrich-Schröder *et al.* (2018). This is emphasised by the fact that, even with the disparate early records, there is no evidence of more than one introduction of *L. invasa sensu stricto* from Australia (Dittrich-Schröder *et al.* 2018).

Gall-forming invasive species recorded on non-native plantation-grown *Eucalyptus* are described below. In most instances there is a paucity of information regarding their biology, distribution and hosts due to the cryptic and complex life cycles of gall-forming Hymenoptera (Csóka *et al.*, 2017). Some genera include gall-formers that have become invasive, as well as species that have not yet become invasive. Comparisons of non-native gall-formers associated with *Eucalyptus* plantations globally have suggested that a combination of adequate available host plants, specialised biological traits (e.g. development within the protected micro-environment of the gall, parthogenesis,) as well as a lack of parasitoids could explain the success of some invasive gall-forming species (Csóka *et al.*, 2017).

Invasive Species

***Aprostocetus* Westwood sp. (Eulophidae, Tetrastichinae)**

The first report of *Aprostocetus* sp. forming galls on *Eucalyptus* outside Australia was from Oahu, Hawaii in 1996 (Beardsley & Perreira, 2000). *Aprostocetus* is one of the largest genera in the Chalcidoidea and this particular wasp was thought to be a new and undescribed species (Beardsley & Perreira, 2000; Noyes, 2003). The galls are “small, elongate and woody” on the midribs of leaves of *Corymbia citriodora* (Beardsley & Perreira, 2000). Based on agreement of morphological characters between Hawaiian and Californian specimens, this species was likely previously also reported from California (Beardsley & Perreira, 2000), while records of gall-forming *Aprostocetus* sp. from Italy were probably misidentifications of *Leptochoye invasa* (Protasov *et al.*, 2007). Eight *Aprostocetus* species have been associated with galls on *Eucalyptus* (*A. auriflavus*, *A. causalis*, *A. consobrinus*, *A. margiscutum*, *A. quinquegrimalaculae*, *A. secus*, *A. tricolor* and *A. nigrithorax*) of which only the latter has been confirmed as a gall-former (Noyes, 2003; Yang *et al.*, 2014). *Aprostocetus* spp. are

additionally reported emerging from eucalypt galls as parasitoids of *Leptocybe* in various countries (Le *et al.*, 2018).

***Epichrysocharis burwelli* Schauff (Eulophidae, Tetrastichinae)**

Epichrysocharis burwelli was discovered emerging from “blister-like” galls on the dorsal and ventral leaf surfaces of *Corymbia citriodora* in California in 1999 (Schauff & Garrison, 2000). It was placed in a genus with three other species: *E. fusca*, *E. nigriventris* and *E. aligherini*, all of which are leaf gallers of *Eucalyptus* (Bouček, 1988; Schauff & Garrison, 2000). Its presence was discovered in Brazil in 2003 when a reduction of up to 80% in essential oil production occurred (Santana & Anjos, 2007). In June 2015 this species, along with the parasitoid *Closterocerus* sp., were reported for the first time in Europe (Portugal) (Franco *et al.*, 2016). Little is known about the biology of *Epichrysocharis* and it is likely that there are several additional undescribed species (Schauff & Garrison, 2000). *Epichrysocharis burwelli* has not been found in Australia.

***Leprosa milga* Kim & La Salle (Eulophidae, Tetrastichinae)**

Leprosa milga was first reported galling the seed capsules of *Eucalyptus* in South Africa, and subsequently became established in Italy (Kim & La Salle, 2008). To date, no specimens of *Leprosa milga* have been found in Australia, but it is assumed that Australia is its country of origin due to its “ambiguous relationship” to the Australian seed gallers *Quadrastichodella nova* and *Moona spermophaga* (Kim & La Salle, 2008). Although *L. milga* was observed probing seed capsules of *E. camaldulensis* in laboratory trials, no gall formation or wasp development occurred. Consequently, this species may be a parasitoid of *Quadrastichodella nova* rather than a primary galler (Klein *et al.*, 2015). Because it may not be a primary galler, it is excluded from invasion pattern descriptions presented in Figure 1-2.

