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Anagonia lasiophthalma (Diptera: Tachinidae): Survey, identification, and biological traits of a new biological control agent of the *Eucalyptus* snout beetle, *Gonipterus platensis* (Coleoptera: Curculionidae)

Cátia Martins[a,*], Catarina Afonso[a,b], Carlos Valente[a], Ana Raquel Reis[c], James O'Hara[d], James Lumbers[e,f], Manuela Branco[b], Catarina I. Gonçalves[a]

[a] *RAIZ, Instituto de Investigação da Floresta e Papel, Quinta de São Francisco, Apartado 15, 3801-501 Eixo, Aveiro, Portugal*
[b] *Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal*
[c] *Altri Florestal, Quinta do Furadouro, 2510-582 Olho Marinho, Óbidos, Portugal*
[d] *Canadian National Collection of Insects, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, Ontario K1A 0C6, Canada*
[e] *Australian National Insect Collection (ANIC), CSIRO Black Mountain, 1 Clunies Ross St, Black Mountain, Canberra, ACT 2601, Australia*
[f] *Research School of Biology, Australian National University, Canberra, ACT 2601, Australia*

HIGHLIGHTS

- *Anagonia* spp. were found parasitizing 7% of field collected *Gonipterus* spp. larvae, in Tasmania.
- One species, identified as *Anagonia lasiophthalma*, was successfully reared on *Gonipterus platensis*.
- Females mated shortly after emergence and started parasitizing a week later.
- On average, females produced 35 offspring and lived up to 84 days.
- Biological traits suggest *A. lasiophthalma* may be effective against *G. platensis*.

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ABSTRACT

The *Eucalyptus* snout beetle, *Gonipterus platensis*, is one of the main pests of eucalypts outside its native range. Among the available control methods for this pest, classical biological control with *Anaphes nitens* (Hymenoptera: Mymaridae) has been the most widely used, but it is not always effective. To identify other natural enemies that might provide further pest control, a field survey was conducted in Tasmania, Australia, in which *Anagonia* spp. were found to parasitize 7 % of *Gonipterus* spp. larvae. One species was subsequently identified as the solitary parasitoid *Anagonia lasiophthalma*, based on direct comparisons with research collection material in Australia. To assess the potential of this species as a biological control agent, *A. lasiophthalma* was reared on *G. platensis* larvae under quarantine conditions, and its biology and reproductive strategy were studied in the laboratory. Global mating success was 58 %, with 66 of the 114 *A. lasiophthalma* females analyzed mating within an established 10-day period. Most females mated within 48 h after emergence and started to parasitize about a week later. Dissections of mated females showed that this species is ovolarviparous, incubating its eggs in an ovisac, before inserting them "ready-to-hatch" directly into the host using a piercing structure derived from the modification of a sternite in the female terminalia. Over the course of their lifetime, females on average produced 35 offspring and *post-mortem* dissections revealed an additional 32 eggs in the ovisac. Longevity of females and males was similar, ranging between 55 and 84 days, but females maintained without host larvae lived longer than females exposed to host larvae. These results provide relevant information to improve laboratory rearing protocols and suggest that *A. lasiophthalma* may be effective against the snout beetle in its invaded range.

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* Corresponding author.
E-mail address: catia.martins@thenavigatorcompany.com (C. Martins).

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1. Introduction

Eucalypts are among the most extensively used exotic tree species in forest plantations worldwide, mostly because of their fast growth, adaptability to a wide range of environmental conditions, and their wood/fiber quality (Campinhos, 1999; Stape et al., 2004; Wingfield et al., 2008). In Europe, eucalypts are mainly cultivated in Portugal and Spain, with an overall area of roughly 845,000 ha and 630,000 ha, respectively (SECF, 2010; ICNF, 2019). *Eucalyptus globulus* Labill. is the most planted species in both countries due to its importance for the pulp and paper industry (Costa e Silva et al., 2009; González-García et al., 2009). Like other exotic species that have been introduced into new environments, eucalypts initially benefited from the absence of their co-evolved pests and pathogens. However, these enemies eventually arrived in the new regions, where they now threaten the health and productivity of the plantations (Wingfield et al., 2008; Kenis and Branco, 2010).

