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 16 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 of identification 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 the initiation of management actions because breeding for resistance and biological control requires 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  gall-forming Hymenoptera, invasive species, plant galls, plantation forestry.
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* utilized in plantations globally (Paine *et al.*, 2011; Hurley *et al.*, 2016). The number of invasive Hymenoptera infesting non-native *Eucalyptus* has 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* fulfills the first condition (host plant availability) favoring 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 (Carnegie *et al.*, 2008) or warranted intervention. It is also likely that only a fraction of Australia’s endemic hymenopteran eucalypt gall-formers has been described (Csóka *et al.*, 2017). An estimate of total numbers of insect species, based on collections examined (Nielsen & West, 1994; Austin *et al.*, 2004), 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 8000 have been described, and where the Chalcidoidea is a major, bio-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* represents 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 1600 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 *Opelimum* 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 are complex, resulting in numerous instances of misidentification, misunderstanding of trophic levels and reclassification. Girault (1913) and later Boucek (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. *Epichyrsoscharis*), seed gall-formers (e.g. *Quadristichodella*) 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* (Boucek, 1988; La Salle, 2005; Kim, 2008). In fact, many were only described after they became invasive elsewhere in the world (Fig. 1).

No hymenopteran gall-formers are significant pests of *Eucalyptus* grown commercially in Australia (Nahrung & Swain, 2015), with only three undescribed species (*Ditripinotella* sp. and two *Opelimum* 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* galls were known from Australia before they were encountered outside that country (Girault, 1915; Timberlake, 1957). These were
Invasive gall-forming wasps

Quadrastichodella nova and Megastigmus eucalypti. Only five species (Leptocybe invasa Haplogroup 2, Ophelimus maskelli, Ophelimus mediterraneus, Megastigmus zebrinus and Moona spermaphaga) 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, 2019), 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 a family level associated with Eucalyptus, only 45 (<0.3%) were Eulophidae and Torymidae. Of the ~1030 species of insects recorded in the APPD associated with Eucalyptus, only two reside in these families. One of them (Chrysonotomia mira) is a likely parasitoid and the other, M. eucalypti, is of uncertain galling/parasitoid/invasive status (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 Ophelimus eucalypti ‘Trans’) were discovered as cryptic species within the first invasive populations recorded of that species. Three (M. eucalypti, M. zebrinus, Leprosa milga) were later considered more likely to be parasitoid species or misidentifications of parasitoid species. Currently, there are 13 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 (Fig. 1). L. invasa Haplogroup
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.

| Species                        | Date Recorded | Country       | Distribution Notes       |
|--------------------------------|---------------|---------------|--------------------------|
| Ophelimus eucalypti            | 1934          | Australia     | invasive                   |
| Ophelimus eucalypti sp.        | 1934          | Australia     | invasive                   |
| Ophelimus *migda-norum*        | 2003          | New Zealand   | invasive                   |
| Ophelimus *zebrinus*           | 1975          | Italy         | invasive                   |
| Leptocybe invasa               | 1977          | Italy         | invasive                   |

Note: A, Q. nova and *O. maskelli* is 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 U.S.A. (Figs 1 and 2), which averaged 4.9 ± 0.3 species per country. However, of these, New Zealand and the U.S.A. had three and two unique species, respectively. Just over half of the invasive species have spread to more than one country. Only *Aprostocetes 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 (Figs 1 and 2). Of the seven species that have spread to more than one continent, two were first detected in Europe and two in the U.S.A.

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 the movement from Australia, or from undiscovered invasive populations elsewhere), and 3.6 new country records for all previously recorded invasive species (representing the movement from bridgehead populations, or additional introductions from Australia) (Fig. 2(B)). 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 S1). 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 occurred contiguously following its discovery in Brazil (2007), with a separate discovery in 2008 in the U.S.A. Thus, apart from inter-regional ‘jumps’, the spread seems to have mainly occurred from previously-invaded countries, a notion supported by scenario modelling in Dittrich-Schröder et al. (2018). This is emphasized 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, specialized biological traits (e.g. development within the protected micro-environment of the gall, parthenogenesis) 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 the 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 *Leptocype invasa* (Protasov et al., 2007). Eight *Aprostocetus* species have been associated with galls on *Eucalyptus* (*Aprostocetus auriflavus*, *Aprostocetus causalis*, *Aprostocetus consobrinus*,...
Aprostocetus margiscutum, Aprostocetus quingnigrimaculacae, Aprostocetus secis. Aprostocetus tricolor and Aprostocetus nigritihora) 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 Schauf (Eulophidae, Tetrastichinaceae)