***Leptocybe* Fisher & La Salle (species complex) (Eulophidae, Tetrastichinae)**

Leptocybe invasa was discovered in Israel in 2000 and described in 2004 (Mendel *et al.*, 2004) in a monotypic genus. This insect has spread rapidly and been recorded from all continents where *Eucalyptus* is planted, although is notably absent from New Zealand. *Leptocybe invasa* is one of the most threatening insect pests of non-native *Eucalyptus* plantation forestry (Mendel *et al.*, 2004).

Leptocybe invasa is thought to have a thelytokous mode of reproduction (Mendel *et al.*, 2004) although rare male specimens have been identified from a few countries (Döganlar, 2004; Chen *et al.*, 2009; Ankita & Poorani, 2009; Akhtar *et al.*, 2012; Sangtongpraow *et al.*, 2011). It forms galls on the midribs, petioles and stems of a number of *Eucalyptus* species in the sections *Exertaria*, *Latoangulata* and *Maidenaria*, where it affects the young growth (Mendel *et al.*, 2004). In severe instances, extensive gall formation may lead to stunted and deformed trees as well as tree death (Mendel *et al.*, 2004). Current feasible control measures include the breeding of tolerant/resistant planting stock as well as biological control (Dittrich-Schröder *et al.*, 2012). Biological control agents, including *Quadrastichus mendeli*, *Selitrichodes kryceri*, *S. neseri*, *Megastigmus lawsoni* and *M. zvimendeli*, have shown much promise in controlling *L. invasa* populations (Kim *et al.*, 2008; Dittrich-Schröder *et al.*, 2014, Mendel *et al.*, 2017; Le *et al.*, 2018).

Specimens collected in Australia in the early 2000's during surveys for potential biological control agents by Mendel and Blumberg (Kim, 2008) suggested the presence of a *Leptocybe* species complex that included at least six species, as well as two species initially described as *Aprostocetus*. Furthermore, Kim (2008) divided the *Leptocybe* species into two main groups based on slight differences of gall morphology, noting that subtle differences in wasp morphology alone made it difficult to separate specimens.

Work by Nugnes *et al.* (2015) and Dittrich-Schröder *et al.* (2018), using molecular markers revealed the presence of two lineages among invasive *Leptocybe* populations, referred to as Haplogroup 1 and Haplogroup 2. Haplogroup 1 has a more global distribution and refers to the first lineage (*L. invasa sensu stricto*) observed in the invasive range (Dittrich-Schröder *et al.*, 2018). Haplogroup 2 has a limited distribution and is predominantly present in Asia and some parts of Africa (Dittrich-Schröder *et al.*, 2018). Haplogroup 1 is thought to be thelytokous and Haplogroup 2 facultatively sexual (Nugnes *et al.*, 2015). Only the latter haplogroup has been found in Australia, despite extensive surveys over several years (Dittrich-Schröder *et al.*, 2018; Otieno *et al.*, 2019). Because of its economic importance *L. invasa* is the best known of the invasive gall-formers, with substantial work having been conducted on its development rates (Mendel *et al.*, 2004), natural enemies (Doğanlar and Hassan, 2010; Kelly *et al.*, 2012; Kim, 2008; Huang *et al.*, 2018) host plant interactions (Dittrich-Schröder *et al.*, 2012; Li *et al.*, 2017) and endosymbionts (Nugnes *et al.*, 2015).

***Megastigmus eucalypti* Girault (Megastigmidae, Megastigminae)**

This species was first described by Girault (1915) from three female Australian specimens that emerged from *Eucalyptus* galls collected in Melbourne, Victoria in 1910 (Girault, 1915). Doğanlar & Hassan (2010) provided a taxonomic revision of various species of *Megastigmus* but only compared morphological characteristics of *M. eucalypti* with other congeners, and no further biological information is available. Records of *M. eucalypti* as an invasive phytophagous species in Paine *et al.* (2011) and Mansfield (2016) arose from the report of Viggiani *et al.* (2002) of a species “probably *Megastigmus eucalypti*” associated with eucalypt galls “similar to those produced by *Aprostocetus* sp.” in Italy. However, the report by Viggiani *et al.* (2002) most likely referred to a misidentification of *L. invasa* as *Aprostocetus*, and may have intended to suggest the *Megastigmus* sp. as a parasitoid associated with those galls (see Protasov *et al.* 2008).

