Accepted Manuscript

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PII: S1049-9644(18)30088-4
DOI: https://doi.org/10.1016/j.biocontrol.2018.06.004
Reference: YBCON 3790

To appear in: Biological Control

Received Date: 16 February 2018
Revised Date: 9 June 2018
Accepted Date: 15 June 2018

Please cite this article as: Le, N.H., Nahrung, H.F., Griffiths, M., Lawson, S.A., Invasive Leptocybe spp. and their natural enemies: global movement of an insect fauna on eucalypts, Biological Control (2018), doi: https://doi.org/10.1016/j.biocontrol.2018.06.004

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Invasive *Leptocybe* spp. and their natural enemies: global movement of an insect fauna on eucalypts.

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Declaration of Interest: None
Abstract

The Eulophidae genus *Leptocybe* Fisher and La Salle and its type species *L. invasa* was first described in 2004 as an invasive galling pest causing serious damage to young eucalypts in the Middle East and Mediterranean. Believed to be of Australian origin, two cryptic species of *Leptocybe* have established throughout the world at an unprecedented rate. Australian parasitoids have been collected and released overseas for classical biocontrol of *Leptocybe* spp., some of which have also established via unassisted movement to other regions. Numerous other gall associates of *Leptocybe* spp. have been reported, both in Australia as the native range of eucalypts, and in countries where eucalypts are non-native. In this review, we describe the range expansion of *Leptocybe* spp. from its discovery to its current range of 43 countries, with reference to data supporting clarification of cryptic species. Records of Australian parasitoids of *Leptocybe* spp., through managed biocontrol programs and unassisted movement, are updated. Data detailing occurrences of potential parasitoids from nine families, of which the richest fauna are found in Australia and in southern Asia, are summarized. Emphasis is given to the Torymidae genus *Megastigmus* Dalman, which is distributed globally and associated with all current known invasive *Leptocybe* spp. Knowledge of the global movement of associates of *Leptocybe* spp. is expected to provide a model in understanding parasitoid - host and insect - host plant relationships. In addition, our summary of available data of potential parasitoids may assist policy makers and researchers in directing and prioritizing resources once alternative or additional biocontrol efforts are needed for management of *Leptocybe* spp.

**Keywords:** invasive pest, parasitoid, galling insect, biological control, Megastigmus

1. Introduction

Eucalypts (*sensu lato*) are almost exclusively native to Australia (CPBR, 2006), with only a few species occurring in Papua New Guinea, Timor Leste, Indonesia and the southern Philippines. During the last two centuries, eucalypt species of advantageous adaptability,
growth rates and wood properties have been grown in more than 90 countries in Asia, Africa, Europe, South America and North America (Bennett, 2010), rendering them one of the world’s major sources of planted biomass (Booth, 2013; Wingfield et al., 2008). Insects have been an integral part in the introduction history of eucalypts: in early periods, the lack of restrictive pests and diseases facilitated eucalypt expansion (Paine et al., 2011), yet gradually insect communities started to establish in eucalypt growing areas, the most destructive of which are native Australian pests finding pathways to join with their usual hosts (Mansfield, 2016; Paine et al., 2011).

The Australian origin of eucalypts and their relatively short history of introduction provide researchers a unique model to investigate migration patterns of insects in pest-host plant relationships. Nahrung and Swain (2014) compared life history traits of eucalypt specialists in Australia and overseas, revealing that characteristics such as diapause, body size, feeding habit or duration of adult active flights were differentially exhibited, and can play a role in the spreading capacity of alien species. Hurley et al. (2016) established a list of “first entry sites” restricted to relatively few countries including the US, Argentina, South Africa and the Europe-Mediterranean region, topped by New Zealand. Paine et al. (2011) noted two main phases of introduction: early pioneers recorded in countries under the former British Empire, and more recent movement associated with the post-1950s expansion of eucalypt plantations and faster international transit times. According to them, among Australian invaders, leaf beetles and psyllids appeared in North America before spreading further to Europe while galling wasps tended to invade Europe before establishing more widely (Paine et al., 2011).

The myrtaceous faunas in South Africa and South America are more diverse than those in the Northern Hemisphere, facilitating host shifts of insects from local plants to eucalypts (Paine et al., 2011). Host shifting to eucalypts is also common in Asia, where there are surprisingly very few examples of serious pests that originated from Australia. In China, except for L.
invasa, key eucalypt pests such as the moths *Biston suppressaria* (Guenée), *Endoclita signifier* Walker and the subterranean termite *Odontotermes formosanus* (Shiraki) (Xie et al., 2017) are all native to Oriental countries. Over time, the rate of introduction of Australian eucalypt pests has increased over time: Hurley et al. (2016) estimated a five-fold increase in introduction rate after 1986, while more recent invaders took less time than their pioneers to reach the same number of non-Australian countries (Mansfield, 2016).