E. burwelli was discovered emerging from ‘blister-like’ galls on the dorsal and ventral leaf surfaces of C. citriodora in California in 1999 (Schauff & Garrison, 2000). It was placed in a genus with three other species: Epichrysocharis fasca, Epichrysocharis nigritenris and Epichrysocharis algheriniti, all of which are leaf gallers of Eucalyptus (Boucek, 1988; Schauf & 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). E. burwelli has not been found in Australia.

Leprosa milga Kim and La Salle (Eulophidae, Tetrastichinaceae)

L. 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 L. 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 Q. nova and M. spermophaga (Kim & La Salle, 2008). Although L. milga was observed probing seed capsules of Eucalyptus camaldulensis in laboratory trials, no gall formation or wasp development occurred. Consequently, this species may be a parasitoid of Q. 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 Figs 1 and 2.

Leptocybe Fisher and La Salle (species complex) (Eulophidae, Tetrastichinaceae)

L. 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 it is notably absent from New Zealand. L. invasa is one of the most threatening insect pests of non-native Eucalyptus plantation forestry (Mendel et al., 2004).

L. 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 (Doğanlar, 2004; Ankità & Poorani, 2009; Chen et al., 2009; Sangtongprao et al., 2011; Akhtar et al., 2012). It forms galls on the midribs, petioles and stems of a number of Eucalyptus species in the sections Exsertaria, 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, Selirichodes krycere, Selirichodes neserti, Megastigmus lawsonii and Megastigmus 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 2000s 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 (Kim, 2008; Doğanlar & Hassan, 2010; Kelly et al., 2012; 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 and 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 M. 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
Megastigmus zebrinus Grissell (Megastigmidae, Megastigminae)

Like M. eucalypti and L. milga, records of M. zebrinus as an invasive primary gall er are likely erroneous. M. 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 gall er (Klein et al., 2015). M. zebrinus has been recorded from Queensland, Australia (Grissell, 2006). Confusion regarding the gall-forming, parasitoid, in quiline 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 gall er, it has been excluded from invasion pattern descriptions (Figs 1 and 2, Table 1).

Moona spermophaga Kim and La Salle (Eulophidae, Tetrastichinae)

The first record of M. spermophaga outside Australia was in Argentina in 2000, as an undescribed species (Kim et al., 2005). M. 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). M. spermophaga was first detected in Corymbia maculata seedlots as part of a shipment sent to 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 C. citriodora (Kim et al., 2005). It was these discoveries that prompted its taxonomic description (Kim et al., 2005).

M. 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 and Withers (Pteromalidae, Ormocerinae)

N. xanthops was discovered in New Zealand in October 1999 and described in 2002 (Berry & Withers, 2002). Wasp s in this genus are predominantly plant gall-formers with a subgroup inducing galls on seeds and fruits (Berry & Withers, 2002). Host plants include species within the Casuarinaceae, Cela tracea, Fabaceae and Myrtaceae (Bouček, 1998). N. xanthops has been recorded from three Eucalyptus species (Eucalyptus nicholii, Eucalyptus globulus, Eucalyptus cinerea); however, E. nicholii is the preferred host (Berry & Withers, 2002). This species induces two morphologically different gall types (Berry & Withers, 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 & Withers, 2002).

Ophelimus migdananorum Molina-Mercader (Eulophidae, Euderinae)

O. migdananorum was first detected in Chile in February 2003 by the Agricultural and Livestock Services (Servicio Agrícola y Ganadero) in Region V (Informativo Fitosanitario Forestal, 2006). Subsequently, it was also detected in regions VI, VII and the Metropolitan (Informativo Fitosanitario Forestal, 2006). The predominant Eucalyptus species affected by O. migdananorum include Eucalyptus 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; Molina-Mercader et al., 2019).