Le *et al.* (2018) considered it likely to be the same species later described as *M. leptocybus* (Doğanlar and Hassan 2010, 2013), a parasitoid of *L. invasa* in the Mediterranean (Protasov *et al.*, 2008, Mendel *et al.*, 2017). There is consequently no clear evidence that *M. eucalypti* is either a primary galler or an invasive species. This species is not considered a gall-inducing invasive hymenopteran in the present review and we have not included it in invasion pattern descriptions (Figure 1-2, Table 1).

***Megastigmus zebrinus* Grissell (Megastigmidae, Megastigminae)**

Like *M. eucalypti* and *L. milga*, records of *M. zebrinus* as an invasive primary galler are likely erroneous. *Megastigmus zebrinus* was described in 2006 as a primary gall-former on *E. camaldulensis* and *Syzygium cordatum* seeds in South Africa (Grissell, 2006). It was presumed to have an Australian origin (Grissell, 2006), but has also been recorded as a larval-pupal ectoparasitoid in *Leptocybe* spp. galls in South Africa, Thailand, and Argentina (Huang *et al.*, 2018). It did not induce galls on *E. camaldulensis* in laboratory trials and is likely to be a parasitoid rather than a primary galler (Klein *et al.*, 2015). *Megastigmus zebrinus* has been recorded from Queensland, Australia (Grissell, 2006). Confusion regarding the ecology (gall-former, parasitoid, inquiline) of the Australian eucalypt-gall associated *Megastigmus* are currently being addressed in a revision of the genus (Le *et al.*, 2020). Because *M. zebrinus* may not be a primary galler, it has been excluded from invasion pattern descriptions (Figure 1-2, Table 1).

***Moona spermophaga* Kim & La Salle (Eulophidae, Tetrastichinae)**

The first record of *Moona spermophaga* outside Australia was in Argentina in 2000, as an undescribed species (Kim *et al.*, 2005). *Moona spermophaga* forms minute galls on *Eucalyptus* and *Corymbia* seed capsules with galls usually having 2 – 4 chambers, and adult wasps emerging over several months (Kim *et al.*, 2005; Kim & La Salle, 2008). Gall-formation in seed capsules is relatively rare for insects in the family Eulophidae (Kim *et al.*, 2005). *Moona spermophaga* was first detected in *Corymbia maculata* seedlots as part of a shipment sent by the Australian Tree Seed Centre (ATSC) for the establishment of *Eucalyptus* plantations in Argentina (Kim *et al.*, 2005). Later this pest was also collected from *Corymbia citriodora* (Kim *et al.*, 2005). It was these discoveries that prompted its taxonomic description (Kim *et al.*, 2005).

Moona spermophaga was observed emerging from seeds that had been fumigated and sealed before shipment (Kim *et al.*, 2005). Fumigation proved to be ineffective against the pupal stage of *M. spermophaga* with this life stage remaining dormant within the gall on the seeds for up to 10 years (Kim *et al.*, 2005). Little is known about the respiration biology of *M. spermophaga* enabling it to remain dormant in *C. maculata* seeds for extended periods and its tolerance to carbon dioxide fumigation (Kim *et al.*, 2005). It is also reported from South Africa (Kim & La Salle, 2008).