The genus *Leptocybe* Fisher and La Salle (Hymenoptera: Eulophidae) is relatively new to science, with first occurrence records dated to 2000. *Leptocybe invasa* was described in 2004 as a serious galling pest causing damage to eucalypt plantations (Mendel et al., 2004). This, and another *Leptocybe* sp., have since expanded their global distributions at an unprecedented rate (Branco et al., 2016; Nugnes et al., 2015). Attempts have been made to collect *L. invasa* in Australia (ACIAR, 2016; Kim, 2008), clarify its species status (Dittrich-Schröder, 2014; Dittrich-Schröder et al., 2018; Nugnes et al., 2015), find native enemies in Australia (Doğanlar and Hassan, 2010; Kelly et al., 2012; Kim, 2008), identify local enemies in its exotic range (Hernández et al., 2015; Ramanagouda and Vastrad, 2015; Zheng et al., 2016) and release biological control agents (ACIAR, 2016; Mendel et al., 2017; Ramanagouda and Vastrad, 2015). The rapid spread of *Leptocybe* spp. and accumulation of knowledge of their biocontrol associates provide a pertinent model in understanding migratory patterns of eucalypt galling insects, and subsequent accidental introductions of, and local (fortuitous) colonisation by, natural enemies. In this study, we facilitate progress towards such an understanding by 1. summarizing information regarding the distribution and spread of *Leptocybe* spp.; and 2. updating recent progress in the study of natural enemies and biocontrol of *Leptocybe* spp.
2. Invasive history of *Leptocybe* spp.

2.1. Cryptic species

DNA barcode data revealed that invasive *Leptocybe* spp. comprise two genetically separate lineages, i.e. cryptic species (Dittrich-Schröder, 2014; Dittrich-Schröder et al., 2018; Nugnes et al., 2015). While all specimens from the Western Palearctic (lineage A) shared a unique DNA barcode, another lineage (lineage B) which grouped with Australian specimens, was found in China, Malaysia, Laos, Thailand and Ghana. Both lineages concurrently exist in Oriental and Afrotropical regions, co-occurring in Thailand, Laos, Vietnam and South Africa (Dittrich-Schröder et al., 2018) ([Figure 1](#)). Nugnes et al. (2015) suggested a link between the presence of males in the invasive populations and lineage identity. CO1 gene and *Rickettsia* endosymbionts of specimens from locations where only females were found (Brazil, Italy, Tunisia) or with a very low male ratio (Turkey, 0.75% male), were different from those from the Chinese lineage where 18% to 48% of examined populations were males (Nugnes et al., 2015). Male individuals have been additionally found in Thailand, Laos, India, Taiwan, Sri Lanka and Malta ([Figure 1](#)).
**Figure 1.** Invasion history of *Leptocybe* spp. by year of first recorded occurrence. DA, DB, DC indicate lineage A, B, C according to Dittrich-Schröder (2014) and Dittrich-Schröder et al. (2018). N1, N2 indicate lineage 1 and lineage 2 according to Nugnes et al. (2015). Blue stars indicate countries with confirmed presence of males.
2.2. Origin of *Leptocybe* spp.

A study using cytochrome oxidase genes allocated eight different Australian *Leptocybe* species including *Leptocybe* “sp. 9” which induced galls of similar morphology to *L. invasa* (Kim, 2008). However, *L. invasa sensu stricto* has not been located in its presumptive Australian origin despite numerous search attempts (Dittrich-Schröder, 2014; Kim, 2008). Efforts hitherto have been made to search in Queensland, New South Wales, and to a limited extent Victoria and Western Australia without finding *L. invasa* (ACIAR, 2016). DNA barcoding data, although failing to find *L. invasa*, identified Australian populations with less than 2% divergence to specimens from China, Malaysia and Thailand. Barcoding sequences illustrated high genetic diversity of *Leptocybe* spp. in Australia, supporting the hypothesis of Australia as the original gene pool for the invasive *Leptocybe* spp. in the rest of the world (Dittrich-Schröder, 2014; Dittrich-Schröder et al., 2018).

2.3. Invasion records

Up to 2018, *Leptocybe* spp. have been found in 45 countries from five zoogeographical regions (Figure 1) The Western Palearctic was the first outbreak site of *Leptocybe* spp. Upon description in 2004, *Leptocybe invasa* had been recorded from 11 countries in the region (Mendel et al., 2004), and two from the Oriental region. Earliest evidence of the pest dated back to 2000 in Italy, and remaining countries in the region subsequently reported finding *Leptocybe* spp. within the following few years, except for Iraq (first record 2010).

Although first reported as early as 2001 in India (Kumar et al., 2015) and 2002 in Vietnam (Pham et al., 2009), *Leptocybe* spp. only seemed to reach an epidemic level of spread in the Oriental region after it successfully invaded almost all the Western Palearctic countries. The insect was found in Thailand (2004), Cambodia and China (2007), Laos (2009), Sri Lanka and Taiwan (2010) and as late as 2012 in Malaysia.
Countries adjacent to the Western Palearctic (Ethiopia, Kenya, Uganda) first reported *Leptocybe* spp. at the same time as their northern neighbours (2002), yet the insect was not found in South Africa until 2007 and evidence of the insect in other Afrotropical countries was only presented after 2010.