Ophelimus mediterraneus Borowiec and Burks (Eulophidae, Euderinae)

O. 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). O. mediterraneus emerges between spring and summer. They are univoltine and have an approximate life span of 2 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 E. globulus (Branco et al., 2016) and Eucalyptus gunnii (Borowiec et al., 2019). No parasitoids have been observed emerging from O. mediterraneus galls in France, Italy or Portugal (Borowiec et al., 2019).
Ophelimum eucalypti (Gahan) (species complex) (Eulophidae, Euderinae)

*O. 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 recognized as two biotypes (Withers et al., 2000; Protasov et al., 2007). The first, *O. eucalypti sensu lato*, was reported from New Zealand in 1921 on *E. globulus* (described as *Rhinocoptella 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 has been recorded from Australia. *O. eucalypti* was first discovered in 2008 in California on *E. globulus* (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 *Quadrostichodella* includes 10 species (Ikeda, 1999) of which nine are native to Australia (*Quadrostichodella aenea, Quadrostichodella bella, Quadrostichodella boudienymi, Quadrostichodella candida, Quadrostichodella cyaneviridis, Quadrostichodella neglecta, Q. nova and Quadrostichodella pilosa*) (Noyes, 2003). *Q. pilosa* is purported to be native to New Zealand (Ikeda, 1999). Species of *Quadrostichodella* have been recorded from Europe, North and South America and are thought to have been adventitiously introduced with *Eucalyptus* seeds (Flock, 1957; Timberlake, 1957; Dumbleton, 1971; de Graham, 1987; Ikeda, 1999). The genus *Quadrostichodella* includes seed gallers of various *Eucalyptus* species. For example, *Q. nova* has been recorded from *Eucalyptus umbellatae* and *Eucalyptus resinifera* (Flock, 1957; Timberlake, 1957; Dumbleton, 1971); *Q. neglecta* from *Eucalyptus neglecta* (Ikeda, 1999) and *Q. pilosa* from an unknown *Eucalyptus* species (Ikeda, 1999).

*Quadrostichodella* nova Girault (Eulophidae, Tetrastichinae)

*Q. 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 adventitiously. 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).

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Ophelimum maskelli (Ashmead) (Eulophidae, Euderinae)

*O. maskelli* was described as *Protoprix maskelli* Ashmead in 1900 from specimens purportedly from New Zealand. The species now recognized as *O. maskelli* was first reported (incorrectly) as *O. eucalypti* from Italy in 2000 (Arzone & Alma, 2000; Protasov et al., 2007; Mansfield, 2016; Durand et al., 2011). As *Eucalyptus* is 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).

*O. 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 the 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, *Stethynomus ophelimi* Huber and *Stethynomus 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).

Gallsofthe*Eucalyptus*speciesisAustralia(Huberet al.,2007;Brancoc et al.,2011). 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 has been recorded from Australia. *O. eucalypti* was first discovered in 2008 in California on *E. globulus* (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 *Quadrostichodella* includes 10 species (Ikeda, 1999) of which nine are native to Australia (*Quadrostichodella aenea, Quadrostichodella bella, Quadrostichodella boudienymi, Quadrostichodella candida, Quadrostichodella cyaneviridis, Quadrostichodella neglecta, Q. nova and Quadrostichodella pilosa*) (Noyes, 2003). *Q. pilosa* is purported to be native to New Zealand (Ikeda, 1999). Species of *Quadrostichodella* have been recorded from Europe, North and South America and are thought to have been adventitiously introduced with *Eucalyptus* seeds (Flock, 1957; Timberlake, 1957; Dumbleton, 1971; de Graham, 1987; Ikeda, 1999). The genus *Quadrostichodella* includes seed gallers of various *Eucalyptus* species. For example, *Q. nova* has been recorded from *Eucalyptus umbellatae* and *Eucalyptus resinifera* (Flock, 1957; Timberlake, 1957; Dumbleton, 1971); *Q. neglecta* from *Eucalyptus neglecta* (Ikeda, 1999) and *Q. pilosa* from an unknown *Eucalyptus* species (Ikeda, 1999).