***Nambouria xanthops* Berry & Withers (Pteromalidae, Ormocerinae)**

Nambouria xanthops was discovered in New Zealand in October 1999 and described in 2002 (Berry *et al.*, 2002). Wasps in this genus are predominantly plant gall-formers with a subgroup inducing galls on seeds and fruits (Berry *et al.*, 2002). Host plants include species within the Casuarinaceae, Celastraceae, Fabaceae and Myrtaceae (Bouček, 1998). *Nambouria xanthops* has been recorded from three *Eucalyptus* species (*E. nicholii*, *E. glaucescens*, *E. cinerea*), however, *E. nicholii* is the preferred host (Berry *et al.*, 2002). This species induces two morphologically different gall types (Berry *et al.*, 2002). One is an oblong gall with an uneven surface, visible on both dorsal and ventral leaf surface. The other gall type is flat and referred to as a “pit gall”. Its geographical distribution is central to southern Auckland (Berry *et al.*, 2002).

***Ophelimus migdanorum* Molina-Mercader (Eulophidae, Euderinae)**

Ophelimus migdanorum was first detected in Chile in February 2003 by the Agricultural and Livestock Services (Servicio Agrocola y Ganadero) in Region V (Informativo Fitosanitario Forestal, 2006). Subsequently, it was also detected in regions VI, VII and the Metropolitana (Informativo Fitosanitario Forestal, 2006). The predominant *Eucalyptus* species affected by *Ophelimus migdanorum* include *E. globulus* and *E. camaldulensis* (Informativo Fitosanitario Forestal, 2006). In severe instances gall formation by *Ophelimus* species may lead to the premature dropping of leaves (Informativo Fitosanitario Forestal, 2006; Molin-Mercader *et al.*, 2019).

***Ophelimus mediterraneus* Borowiec & Burks (Eulophidae, Euderinae)**

Ophelimus mediterraneus was first observed in 2010 in France (Borowiec *et al.*, 2012), with subsequent surveys revealing its presence in Italy and Portugal (Branco *et al.*, 2016), as well as Australia (Borowiec *et al.*, 2019). Galls caused by this species are smaller but generally similar to those of *O. maskelli* (Branco *et al.*, 2016). Differences include the brown-grey colour of the galls, the smoother surface texture as well as the orientations of gall-formation in relation to the leaf surface (Branco *et al.*, 2016). *Ophelimus mediterraneus* emerges between spring and summer. They are univoltine and have an approximate life span of two weeks, although this is greatly reduced when high temperatures occur (Branco *et al.*, 2016; Borowiec *et al.*, 2019). Hosts include *Eucalyptus* species from the Maidenaria section with severe galling occurring on *Eucalyptus globulus* (Branco *et al.*, 2016) and *E. gunnii* (Borowiec *et al.*, 2019). No parasitoids have been observed emerging from *O. mediterraneus* galls in France, Italy or Portugal (Borowiec *et al.*, 2019).

***Ophelimus eucalypti* (Gahan) (species complex) (Eulophidae, Euderinae)**

Ophelimus eucalypti has recently been confirmed to comprise two cryptic invasive lineages ("Trans." and "Maid.") (Borowiec *et al.*, 2019). Both cryptic invasive lineages are found in New Zealand (Borowiec *et al.*, 2019) and were previously recognised as two biotypes (Withers *et al.*, 2000; Protosov *et al.*, 2007). The first, *Ophelimus eucalypti sensu lato*, was reported from New Zealand in 1921 on *E. globulus* (described as *Rhichnopeltella eucalypti* Gahan in 1922). In 1987 *O. eucalypti* was reported galling *Eucalyptus botryoides* (Raman & Withers, 2003) and this discovery likely represents the undescribed cryptic 'Trans' lineage

(Withers *et al.*, 2000; Protasov *et al.*, 2007; Borowiec *et al.*, 2019). The ‘Maid’ lineage is uniparental (thelytokous) and attacks *Eucalyptus* in the section *Maidenaria*, while the ‘Trans’ lineage is biparental and male biased (Withers *et al.*, 2000) and attacks *Eucalyptus* in the section *Tranversaria* (Protasov *et al.*, 2007).