South America was the most recent expansion range of *Leptocybe* spp. Brazil, as one of the world’s largest growers of Eucalypts, recorded the first occurrence of *Leptocybe invasa* in 2007 (Costa et al., 2008) and an epidemic outbreak in 2009 (Fernandes et al., 2014). *Leptocybe invasa* specimens were successively collected for the first time in Argentina (2009), Chile (2010), Paraguay (2012), Uruguay (2013) and as far north as Mexico (2014). Florida (2008) is so far the only recorded *Leptocybe* sp. location in the US.

3. Classical biocontrol agents from Australia

3.1. Worldwide use of Australian parasitoids

The first agents identified for biocontrol of *Leptocybe* spp. were the eulophid species *Quadrastichus mendeli* Kim and La Salle and *Selitrichodes kryceri* Kim and La Salle (Kim et al., 2008), followed by *Megastigmus zvimendeli* Doğanlar and Hassan, *M. lawsoni* Doğanlar and Hassan and *S. neseri* Kelly and La Salle (Kelly et al., 2012; Lawson et al., 2017). Those species have been used in other parts of the world as classical biological control agents for *Leptocybe* spp., as illustrated in Table 1. As yet, no attempt has been made to compare the two cryptic *Leptocybe* species in regard to their association with biocontrol agents.
Table 1. Worldwide records of Australian-origin biocontrol agents of *Leptocybe* spp.

<table>
<thead>
<tr>
<th>Agent/Country</th>
<th>Establishment status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Quadrastichus mendeli</strong> Kim and La Salle [Eulophidae: Tetrastichinae]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>SA</td>
<td>Zheng et al. (2014)</td>
</tr>
<tr>
<td>Laos, Cambodia, Vietnam, Thailand</td>
<td>SA</td>
<td>ACIAR (2016)</td>
</tr>
<tr>
<td>India</td>
<td>RE</td>
<td>Jacob et al. (2015), Jacob (2018, pers. comm.)</td>
</tr>
<tr>
<td>Israel, Turkey</td>
<td>RE</td>
<td>Branco et al. (2016, Mendel et al. (2017)</td>
</tr>
<tr>
<td>Italy</td>
<td>SA</td>
<td>Nugnes et al. (2016)</td>
</tr>
<tr>
<td>Kenya</td>
<td>RQ</td>
<td>Dittrich-Schröder et al. (2014)</td>
</tr>
<tr>
<td>South Africa</td>
<td>SA, RQ</td>
<td>Bush et al. (2017), Hurley et al. (2013)</td>
</tr>
<tr>
<td>Argentina</td>
<td>SA</td>
<td>Aquino et al. (2017)</td>
</tr>
<tr>
<td><strong>II. Selitrichodes kryceri</strong> Kim and La Salle [Eulophidae: Tetrastichinae]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>RQ</td>
<td>Jacob (2018, pers. comm.), Shylesha (2008)</td>
</tr>
<tr>
<td>Israel, Turkey</td>
<td>RE</td>
<td>Branco et al. (2016), Mendel et al. (2017)</td>
</tr>
<tr>
<td>South Africa</td>
<td>RQ</td>
<td>Dittrich-Schröder et al. (2014, Hurley et al. (2013)</td>
</tr>
<tr>
<td>Kenya</td>
<td>RQ, SA</td>
<td>Dittrich-Schröder et al. (2014), Le et al. (2018, unpublished data)</td>
</tr>
<tr>
<td><strong>III. Selitrichodes neseri</strong> Kelly and La Salle [Eulophidae: Tetrastichinae]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>RE</td>
<td>Masson et al. (2017), Schühli et al. (2016)</td>
</tr>
<tr>
<td>Chile</td>
<td>RN</td>
<td>Bush et al. (2017)</td>
</tr>
<tr>
<td>Laos</td>
<td>RQ</td>
<td>ACIAR (2016), Griffiths (2018, unpublished data)</td>
</tr>
<tr>
<td>Mauritius</td>
<td>RN</td>
<td>Bush et al. (2017)</td>
</tr>
<tr>
<td>South Africa</td>
<td>RE</td>
<td>Bush et al. (2017, Dittrich-Schröder et al. (2014), Hurley et al. (2013)</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>RN</td>
<td>Bush et al. (2017), FAO (2016)</td>
</tr>
<tr>
<td><strong>IV. Megastigmus zvimendeli</strong> Doğanlar and Hassan [Torymidae: Megastigmidae]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>RE</td>
<td>Mendel et al. (2017)</td>
</tr>
<tr>
<td><strong>V. Megastigmus lawsoni</strong> Doğanlar and Hassan [Torymidae: Megastigmidae]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>RE</td>
<td>Mendel et al. (2017)</td>
</tr>
</tbody>
</table>