**Challenges in dealing with the 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 (Waldchen & Maeder, 2018), is severely hampered. Some of the key challenges with 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 L. invasa Haplogroup 1 and Haplogroup 2. L. 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 hapeata ogs 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 interactions in gall-forming insects result 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 gallsa (M. zebrinus and L. milga). These highlight the need for biological as well as the taxonomic resolution in determining species identities and roles. Species identification may be difficult to formalize 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, O. 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 (Schaff & 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**

L. 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 the 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 (one insect pest species on Eucalyptus every 1.4 years in general (Hurley et al., 2016) – and one new hymenopteran gall-former every 2 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 cannot 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 recognize cryptic species influences the efficacy of implemented control measures as different species to
respond differently to control agents (Bickford et al., 2006). This has been seen particularly in studies conducted on entomopathogenic nematodes (Bidocha et al., 2001) and parasitoids (Hafez & Doutt, 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, Gomipterus 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 the 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 is 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 subpopulations interbreeding, the response to the 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 L. 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 were 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 & Wieczorek, 2009; Hornoy et al., 2013) and perhaps more importantly to trace pathways of invasion. Current examples illustrating this point can be found within species of Ophelinus, 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 Ophelinus species is best resolved using molecular data (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 landowners to plant alternative Eucalyptus species or genotypes.

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 the rapid implementation of species-appropriate control measures. In addition, molecular data can be used to elucidate invasion pathways and characterize genetic diversity, which becomes important for the successful implementation of biological control and phytosanitary regulations. To fully exploit this potential for increased efficiency and precision in the management of these invasive pests 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 (McFarlane et al., 2018).

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Data availability statement

The data that support the findings of this study are openly available from published articles cited in the text.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Data used to estimate the spread of Leptocybe invasa between neighbouring countries. Simultaneous discoveries in neighbouring countries (e.g. Ethiopia, Kenya, Uganda) were considered a single discovery.

References

Akhtar, M.S., Patankar, N.V. & Gaur, A. (2012) Observations on the biology and male of Eucalyptus gall wasp Leptocybe invasa Fisher and La Salle (Hymenoptera: Eulophidae). Indian Journal of Entomology, 74, 173–175.

Ankita, G. & Poorani, J. (2009) Taxonomic studies on a collection of Chloridiodae (Hymenoptera) from India with new distribution records. Journal of Threatened Taxa, 1, 300–304.

APPD. (2019) Australian Plant Pest Database. Plant Health Australia, licenced under Creative Commons Attribution 3.0 Australia License. https://appd.ala.org.au/appd-hubindex [accessed on June 2020].

Arzone, A. & Alma, A. (2000) A gall Eupholid of Eucalyptus in Italy. Informatore Fitopatologico, 50, 43–46.

Austin, A.D., Yeates, D.K., Cassis, G. et al. (2000) ‘down under’ – diversity, endemism and evolution of the Australian insect fauna: examples from select orders. Australian Journal of Entomology, 43, 216–234.

Bain, J. (1977) Rhinopeltella eucalypti Gahan (Hymenoptera: Chalcidoidea: Eulophidae), blue-gum chalcid. Forest and Timber Insects in New Zealand, New Zealand Forest Service, 15.

Bauhus, J., van der Meer, P.J. & Kanninen, M. (2010) Ecosystem Goods and Services from Plantation Forests, p. 254. Washington, DC 20036, USA: Earthscan.

Beardsley, J.W. & Perreira, W.D. (2000) Aprostocetus sp. (Hymenoptera: Eulophidae: Tetrarchitinae), a gall wasp new to Hawaii. Proceedings of the Hawaiian Entomological Society, 34, 183.

Berry, J.A. & Withers, T.M. (2002) New gall-inducing species of ormeccerine pteromalid (Hymenoptera: Ormocerinae) described from New Zealand. Australian Journal of Entomology, 4, 18–22.

Bickford, D., Lohman, D.J., Sodhi, N.S. et al. (2006) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution, 22, 148–155.

Bidochna, M.J., Kamp, A.M., Lavender, T.M., Dekoning, J. & De Croo, J.N.A. (2001) Habitat association in two genetic groups of the insect-pathogenic fungus Metarhizium anisopliae: uncovering cryptic species? Applied and Environmental Microbiology, 67, 1335–1342.

Borowiec, N., Thao, M., Brancaccio, L., Warot, S., Ris, N. & Malausa, J.-C. (2012) L’eucalyptus menacé par une nouvelle espèce d’Ophelius en France. Phytophama, 656, 42–44.

Borowiec, N., La Salle, J., Brancaccio, L. et al. (2019) Ophelius mediterraneus sp. n. (Hymenoptera: Eulophidae): a new Eucalyptus gall wasp in the Mediterranean Region. Bulletin of Entomological Research, 109, 679–694.

Boucek, Z. (1988) Australasian Chalcidoidea (Hymenoptera): A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species, p. 832. CAB International, Wallingford, U.K.