Galls of the *O. eucalypti* ‘Maid’ are formed on leaf midribs and shoot axes (Raman & Withers, 2003). Gall morphology is different between males and females and determined by the development of male or female specimens within the gall (Raman & Withers, 2003). Galls containing female wasps develop on both dorsal and ventral leaf surface and are between 2-3 mm in size, whereas galls containing male specimens are smaller (1-1.15 mm) and “non-protruding” (Raman & Withers, 2003). The duration of its life cycle is approximately six months resulting in two generations per year (Withers *et al.*, 2000).

Ophelimus maskelli was confused with *O. eucalypti* in Europe (Protasov *et al.*, 2007; Borowiec *et al.*, 2019) so it is unclear whether either of the *O. eucalypti* biotypes are invasive outside New Zealand. *Ophelimus eucalypti* is only listed in New Zealand in recent reviews by Hurley *et al.*, (2016) and Mansfield (2016; both biotypes) and it has recently caused serious damage to *E. urophylla* and hybrids of this species with *E. grandis* in Sumatra (Indonesia) although the biotype has not been determined for that invasion (M. J. Wingfield, unpublished). Neither biotype has been recorded from Australia.

***Ophelimus maskelli* (Ashmead) (Eulophidae, Euderinae)**

Ophelimus maskelli was described as *Pteroptrix maskelli* Ashmead in 1900 from specimens purportedly from New Zealand. The species now recognised as *O. maskelli* was first reported (incorrectly) as *O. eucalypti* from Italy in 2000 (Arzone & Alma, 2000; Protasov *et al.*, 2007; Mansfield, 2016). As *Eucalyptus* are non-native to New Zealand, it is likely that the origin of this species is Australia (Huber *et al.*, 2006; Mendel *et al.*, 2007; Durand *et al.*, 2011).

Ophelimus maskelli is a uniparental species and oviposits near the petiole on the leaf blade of immature leaves on the lower canopy of the tree (Protasov *et al.*, 2007; Branco *et al.*, 2016). Galls initiated by *O. maskelli* are blister-like with a single individual per gall and leaves may have galls initiated by different aged wasps (Protasov *et al.*, 2007). Three generations per year have been recorded in Israel (Protasov *et al.*, 2007). Exposure of the galls to sun changes the colour from green to ranges of purple and heavy galling may cause leaves to drop prematurely (Protasov *et al.*, 2007). Its current adventive range spans 25

countries, making it the second-most widely distributed invasive gall-former on *Eucalyptus*. Three biological control agents, *Closterocerus chamaeleon* Girault, *Stethynium ophelimi* Huber and *Stethynium brevipositor* Huber, have been unintentionally introduced to the Mediterranean region as well as Africa to control *O. maskelli* (Bush *et al.*, 2016; Wondafrash *et al.*, 2020).

***Quadrastichodella nova* Girault (Eulophidae, Tetrastichinae)**

Quadrastichodella nova was described from Australia 22 years before it became invasive outside that country. This is the only confirmed gall-former to be described from its native habitat before becoming invasive, although it was described as a new species when found adventively. The first report of *Q. nova* as an alien invasive was from California in 1957 (Flock, 1957) where it was described as *Flockiella eucalypti* Timberlake, and later reduced to synonymy with *Q. nova*. It forms galls on the seed capsules of a number of *Eucalyptus* and *Corymbia* species (Kim & La Salle, 2008). Galls in the seed capsules are formed as a result of the female ovipositing into the flower buds (Kim *et al.*, 2005). These galls then appear similar to the seeds observed in the seed capsule (Kim *et al.*, 2005).