RQ: Attempted rearing in quarantine but failed to establish, except for an on-going trial in Laos with *S. neseri*.
RN: Released, no further update on long term establishment
RE: Released, established
SA: Spread accidentally without record of deliberate release

3.1.1. The first parasitoids

Located within the initial outbreak region, Israel was the first country to launch a biological control program against *Leptocybe invasa* using Australian parasitoids, (*M. zvimendeli*, *M. lawsoni*, *S. kryceri*, and *Q. mendeli*), with a considerable level of success. By 2014, all of the released species had successfully established, fewer galls were found, and gall occurrence had decreased seven years after release (Mendel et al., 2017).
Several other countries have also released a set of parasitoids similar to that in Israel for *Leptocybe* spp. biocontrol. *S. kryceri* and *Q. mendeli* were released and established in Turkey (Branco et al., 2016). A review report was published in Kenya in 2009 in preparation for the importation of *Q. mendeli* and *S. kryceri* (Nyeko et al., 2009b) and the two parasitoids have been reared in quarantine in Kenya and South Africa but failed to establish (Dittrich-Schröder et al., 2014). *Q. mendeli*, *S. kryceri* and an Australian *Megastigmus* species were transferred to India in 2008 (Shylesha, 2008). Preliminary evidence suggested successful establishment of *Q. mendeli* and the *Megastigmus* sp. and a decline in gall incidence 40 days after release (Jacob et al., 2015) and recovery of both parasitoids at 6 years after release (Jacob 2018, pers. comm.).

3.1.2. *Selitrichodes neseri* Kelly and La Salle

In contrast to the Israeli program, *Q. mendeli* and *S. kryceri* were not able to be reared successfully under quarantine conditions in South Africa and Kenya (Dittrich-Schröder et al., 2014; Hurley et al., 2013) and additional agents were sought. The Australian species *S. neseri* was described in 2012 (Kelly et al. 2012) and subsequently imported to South Africa, reared in quarantine and then released at over 300 sites countrywide (Hurley et al., 2013; SAF, 2013). This species has also been recorded emerging from the galls of the Australian-origin wasp *Ophelimus maskelli* (Ashmead) in South Africa (Bush et al., 2016), but its in-gall role has not been clarified. Studies on the establishment and spread of *S. neseri* are underway (Hurley et al., 2013).

From South Africa, *S. neseri* was exported to Brazil, reared in quarantine (Schühli et al., 2016), and released (Masson et al., 2017). *S. neseri* was also transferred to and released in Zimbabwe (FAO, 2016), Mauritius and Chile (Bush et al., 2017). Laos has recently imported and attempted rearing in quarantine facilities, in preparation for biocontrol use in the Mekong countries (Lawson 2018, unpublished data).
3.1.3. Unassisted dispersal of *Q. mendeli* and *S. kryceri*

After the release in Israel, unassisted spread of *Q. mendeli* has been confirmed in numerous countries. The species has been noted in Italy since 2013, with mean parasitism ranging from 30.2 to 50.5%, exerting considerable control over *Leptocybe* spp. (Nugnes et al., 2016). *Quadrastichus mendeli* was also reported in association with *Leptocybe* spp. in China, Laos, Cambodia, Vietnam and Thailand without any official recorded release (ACIAR, 2016; Zheng et al., 2016). Even in South Africa where human-facilitated establishment was unsuccessful, natural occurrence of the species was confirmed in 2016 (Bush et al., 2017).

Similar to *Q. mendeli*, dispersal after unsuccessful human assisted establishment has been recorded for *S. kryceri* in Kenya. Although initial pre-release rearing failed to generate a sustainable population in quarantine (Dittrich-Schröder et al., 2014), *S. kryceri* was recorded co-occurring with *Megastigmus* sp. in *Leptocybe* spp. galls collected in Kenya (Le et al., 2018, unpublished data).