Gonipterus platensis Marelli (Coleoptera: Curculionidae), along with two other species of *Gonipterus*, were accidentally introduced from Australia into other parts of the world and became severe eucalypt pests (Tooke, 1995; Mapondera et al., 2012; Hurley et al., 2016). Continuous feeding, both in the larval and adult stages, leads to partial or total defoliation, reducing the photosynthetic capacity of plants, loss of apical dominance, stunted growth, and wood losses (Tooke, 1995; Loch, 2006; Reis et al., 2012; Valente et al., 2018). In Portugal, *G. platensis* was detected in 1995 and quickly became a key pest of *E. globulus* (Valente et al., 2018). Among the available methods for the control of *G. platensis*, classical biological control with the egg parasitoid *Anaphes nitens* (Girault) (Hymenoptera: Mymaridae) is the most widely used and cost-effective (Tooke, 1995; Cordero-Rivera et al., 1999; Hanks et al., 2000; Reis et al., 2012; Valente et al., 2018). Despite good results in many countries, *A. nitens* has not always achieved complete success, particularly in regions with temperate climate in South America, Southwestern Europe, South Africa, and Western Australia (Cordero-Rivera et al., 1999; Loch and Floyd, 2001; Reis et al., 2012; Valente et al., 2018). Two other *Anaphes* species are known to parasitize *Gonipterus* spp., namely *Anaphes tasmaniae* Huber and Prinsloo and *Anaphes inexpectatus* Huber and Prinsloo (Huber and Prinsloo, 1990). In 2009, *A. tasmaniae* was released in Chile and is considered to have established there as of 2013, albeit in small numbers (Mayorga et al., 2013; SAG, 2014). In Portugal, extensive work was done with *A. inexpectatus* between 2008 and 2020 (Valente et al., 2017a, 2017b, 2019). The parasitoid was released and appears to have been established, with limited success (unpublished data), likely due to its limited ability to compete with the incumbent *A. nitens* (Valente et al., 2019).

After the biological control programs with *A. nitens* and *A. inexpectatus*, the focus in Portugal shifted to larval parasitoids, as this life stage of *G. platensis* is currently free from natural enemies. Three larval parasitoids have been previously identified from *Gonipterus* spp. larvae: *Anagonia* spp. (Diptera: Tachinidae), *Oxyserphus* sp. (Hymenoptera: Proctotrupidae), and *Entedon magnificus* (Girault and Dodd) (Hymenoptera: Eulophidae) (Valente et al., 2017b; Garcia et al., 2019; Gonçalves et al., 2020). Despite efforts to establish laboratory populations of these three larval parasitoids, only one tachinid species reproduced successfully on *G. platensis* under laboratory conditions and was therefore selected for further studies (Gonçalves et al., 2020).

Tachinidae is a large and ubiquitous family of true flies in the order Diptera, with over 8,500 described species worldwide (O'Hara et al., 2020). They are highly diverse in their size and morphology, and all are endoparasitoids of insects and a few other arthropods during their larval stage (Stireman et al., 2006; O'Hara, 2008). Hosts include important pests among the Lepidoptera, Hemiptera, Coleoptera, and Hymenoptera (Symphyta), hence their use as biological control agents against multiple pests worldwide (Grenier, 1988; Belshaw, 1993; Stireman et al., 2006). However, although tachinids are good candidates for the biological control of target insect pests, their use as biocontrol agents has been underestimated, mostly due to the lack of specific knowledge about the biology, behavior and ecology of these parasitoids (Grenier, 1988; Dindo and Grenier, 2023). Of interest during this study was *Anagonia lasiophthalma* (Malloch), a species belonging to the tribe Blondeliini in the subfamily Exoristinae. This tribe comprises nearly 170 genera and 1,000 species and is present on all continents (Wood, 1985; O'Hara et al., 2020). Among Australian blondeliines, the most common hosts are Coleoptera (especially Chrysomelidae but also Curculionidae), various families of Lepidoptera, and Hymenoptera (Symphyta) (Crosskey, 1973; Cantrell, 1986).

Colless (2012) revised the *Froggattimyia-Anagonia* genus group and recognized 37 species, with 12 belonging to *Froggattimyia* Townsend and 25 to *Anagonia* Brauer and Bergenstamm. Almost all are known only from Australia. Females of the *Froggattimyia-Anagonia* group, and more generally the vast majority of blondeliines, are ovolarviparous, i.e., eggs are fertilized internally and retained in a distensible ovisac until they are ready to hatch. There are four species belonging to the *A. lasiophthalma* species complex (Colless, 2012: 195). All of these species are particularly advanced in respect to their oviposition apparatus which is characterized by a "remarkable development of sternite 7 of the female terminalia, as a sharp-pointed, highly sclerotized, downcurved 'piercer'" (Colless, 2012: 195).

In this study, parasitism of *Gonipterus* spp. by *Anagonia* spp. in its natural habitat in Tasmania is documented. The identification of the parasitoid as *A. lasiophthalma* was investigated by comparing laboratory-reared specimens with reference specimens at the Australian National Insect Collection (ANIC), Canberra, Australia. Furthermore, a series of laboratory studies were performed to characterize basic biological traits of *A. lasiophthalma*, including female reproductive biology, mating behavior, fecundity, and longevity. The information generated through this work is expected to contribute to the assessment of the potential efficacy of *A. lasiophthalma* as a biological control agent against *G. platensis* in a new environment. In addition, an understanding of the reproductive behavior and life history traits of *A. lasiophthalma* provides valuable information towards the optimization of laboratory rearing protocols and the development of future biological assays.