The genus *Quadrastichodella* includes 10 species (Ikeda, 1999) of which nine are native to Australia (*Q. aenea*, *Q. bella*, *Q. boudiennyi*, *Q. candida*, *Q. cyaneivirdis*, *Q. neglectae*, *Q. nova* and *Q. pilosa*) (Noyes, 2003). *Quadrastichodella pilosa* is purported to be native to New Zealand (Ikeda, 1999). Species of *Quadrastichodella* have been recorded from Europe, North and South America, and South Africa and are thought to have been accidentally introduced with *Eucalyptus* seeds (Timberlake, 1957; Flock 1957; Dumbleton, 1971, Graham, 1987; Ikeda, 1999). The genus *Quadrastichodella* includes seed galls of various *Eucalyptus* species. For example, *Q. nova* has been recorded from *Eucalyptus umbellatae* and *E. resinifera* (Timberlake 1957, Flock 1957, Dumbleton, 1971); *Q. neglecta* from *E. neglecta* (Ikeda, 1999) and *Q. pilosa* from an unknown *Eucalyptus* species (Ikeda, 1999).

***Selitrichodes globulus* La Salle & Gates (Eulophidae, Tetrastichinae)**

Selitrichodes globulus was first discovered in 2008 in California on *E. globulus* (La Salle *et al.*, 2009). Galls induced by this insect are multi-chambered and predominant on the branches of the trees causing them to hang (La Salle *et al.*, 2009). As with many other gall-forming

species associated with *Eucalyptus* species, *S. globulus* has not been recorded in Australia, although it is assumed to have originated in that country (La Salle *et al.*, 2009).

Challenges in dealing with identification of invasive gall-formers on *Eucalyptus*

The ability to accurately identify and detect invasive *Eucalyptus* gall-forming insects is imperative for management. Yet these tasks are hampered by a shortage of experienced taxonomists (Valan *et al.*, 2019). As a result, research concerning the ecology, evolution, population monitoring, global movement and management (Wäldchen & Mäder, 2018), is severely hampered. Some of the key challenges with the taxonomy and identification are discussed here.

Taxonomy and Identification

Identification of insects can be difficult due to considerable species diversity and within-species variation (e.g. sex, colour morph, life stage) (Valan *et al.*, 2019). Furthermore, identification can be challenging if insects have few or minute morphologically distinguishable characteristics, which is often the case with gall-forming wasps (Austin *et al.*, 2004). Due to phenotypic variation within a single species it is not always possible to base identifications solely on morphology (Hebert *et al.*, 2003). The challenges associated with the shortage of taxonomists and the growing discovery of new species have highlighted the need for alternative methods of species identification (Jörger & Schrödl, 2013).

Molecular data can be combined effectively with traditional taxonomic data by the establishment of databases or “reference sequence libraries” (Hajibabaei *et al.*, 2005), which contain sequences of specimens that have been identified based on morphology. An example of this is the Forest Insect Mitochondrial Database (FIMT) (<http://fimt.bi.up.ac.za>) where the identity of specimens can be established by linking or matching to sequences within the database to identified species. The advantage of DNA barcoding is that it can be applied to a range of organisms rapidly, unlike traditional taxonomy that requires expert knowledge on morphological characteristics of individual groups (Hajibabaei *et al.*, 2005).

The challenges of morphological identification and the use of DNA barcoding as a means to resolve taxonomic uncertainty can be illustrated by an example using *Leptocybe invasa* Haplogroup 1 and Haplogroup 2. *Leptocybe invasa* Haplogroup 1 was present in South

African *Eucalyptus* plantations since 2007. Morphologically similar galls were observed in a *Eucalyptus* plantation in 2015. Dissection of these galls indicated the presence of specimens that were morphologically similar to *L. invasa* Haplogroup 1. However, when DNA barcoding was employed to confirm this preliminary identification, the specimens were subsequently identified as *L. invasa* Haplogroup 2 (Dittrich-Schröder *et al.*, 2018). To date no robust morphological characteristics are available to separate the two haplogroups of *L. invasa*. Current identification of these two haplogroups relies on the use of molecular techniques, yet it is likely that the two lineages differ in biological aspects influencing their host and natural enemy interactions, susceptibility to control and invasion dynamics.