3.2. Local *Leptocybe* spp. parasitoids

Apart from classical biological control, numerous countries have attempted to source *Leptocybe* spp. enemies from local parasitoids. Several studies in Australia also established a list of *Leptocybe* spp. associates which may be used in the future in addition to current biocontrol agents. Table 2 summarizes hymenopteran gall associates of *Leptocybe* spp., reported in different countries including Australia, but not yet used in worldwide biocontrol programs.
Table 2. Local associates of *Leptocybe* spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Country of occurrence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Family Torymidae Walker</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Megastigmus erolhasani</em> Doganlar and Hassan</td>
<td>Australia</td>
<td>Doğanlar (2015)</td>
</tr>
<tr>
<td><em>M. eucalypti</em> Girault</td>
<td>Australia</td>
<td>Doğanlar (2010)</td>
</tr>
<tr>
<td><em>M. flavivariegatus</em> Girault</td>
<td>Australia</td>
<td>Doğanlar (2015)</td>
</tr>
<tr>
<td><em>M. fieldingi</em> Girault</td>
<td>Australia</td>
<td>Doğanlar (2010)</td>
</tr>
<tr>
<td><em>M. hilli</em> Dodd</td>
<td>Australia</td>
<td>Doğanlar (2010)</td>
</tr>
<tr>
<td><em>M. judikingae</em> Doganlar and Hassan</td>
<td>Australia</td>
<td>Doğanlar (2015)</td>
</tr>
<tr>
<td><em>M. zebrinus</em> Grissell</td>
<td>Australia</td>
<td>Grissell (2006)</td>
</tr>
<tr>
<td></td>
<td>Thailand</td>
<td>Doğanlar (2015)</td>
</tr>
<tr>
<td></td>
<td>South Africa</td>
<td>Grissell (2006)</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
<td>Hernández et al. (2015)</td>
</tr>
<tr>
<td><em>M. dharwadicus</em> Narendran and Vastrad</td>
<td>India</td>
<td>Narendran et al. (2010), Ramanagouda (2011)</td>
</tr>
<tr>
<td><em>M. sichuanensis</em>³ Doganlar and Zheng</td>
<td>China</td>
<td>Doğanlar et al. (2017)</td>
</tr>
<tr>
<td><em>M. thailandensis</em> Doganlar and Hassan</td>
<td>Thailand</td>
<td>Doğanlar (2015), Mendel et al. (2017)</td>
</tr>
<tr>
<td><em>M. thitipornae</em> Doganlar and Hassan</td>
<td>Thailand</td>
<td>Doğanlar (2015), Mendel et al. (2017)</td>
</tr>
<tr>
<td><em>M. viggianii</em> Narendran and Sureshan</td>
<td>India</td>
<td>Gupta and Poorani (2008)</td>
</tr>
<tr>
<td><em>M. leptocybus</em>⁴ Doganlar and Hassan</td>
<td>Israel, Italy, Turkey</td>
<td>Doğanlar (2015), Mendel et al. (2017)</td>
</tr>
<tr>
<td></td>
<td>South Africa</td>
<td>Doğanlar (2015)</td>
</tr>
<tr>
<td><em>M. pretorianensis</em> Doganlar</td>
<td>India</td>
<td>uhaner &amp; Sureshan (2008)</td>
</tr>
<tr>
<td><em>M. brasiliensis</em> Doganlar, Zache and Wilcken</td>
<td>Brazil</td>
<td>Doğanlar et al. (2013)</td>
</tr>
<tr>
<td><em>Megastigmus sp.</em></td>
<td>Vietnam</td>
<td>Lê and Phạm (2016)</td>
</tr>
<tr>
<td><em>Megastigmus sp.</em></td>
<td>South Africa</td>
<td>Dittrich-Schröder et al. (2014)</td>
</tr>
<tr>
<td><strong>Family Eulophidae Westwood</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cirrospilus</em> sp.</td>
<td>Australia</td>
<td>Souza (2016)</td>
</tr>
<tr>
<td><em>Clostocerus</em> sp.</td>
<td>Australia</td>
<td>Souza (2016)</td>
</tr>
<tr>
<td><em>Epichrysocharis</em> sp</td>
<td>Australia</td>
<td>Souza (2016)</td>
</tr>
<tr>
<td><em>Ophiilimus</em> sp.</td>
<td>Australia</td>
<td>Souza (2016)</td>
</tr>
<tr>
<td><em>Quadrastichus</em> sp.</td>
<td>Australia</td>
<td>Souza (2016)</td>
</tr>
<tr>
<td><em>S ėltirichodes</em> sp.</td>
<td>Australia</td>
<td>Souza (2016)</td>
</tr>
<tr>
<td><em>Aprostocetus causalis</em> La Salle and Wu</td>
<td>Thailand</td>
<td>Yang et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>Zheng et al. (2016)</td>
</tr>
<tr>
<td><em>A. gala</em>⁶(Walker)</td>
<td>India</td>
<td>Kulkarni et al. (2010)</td>
</tr>
<tr>
<td><em>Aprostocetus sp.</em></td>
<td>India</td>
<td>Kulkarni et al. (2010)</td>
</tr>
<tr>
<td><em>Aprostocetus sp.</em></td>
<td>Vietnam</td>
<td>Lê and Phạm (2016)</td>
</tr>
<tr>
<td><strong>Family Eurytomidae Walker</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eurytoma</em> sp.</td>
<td>Australia</td>
<td>Souza (2016)</td>
</tr>
<tr>
<td><strong>Family Mymaridae Haliday</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Erythmelus</em> sp.⁷</td>
<td>India</td>
<td>Kulkarni et al. (2010)</td>
</tr>
</tbody>
</table>
3.2.1. Diversity of *Leptocybe* spp. local associates

As the country of origin of eucalypts, Australia hosts the highest number of *Leptocybe* spp. associates: 21 species in five families, including those exported to other parts of the world.