Branco, M., Battisti, A. & Mendel, Z. (2016) Foliage feeding invasive insects: defoliators and gall makers. Insects and Diseases of Mediterranean Forest Systems (ed. by T. Paine and F. Lieutier), pp. 211–238. Springer, Cham, Switzerland.

Bush, S.J., Slippers, B., Nesen, S., Harney, M., Dittrich-Schröder, G. & Hurley, B.P. (2016) Six recently recorded Australian insects associated with Eucalyptus in South Africa. African Entomology: Journal of the Entomological Society of Southern Africa, 24, 539–544.

Carnegie, A.J., Lawson, S.A., Smith, T., Pegg, G.S., Stone, C. & McDonald, J. (2008) Healthy Hardwoods. A Field Guide to Pests, Diseases and Nutritional Disorders in Subtropical Hardwoods. p. 140. Forest & Wood Products Australia, Melbourne, Australia.

Chen, H.Y., Yao, J.M. & Xu, Z.F. (2009) First description of the male of Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae) from China. Journal of Environmental Entomology, 31, 285–287.

Ciesla, V.M. (2015) The role of human activities on forest insect outbreaks worldwide. International Forestry Review, 17, 269–281.

Cranston, P.S. (2017) Biodiversity of Australasian insects. Insect Biodiversity: Science and Society (ed. by R. G. Footitt and P. H. Adler), pp. 111–139. John Wiley and Sons Incorporated, Hoboken, USA.

Csokak, G., Stone, G.N. & Melika, G. (2017) Non-native gall-inducing insects on forest trees: a global review. Biological Invasions, 19, 3161–3181.

Dittrich-Schröder, G., Wingfield, M.J., Hurley, B.P. & Slippers, B. (2012) Diversity in Eucalyptus susceptibility to the gall-forming wasp Leptocybe invasa. Agricultural and Forest Entomology, 14, 419–427.

Dittrich-Schröder, G., Harney, M., Nesen, S. et al. (2014) Biology and host preference of Selirichoides neseri: a potential biological control agent of the eucalyptus gall wasp. Leptocybe invasa. Biological Control, 78, 33–41.

Dittrich-Schröder, G., Hoareau, T.B., Hurley, B.P., Wingfield, M.J., Lawson, S., Nahrung, H.F. & Slippers, B. (2018) Population genetic analyses of complex global insect invasions in managed landscapes: a Leptocybe invasa (Hymenoptera) case study. Biological Invasions, 20, 2395–2420.

Dögnlar, O. (2004) Occurrence of Leptocybe invasa Fisher & La Salle, J. (Hymenoptera: Chalcidoidea) on Eucalyptus camaldulensis in Turkey, with a description of the male sex. Zoology in the Middle East, 35, 112–114.

Dögnlar, M. & Hassan, E. (2010) Review of Australian species of Megastigmus (Hymenoptera: Torymidae) associated with eucalyptus, with descriptions of new species. Australian Journal of Basic and Applied Sciences, 4, 5059–5120.

Dögnlar, O. & Hassan, E. (2013) Availability and type depository of four species of Megastigmus (Hymenoptera: Torymidae). Munis Entomology & Zoology Journal, 1, 505–506.

Dögnlar, O. & Mendel, Z. (2007) First record of the Eucalyptus gall wasp Ophelius maskelli and its parasitoid, Closterocerus chamaeleon, in Turkey. Phytoparasitica, 35, 333–335.

Dumbleton, L.J. (1971) Flockiella sp. (Hym., Eulophidae) from Eucalyptus. New Zealand Journal of Science, 14, 267–269.

Durand, N., Rodrigues, J.C., Mateus, E., Boavida, C. & Branco, M. (2011) Susceptibility variation in Eucalyptus spp. in relation to Leptocybe invasa and Ophelius maskelli (Hymenoptera: Eulophidae), two invasive gall wasps occurring in Portugal, Silva Lusitana, 19, 19–31.

Elliott, H.J., Ohmart, C.P. & Wylie, F.R. (1998) Insect Pests of Australian Forests: Ecology and Management, p. 214. Inkata Press, Melbourne, Australia.

Fleck, R.A. (1957) Biological notes on a new chalcid-fly from seed-like Eucalyptus galls in California. Pan-Pacific Entomologist, 33, 153–155.