Understanding trophic-level associations in gall-forming insects results in additional confusion. The guild status of three species initially considered primary invasive gall-formers were later revised as misidentifications (in the case of *M. eucalypti*) or were likely parasitoids mistaken as primary gallers (*M. zebrinus* and *L. milga*). These highlight the need for biological as well as taxonomic resolution in determining species identities and roles. Species identification may be difficult to formalise accurately and may take years to resolve. For example, although the genus *Ophelimus* contains more than 50 species, to date only an incomplete draft key, constructed by Girault (1913) is available to separate these species (Protasov *et al.*, 2007). As a result, *Ophelimus maskelli* and *O. eucalypti* are commonly misidentified, but can be separated based on the presence or absence of a single seta (Protasov *et al.*, 2007). Completion or revision of the available key is hampered by the presence of a single, damaged lectotype specimen (Protasov *et al.*, 2007).

A result of the challenge of species identification and description is the time lag associated with the first report of a new species and the subsequent publication of the taxonomic description. Species are often described for the first time once they become invasive and are of economic concern, as is evident by the invasive gall-formers associated with *Eucalyptus*. Invasive hymenopteran species associated with *Eucalyptus* were described within one (e.g. *O. eucalypti sensu lato*; *E. burwelli*, *S. globulus*) to sixteen (*O. migdanorum*) years after their first detection (Schauff & Garrison, 2000; La Salle *et al.*, 2009; Borowiec *et al.*, 2012; Borowiec *et al.*, 2019; Molina-Mercader *et al.*, 2019). Even the two that were described prior to detection as invasive species (*Q. nova* and *O. maskelli*) were challenged with various taxonomical difficulties (see above) (Flock, 1957; Huber *et al.*, 2006).

Cryptic species and population level variation

Leptocybe invasa was first described 19 years ago and only recently a second lineage (cryptic species) of the insect was discovered (Nugnes *et al.*, 2015; Dittrich-Schröder *et al.*, 2018). Similarly, *O. eucalypti* was recently confirmed to represent two cryptic lineages (Borowiec *et al.*, 2019) and it is impossible to say how long the second haplogroup of *O. eucalypti* has been present globally, and in retrospect which lineage was used in studies where two haplotypes occur in sympatry. It is consequently imperative that the time from discovery to description of new species and identification of cryptic species be greatly reduced. This is especially true considering the exponential rate at which new insect pests are recorded annually (1 insect pest species on *Eucalyptus* every 1.4 years in general (Hurley *et al.*, 2016) – and one new hymenopteran gall former every two years since 1999). A further concern regarding this time lag between first detection and species description is that cryptic species are not included in this estimate of the number of invasive gall-forming hymenopterans associated with *Eucalyptus*.

Cryptic species can be defined as “two or more species that have been classified as a single nominal species because they are at least superficially morphologically indistinguishable” (Bickford *et al.*, 2006). In the last decade, research on cryptic species has increased due to the use of DNA sequences to identify specimens (Bickford *et al.*, 2006). As species boundaries can not always be discerned based on morphology, traditional (alpha) taxonomy has limitations, resulting in the number of species often being underestimated (Bickford *et al.*, 2006). Molecular tools are therefore ideal to identify cryptic species (Bickford *et al.*, 2006) and they are likely to impact increasingly on the identification of species.

The inability to recognise cryptic species influences the efficacy of implemented control measures as different species respond differently to control agents (Bickford *et al.*, 2006). This has been seen particularly in studies conducted on entomopathogenic nematodes (Bidochka *et al.*, 2001) and parasitoids (Hafez, 1954) used for biological control (Bickford *et al.*, 2006). Due to the nature of biological control and the specificity of the interaction between pest and biological control agent, unidentified cryptic species, either the pest or biocontrol agent, could decrease the efficacy and success of the programmes (Bickford *et al.*, 2006). For example, the *Eucalyptus* weevil, *Gonipterus scutellatus* (*sensu lato*) has been a pest of *Eucalyptus* species for many years, with varying levels of control by a

mymarid parasitoid, *Anaphes nitens* (Mapondera *et al.*, 2012; Schröder *et al.*, 2020). In the last decade, this weevil has reached pest status in Western Australia where it is also invasive (Mapondera *et al.*, 2012). Recent taxonomic studies suggested that ineffectiveness of the parasitoid in some regions may be due to the presence of cryptic species in the pest genus (Mapondera *et al.*, 2012; Schröder *et al.*, 2020).