In total, nine different hymenopteran families have been found having *Leptocybe* spp. associates. Outside Australia, the South Asian countries Sri Lanka (six species, five families) and India (six species, four families) contain the most diverse *Leptocybe* spp.-associated fauna. Thailand (four species), China and Vietnam (two species) were reported with representatives from two families, Eulophidae Westwood and Torymidae Walker. Unlike the diverse *Leptocybe* spp.-associated fauna in the Oriental region, potential fortuitous parasitoids in the Western Palearctic (Israel, Turkey, Italy), Nearctic (Argentina, Brazil) and Afrotropical (South Africa, Kenya) come solely from the genus *Megastigmus* Dalman (family Torymidae).

In addition, except for South Africa, India and Thailand, no country outside Australia has recorded more than one local *Megastigmus* associate of *Leptocybe*.
The diversity of Asian associates of *Leptocybe* spp. is in line with the findings of Paine et al. (2011) that the Asian region has observed the highest number of local insects shifting to eucalypts. Considering the presence of two cryptic *Leptocybe* species, the rich fauna in Asian countries seems to agree with the occurrence of a Chinese lineage (Nugnes et al., 2015) or lineage B (Dittrich-Schröder, 2014; Dittrich-Schröder et al., 2018), which closely groups with Australian specimens. The other *Leptocybe* species, namely the Western line (Nugnes et al., 2015) or lineage A (Dittrich-Schröder, 2014; Dittrich-Schröder et al., 2018) has not been found in Australia, and is only associated with Eulophidae and Torymidae overseas.

Proper identification of cryptic species and matching of host-associates are prerequisite factors for successful biocontrol programs, as different parasitoids may selectively interact with different hosts bearing identical morphology, and unresolved identification of cryptic species leads to potential host-parasitoid mismatches. For example, the invasive eucalypt weevil *Gonipterus scutellatus* Gyllenhal has recently been revealed as a species-rich complex, with host-parasitoid mismatch considered a factor in its variable biocontrol success (Mapondera et al., 2012). In the case of *Leptocybe* spp., further comparative studies linking gall associates with molecular identification of each local population would confirm whether the cryptic species display different gall associate profiles.

### 3.2.2. Fortuitous parasitism

Most of the studies on local (fortuitous) parasitoids of *Leptocybe* spp. have focused on establishing lists of *Leptocybe* spp. associates as presumptive natural enemies, including parasitism rates but without further biological data. *Aprostocetus causalis* and *Megastigmus sichuanensis* (named *M. viggianii* in the original publication) are known parasitoids in China, with parasitism rates reaching as high as 26% and 25%, respectively (Zheng et al., 2016). Six species from two genera, *Megastigmus* Dalman and *Telenomus* Haliday, have been found associated with *Leptocybe* sp. galls in Sri Lanka.
To a limited extent, trials have been conducted to assess fortuitous parasitism of local parasitoids. In India, a trial using *Aprostocetus gala* and *Megastigmus dharwadicus* did not lower gall incidence at 3 months after release but did so successfully eight months after release (Ramanagouda and Vastrad, 2015). Long term follow-up reports have not been published, hence it is difficult to estimate effectiveness and exclude/calibrate seasonal impact. Under greenhouse conditions, field-collected *A. gala* and *Megastigmus* sp. were released in small numbers in 2008 and 2009, resulting in total parasitism rates of 21% to 34% (Kulkarni et al., 2010). It should be noted that *A. gala* was possibly a misidentified Indian *Aprostocetus* species (Yang et al., 2014), while *Megastigmus* sp. was later described as *M. dharwadicus* (Narendran et al., 2010).

Study of galling roles (parasitoid/hyperparasitoid/inquiline/gall former) of local associates of *Leptocybe* spp. were examined for *M. thitipornae* in Thailand (Sangtongpraow and Charernsom, 2013), and *M. pretorianensis* in South Africa (Hurley et al. 2017). In Thailand, *M. thitipornae* was reared and assessed for parasitism capacity and other life history traits (Sangtongpraow and Charernsom, 2013). *Megastigmus thailandiensis* and *M. zebrinus* were also reported to occur in Thailand (Doğanlar, 2015), but no *Megastigmus* species were detected during subsequent *Leptocybe* spp. surveys in 2015 (ACIAR, 2016). In South Africa, *M. pretorianensis* may be an inquiline and/or parasitoid (Hurley et al., 2017).