Franco, J.C., Garcia, A. & Branco, M. (2016) First report of Epichrysocharis burwelli in Europe, a new invasive gall wasp attacking eucalypts. Phytoparasitica, 44, 443–446.
Garnas, J.R., Hurley, B.P., Slippers, B. & Wingfield, M.J. (2013) Biological control of forest plantation pests in an interconnected world requires greater international focus. *International Journal of Pest Management*, 58, 211–223.

Garnas, J.R., Auger-Rozenberg, M., Roques, A. *et al.* (2016) Complex patterns of global spread in invasive insects: eco-evolutionary and management consequences. *Biological Invasions*, 18, 935–952.

Girault, A.A. (1913) Some new genera and species of Chalcidoidea. *Hymenoptera of the family Eulophidae from Australia*. *Journal of Entomology and Zoology*, 5, 104–105.

Girault, A.A. (1915) Some new genera and species of Chalcidoidea. *The Australian Hymenoptera Chalcidoidea, XII*. *Memoirs of the Queensland Museum*, 4, 300.

de Graham, M.W.R.V. (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.

Grissell, E.E. (2006) A new species of *Megastigmus* Dalman (Hymenoptera: Torymidae), galling seed capsules of *Eucalyptus camaldulensis* Dehnhardt (Myrtaceae) in South Africa and Australia. *African Entomology: Journal of the Entomological Society of Southern Africa*, 14, 87.

Haefz, M. & Dott, R.L. (1954) Biological evidence of sibling species in *Aphysis maculicornis* (Masi) (Hymenoptera: Aneaphilidae). *Canadian Entomologist*, 86, 90–96.

Hajibabaei, H.M., deWaard, J.R., Ivanova, N.V. *et al.* (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B*, 360, 1959–1967.

Hebert, P.D.N., Ratnasingham, S. & de Waard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society of London B*, 270, S96–S99.

Hill, K.D. & Johnson, L.A.S. (2000) Systematic studies in the eucalypts. 10. New tropical and subtropical eucalypts from Australia and New Guinea (Eucalyptus, Myrtaceae). *Telopea*, 8, 503–539.

Hornoy, B., Atlan, A., Roussel, V., Buckley, Y.M. & Tarayre, M. (2013) Two colonisation stages generate two different patterns of genetic diversity within native and invasive ranges of *Ulex europaeus*. *Hereditas*, 111, 355–363.

Huang, Z.Y., Li, J., Liu, W., Zheng, X.L. & Yang, Z.D. (2018) Parasitoids of the eucalypt gall wasp *Leptocybe invasa*: a global review. *Environmental Science and Pollution Research*, 25, 29983–29995.

Haber, J.T., Mendel, Z., Protasov, A. & La Salle, J. (2006) Two new Australasian species of *Stethyma* (Hymenoptera: Mymaridae), larval parasitoids of *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) on *Eucalyptus*. *Journal of Natural History*, 40, 1909–1921.

Hurley, B.P., Garnas, J., Wingfield, M.J., Branco, M., Richardson, D.M. & Slippers, B. (2016) Increasing numbers and intercontinental spread of invasive insects on eucalypts. *Biological Invasions*, 18, 921–933.

Ikeda, E. (1999) A revision of the world species of *Quadrastichodella* Girault, with descriptions of four new species (Hymenoptera, Eulophidae). *Insecta Matsumurana*, 55, 13–35.

Janes, J.K. & Batista, P.D. (2016) The role of population genetic structure in understanding and managing pine beetles. *Advances in Insect Physiology* (ed. by C. Tittiger and G. J. Blomquist), pp. 75–100. UK Academic Press: Elsevier.

Jörgen, K.M. & Schrödl, M. (2013) How to describe a cryptic species? Practical challenges of molecular taxonomy. *Frontiers in Zoology*, 10, 59–86.

Kelly, J., La Salle, J., Harney, M., Dittrich-Schröder, G. & Hurley, B.P. (2012) *Selitrichodes neseri* n. sp., a new parasitoid of the eucalypt gall wasp *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). *Zootaxa*, 3333, 50–57.

Kim, I. (2008) Evolution of gall inducing Eulophidae (Hymenoptera: Chalcidoidea) on *Myrtaceae* in Australia. PhD thesis, Australian National University.

Kim, I.K. & La Salle, J. (2008) A new genus and species of Tetrastichinae (Hymenoptera: Eulophidae) inducing galls in seed capsules of *Eucalyptus*. *Zootaxa*, 1745, 63–68.