Understanding population level variation in insect pests is important to inform management approaches (Janes & Batista, 2016). Information such as the geographic structure of populations, the presence or absence of gene flow between populations and the genetic diversity present within populations are important to consider when selecting appropriate control methods (Janes & Batista, 2016). These measures could inform predictions of the future range of an invasive pest, the possibility of different sub populations interbreeding, the response to breeding of resistant plant material and the emergence and spread of resistance. Admixture between closely related species is important to consider as this may lead to unique genetic diversity within the admixed population, resulting in the ability to invade previously unsuitable environments or overcoming control measures (Garnas *et al.*, 2016).

A recent case study of the invasion pathways and population structure of the *Eucalyptus* gall-forming wasp *Leptocybe invasa*, highlighted the complexity of such invasive introductions (Dittrich-Schröder *et al.*, 2018). This study indicated that the distribution of two global lineages was very different. The dominant, mostly clonal group (Haplogroup 1), originated from a small, unidentified source population, yet became highly invasive and in < 10 years was recorded globally. Haplogroup 2 of the pest had a limited distribution in Asia and parts of Africa, but a greater genetic diversity in the native population. Population genetic analyses indicated the presence of admixture between the two haplogroups in Asia as well as 'bridgehead populations' which could promote admixture between haplogroups resulting in increased genetic diversity (Garnas *et al.*, 2016).

Specimens in collections, either from diverse geographical locations or from different time points, provide a valuable resource to study diversity at the population level. These specimens may, for example, be used to compare the genetic diversity of a native population and the corresponding invasive population (Le Roux *et al.*, 2011; Hornoy *et al.*, 2013) and perhaps more importantly to trace pathways of invasion. Current examples illustrating this point can be found within species of *Ophelimus*, which are spreading

globally. Certainty regarding the various species within this genus is difficult when no molecular data are available for comparison and type specimens can either not be found, as is the case with *O. maskelli* (Protasov *et al.*, 2007), or they are difficult to access from personal collections. In this instance, confusion regarding the different *Ophelimus* species is best resolved using molecular data (e.g. Borowiec *et al.*, 2019). Molecular data, such as DNA barcodes, can be used to group specimens based on contrasts between intra- and inter-specific variation, thus delineating species.

Conclusions

The increase in global travel and trade, especially the importation of live plants and plant material, has driven the increase in invasive alien insect pests, including gall-forming hymenopterans. Sixteen gall-forming hymenopterans have become invasive on *Eucalyptus* within the last two decades, with more than 80% of these hymenopterans first being described from the invasive range. In addition, this group of invasive insects is gaining in economic importance, where some recent introductions have resulted in substantial economic losses, thereby forcing land owners to plant alternative *Eucalyptus* species or genotypes.

A rapid response to the increasing number of invasions by gall-forming hymenopterans is imperative. This must include more effective quarantine measures and continuous monitoring to enable early detection. However, when invasive gall-forming hymenoptera are detected, an ability to respond rapidly is hindered by the lack of taxonomic expertise and the shortage of taxonomists. Species identity of gall-forming hymenopterans is further hampered by morphological species variation, phenotypic variation, the presence of cryptic species and the growing number of invasive species.

The application of molecular techniques can fast-track species identifications by comparing molecular data with curated databases. This in turn allows for a rapid implementation of species-appropriate control measures. In addition, molecular data can be used to elucidate invasion pathways and characterise genetic diversity, which become important for the successful implementation of biological control and phytosanitary regulations. To fully exploit this potential for increased efficiency and precision in management of these invasive pest will require a concerted effort from researchers working

in the field globally to share resources and information to populate open access databases (Wingfield *et al.*, 2015). Additional future prospects include whole genome sequencing and comparative genomics for purposes of species delineation, gene function characterization, and the development of new population management tools through gene editing technologies (Mc Farlane *et al.*, 2018).

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