3.3. *Megastigmus* Dalman 1820

The Torymidae genus *Megastigmus* Dalman contains 143 species (Noyes, 2018), and occurs worldwide but is most species-rich in Australia (Doğanlar et al., 2013; Grissell, 1999; Roques et al., 2016). Before *Leptocybe* spp. emerged as significant pests, research focused mainly on species associated with plants of the families Pinaceae and Rosaceae (Grissell, 1999). Grissell estimated about one-third of species are parasitoids, many of which are associated with various oak galls and later removed from the genus (Doğanlar, 2011).
Unlike other local Hymenoptera that only associate with *Leptocybe* spp. in Asia, *Megastigmus* species occur worldwide and apparently associate with both invasive *Leptocybe* species. At least eleven *Megastigmus* species have been recorded to associate with *Leptocybe* spp. galls outside Australia, including Australian species released in biocontrol programs (*M. lawsoni* and *M. zvimendeli*) or accidentally introduced (*M. zebrinus*) (Table 1, 2).

“*Megastigmus* sp. prob. *eucalypti*” (Viggiani et al., 2002) was the first mentioned non-Australian *Leptocybe* sp. gall associate and is likely the same species described as *M. leptocybus* by Doğanlar and Hassan (2010, 2013). The species is believed to occur naturally in Italy, Israel and Turkey (Doğanlar, 2015), but local hosts have not been identified. Seven other local *Megastigmus* species are thought to have host-shifted to *Leptocybe*: *M. thailandiensis* and *M. thitipornae* in Thailand, *M. pretorianensis* in South Africa (Doğanlar, 2015), *M. brasiiliensis* in Brazil (Doğanlar et al., 2013), *M. viggiani* (Gupta and Poorani, 2008) and *M. dharwadicus* (Narendran et al., 2010) in India, and *M. sichuanensis* in China (initially misidentified as *M. viggianii*) (Doğanlar et al., 2017). In addition to that, *Megastigmus* species of unresolved identity were also reported in Vietnam, India and Sri Lanka (Dittrich-Schröder, 2014; Lê and Phảm, 2016; Udagedara and Karunarathne, 2014).

Yet of all the newly described local fortuitous *Megastigmus, M. viggianii* was the only species that was described before the age of *Leptocybe* spp. as well as the only species with a specified local host. *M. viggianii* was found associated with *Leptocybe* spp. galls in India (Gupta and Poorani, 2008) but it was initially described as an associate of the galling thrips *Austrothrips cochinchinensis* Karny on the Combretaceae plant *Calycopterys floribunda* (Narendran and Sureshan, 1988). Further study of these *Megastigmus* species, including their phylogenetic relationships and native hosts, are important to understand *Leptocybe* spp. population regulation, and host-specificity and host-shifts in parasitoids more generally.
The Australian species, *M. lawsoni* and *M. zvimendeli* successfully parasitized the Western lineage (lineage A) in Israel (Mendel et al., 2017). *Megastigmus zebrinus* was also reported to occur widely: it was first described outside of Australia (Grissell, 2006), and has so far been found in South Africa, Argentina and Thailand without introduction record. A further six species are thought to associate with *Leptocybe* galls in Australia, including *M. judykingae* and *M. fielding* from *Corymbia tessellaris* (Doğanlar), which is recorded as resistant to *Leptocybe* (Phảm et al., 2009).

4. Discussion

Considerable knowledge has been generated on the spread of *Leptocybe* spp., their natural enemies and impacts of biocontrol programs. Reviews of the progress in worldwide biocontrol of *Leptocybe* spp. have raised several issues, for which further studies may lead to a more comprehensive understanding of the interaction between eucalypts and their insect associates as a dynamic model.

Since *Leptocybe* spp. populations around the world have been confirmed to comprise at least two cryptic species, DNA data should be employed to understand precisely the species present in an invasive region. Further study of male: female sex ratios can verify the sex-lineage association: if this hypothesis is true, male specimens are expected in Malaysia and Ghana, while none or few males should be present in Brazil and other Nearctic countries, at least before multiple introduction and spread complicates the issue. In the opposite direction, once verified the presence of males in a colony could be an indicator of the specific lineage in a region, which may consequently redraw the distribution map of *Leptocybe* spp.

Hybridization between the two lineages has been reported (Dittrich-Schröder et al., 2018). Theoretically, hybridization of the two lineages, if widespread, would result in lineage A genotypes in CO1 sequences and sex ratio. Because of the scarcity, non-functionality or
absence of males in lineage A, mating is most probable between males from lineage B and females from lineage A producing offspring with *Rickettsia* endosymbionts and mitochondria inherited from maternal ovaries and eggs.

Available literature allows a brief understanding of the effectiveness of biocontrol programs in numerous locations, particularly Israel. Follow-up studies would be expected to reveal changes in the fauna associated with *Leptocybe* after release and following interactions between different agents, both exotic and endemic. Spread of biocontrol agents could be a factor of interest. So far, *Q. mendeli* has been known to have travelled (apparently) unassisted to Oriental and Afrotropical countries, although whether from its native Australia, or from its adventive range is unknown. Monitoring of its introduction to South Africa and regular surveys of other exotic agents within the distribution of *Leptocybe* spp. will give more insight into the spread of eucalypt galling associates.