Kim, I.K., McDonald, M. & La Salle, J. (2005) *Moona*, a new genus of tetrachistine gall inducers (Hymenoptera: Eulophidae) on seeds of *Corymbia* (Myrtaceae) in Australia. *Zootaxa*, 899, 1–10.

Klein, H., Hoffmann, J.H., Neser, S. & Dittrich-Schröder, G. (2015) Evidence that Quadrastichodella nova (Hymenoptera: Eulophidae) is the only gall inducer among four Hymenopteran species associated with seed capsules of *Eucalyptus camaldulensis* (Myrtaceae) in South Africa. *African Entomology: Journal of the Entomological Society of Southern Africa*, 23, 207–223.

La Salle, J. (2005) Chalcidoidea (Hymenoptera: Eulophidae, Eurytominae, Pteromalinae, Tanaostigmatidae, Torymidae). *Biology, Ecology, and Evolution of Gall-Inducing Arthropods* (ed. by A. Raman, C.W. Schafer and T. M. Withers), pp. 507–537. Science Publishers, Inc, Enfield, New Hampshire.

La Salle, J., Arakelian, G., Garrison, R.W. & Gates, M.W. (2009) A new species of invasive gall wasp (Hymenoptera: Eulophidae: Tetrastichinae) on blue gum (*Eucalyptus globulus*) in California. *Zootaxa*, 2121, 35–43.

Le Roux, J. & Wieczorek, A.M. (2009) Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management. *Annals of Applied Biology*, 154, 1–17.

Le Roux J.J., Brown G.K., Byrne M., Ndlovu J., Richardson D.M., Thompson G.D., Wilson J.R.U. (2011) Phylogeographic consequences of different introduction histories of invasive Australian Acacia species and Paraserianthes lophantha (Fabaceae) in South Africa. *Divers Distrib* 17, 861–871.

Le, N.H., Nahrung, H.F., Griffiths, M. & Lawson, S.A. (2018) Invasive *Leptocybe* spp. and their natural enemies: global movement of an insect fauna on eucalypts. *Biological Control*, 125, 7–14.

Le, N.H., Nahrung, H.F., Morgan, J.A. & Lawson, S.A. (2020) Multivariate ratio analysis and DNA markers reveal a new Australian species and three synonyms in eucalypt-gall-associated *Megastigmus* (Hymenoptera: Megastigmidae). *Bulletin of Entomological Research*, 1–16. https://doi.org/10.1017/S000748532000002X.

Li, X.Q., Liu, Y.Z., Guo, W.F. *et al.* (2017) The gall wasp *Leptocybe invasa* (Hymenoptera: Eulophidae) stimulates different chemical and phytohormone responses in two *Eucalyptus* varieties that vary in susceptibility to galling. *Tree Physiology*, 37, 1208–1217.

Mansfield, S. (2016) New communities on eucalypts grown outside Australia. *Frontiers in Plant Science*, 7, 1812.

Mapondora, T.S., Burgess, T., Matsuki, M. & Oberprieler, R.G. (2012) Identification and molecular phylogenetics of the cryptic species of *Gonipiterus scutellatus* complex (Coleoptera: Curculionidae: Gonipiterini). *Australian Journal of Entomology*, 51, 175–188.

McFarlane, G.R., Whitelaw, C.B.A. & Lillioc, S.G. (2018) CRISPR-based gene drives for pest control. *Trends in Biotechnology*, 36, 130–133.

Mendel, Z., Protasov, A., Fisher, N. & La Salle, J. (2004) Taxonomy and biology of *Leptocybe invasa* gen. & sp. n. (Hymenoptera: Eulophidae), an invasive gall inducer on *Eucalyptus*. *Australian Journal of Entomology*, 43, 101–113.

Mendel, Z., Protasov, A., Blumberg, D., Brand, D., Saphir, N., Madar, Z. & La Salle, J. (2007) Note: release and recovery of parasitoids of the eucalypt gall wasp *Ophelimus maskelli* in Israel. *Phytoparasitica*, 35, 330–332.

Mendel, Z., Protasov, A., La Salle, J., Blumberg, D., Brand, D. & Branco, M. (2017) Classical biological control of two *Eucalyptus* gall wasps; main outcome and conclusions. *Biological Control*, 105, 66–78.