Knowledge on the host range of *Leptocybe* spp. natural enemies is important in predicting potential non-target impact to local insect fauna and understanding interactions of among eucalypt gall associates. Although data on host range of *Q. mendeli*, *S. neseri* and *S. kryceri* are limited due to their relatively short research history, there have been records of association of *Q. erythrinae* and *Q. rosarum* to other chalcidoid hosts in non-eucalypt models (Noyes, 2018). In eucalypt galls, preliminary data could be drawn from further investigation of *S. neseri* roles in *O. maskelli* galls (Bush et al., 2016) and comparison with *S. neseri* behavior in Australia. Interactions of the introduced agents with local fortuitous insects (Kulkarni et al., 2010; Udagedara and Karunaratne, 2014; Zheng et al., 2016) in several countries also provide a model for host range studies. *Megastigmus* seem to be better understood, with records of 28 chalcid species (excluding *Leptocybe*) from four largely phytophagous families associated with different *Megastigmus* species (Noyes, 2018).
Regarding *Megastigmus*, consensus exists over the difficulty of species discrimination, and a revision of the genus is required (Bouček, 1988; De Marzo, 2016; Protasov et al., 2008). Bouček (Bouček, 1988) mentioned challenges in aligning the Australian species with European *Megastigmus* and believed that substantial changes in the species list could be expected from a thorough revision of the genus. Protasov et al. (2008) admitted that revision of European species is prerequisite to identifying species associated with *Eucalyptus* in Turkey and Israel. Morphological keys have been published for species in the Nearctic (Milliron, 1949), India (Narendran et al., 2010), and Australia (Doğanlar and Hassan, 2010), and for seed-feeding species in Western Palearctic and Afrotropical regions (Roques et al., 2016; Roques and Skrzypczyńska, 2003), but taxonomy remains a challenge to researchers as many traits used in morphological taxonomy such as size, sculpture and colour vary widely (Bouček, 1988).

A more exhaustive study involving both molecular and morphological data, which have recently been used in discrimination of seed-feeding *Megastigmus* (Auger-Rozenberg et al., 2006; Roques et al., 2016), is required to resolve the taxonomic difficulty over *Megastigmus* and assist in clarification of both identity and native range of *Leptocybe* spp. associates. Once made available, distribution data of species can be employed to verify the spread pathways of Australian species, as demonstrated in the case of *M. transvaalensis* (Scheffer and Grissell, 2003), and host-shifting of local parasitoids to eucalypt gallers.

Given the long history of coevolution between *Megastigmus* and eucalypts in Australia, the existence (or lack) of gall-forming *Megastigmus* in the region may lead to a more affirmative understanding of gall forming between *Megastigmus* and eucalypts as a model. Particular attention should be paid to *M. zebrinus*, considering its early association with eucalypts in the introduced range, its global distribution and controversy over its gall induction capability. Upon its description in 2006 the species was identified as the first known gall former in the
genus (Grissell, 2006), but further study testing gall induction cast doubt on its gall former status (Klein et al., 2015). Further research on biology and gall forming ability of Australian Megastigmus, particularly globally distributed species, would be of essential value if the genus is to be used as a source of additional parasitoids for further biocontrol programs.

The Mediterranean Megastigmus sp. referred to as “probably Megastigmus eucalypti” in Viggiani et al. (2002) was believed to induce galls by several authors. Grissell (2006) mentioned M. eucalypti as an invasive Australian gall forming chalcidoid on eucalypts, while Paine at al. (2011) and Mansfield (2016) listed it as an invasive gall former in Europe. There is a suggestion of a phytophagous Megastigmus sp. associated with eucalypt capsules and buds in Italy (De Marzo, 2016), but the Mediterranean species referred to as M. eucalypti in Viggiani et al. (2002) was likely the same species described as M. leptocybus by Doğanlar and Hassan (2010, 2013). Specimens provided by Viggiani were identified as M. leptocybus, while M. eucalypti was not recorded outside of Australia by (Doğanlar and Hassan, 2010; Doğanlar and Hassan, 2013). According to the authors, M. leptocybus may feed on gall tissue after consuming the primary host in Leptocybe spp. galls. The in-gall role of M. leptocybus should be clarified once further data of galling roles of Megastigmus are available.

5. Acknowledgements

We express our thanks to Dr. John Prasanth Jacob and Dr. Zvi Mendel for professional advice during the preparation of the manuscript, and Dr Beryn Otieno for providing specimens from Kenya.

Funding: This study was supported by the ACIAR project FST/2012/091 (Australian Centre for International Agricultural Research) and Australian Endeavour Scholarships and Fellowships.
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Phytoparasitica 36, 449-459.


• Invasion reports of *Leptocybe* spp. cryptic species in 44 countries are listed
• Known associates of invasive *Leptocybe* are summarised for the first time
• Use and distribution of Australian-origin biocontrol agents are summarized
• Records of local hymenopteran parasitoids of *Leptocybe* spp. are updated
• Future research into *Megastigmus* is discussed