2. Material and methods

With the exception of the surveys in Tasmania, *A. lasiophthalma* rearing and all studies took place under quarantine conditions in the Forest Protection laboratory at RAIZ - Forest and Paper Research Institute, Aveiro, Portugal. *Gonipterus platensis* and *A. lasiophthalma* were reared under the conditions described below. All trials were performed at 20 °C, 14:10 L:D photoperiod and 70 % relative humidity.

2.1. Survey of Gonipterus spp. Larvae in Tasmania

Field surveys of *Gonipterus* spp. larvae were performed in 14 locations in Tasmania in November and December 2017 (Fig. 1; Table 1), with the aim of collecting larvae that were parasitized by natural enemies that could be used in a classic biological control program. Sampling locations had been identified in previous surveys (Valente et al., 2017b; Garcia et al., 2019) and typically consisted of small eucalypt trees, isolated or in small groups, by the roadside, preferably *E. globulus* and *E. ovata* Labill. Trees were inspected and all *Gonipterus* spp. larvae were collected and stored in plastic containers, sorted by location. Larvae were shipped to the Forest Protection laboratory at RAIZ - Forest and Paper Research Institute, Aveiro, Portugal, where they were placed in plastic Petri dishes (1 cm height x 90 cm diameter), either individually or in small groups from the same sampling site, together with *E. globulus* leaves as food source. Larvae were incubated at 20 °C and fresh leaves were provided as necessary, until parasitoid puparia were detected. Puparia were individualized and incubated until the emergence of *Anagonia* spp. or hyperparasitoids. Upon emergence, adult flies were sexed and used to establish laboratory populations: one from Ferny

Fig. 1. Map of 14 locations in Tasmania where *Gonipterus* spp. larvae were surveyed.

Table 1
Details of the sites where *Gonipterus* spp. larvae were collected.

Sampling site	Sampling date	Latitude	Longitude	*Eucalyptus* species	Nr. of *Gonipterus* spp. larvae collected
Channel Highway	11–28-2017	−43.268	147.242	*Eucalyptus* spp.	60
Cygnet	11–28-2017	−43.163	147.083	*E. globulus, E. ovata, Eucalyptus* sp.	354
Deddington	12–05-2017	−41.627	147.397	*E. ovata, E. pulchella*	286
Ferny Hill Road	12–04-2017	−41.152	147.301	*E. globulus, E. ovata, E. pulchella, E. viminalis, Eucalyptus* sp.	567
Grindelwald	12–05-2017	−41.356	147.014	*E. globulus*	142
Hobart	11–23-2017	−42.883	147.301	*E. globulus, E. viminalis, Eucalyptus* sp.	35
Leslie Road	11–24-2017	−42.973	147.271	*E. globulus, E. delegatensis, Eucalyptus* sp.	58
Missing Link Road	11–27-2017	−43.169	147.036	*Eucalyptus* sp.	8
Mount Nelson	11–22-2017	−42.912	147.322	*E. globulus*	40
Snug	11–28-2017	−43.071	147.242	*E. globulus, E. ovata*	33
The Glen Road	12–05-2017	−41.170	147.059	*E. globulus, E. ovata, E. pulchella, Eucalyptus* sp.	448
Total					2043

Hill Road, one from The Glen Road, and a mixed population from the remaining provenances.

2.2. Rearing of *G. platensis* and *A. lasiophthalma*

Gonipterus platensis larvae were obtained from a population established with adults collected in infested *E. globulus* plantations in Central Portugal. Groups of 40 adults were kept in plastic containers (take-away type, 1 L) with fresh *E. globulus* shoots as food source and oviposition substrate. Egg capsules were collected, placed in Petri dishes in groups of 50, and incubated at 20 °C for approximately seven days. After the eggs hatched, the larvae were transferred to plastic containers (take-away type, 1 L), by placing fresh *E. globulus* shoots in contact with infested shoots and/or using a moistened small paintbrush. Shoots were replaced twice a week. Rearing temperature was 20 °C, with lower temperatures (10 °C and 15 °C) being used whenever it was necessary to slow down development. Larvae were reared to the fourth and final instar before they were offered to *A. lasiophthalma* adults, as the late instars were found to produce the most progeny (unpublished data).

The methods used to rear *A. lasiophthalma* were adapted from general information on tachinid rearing (e.g. Dindo and Grenier, 2023). Adults of *A. lasiophthalma* were maintained at 15 °C in BugDorm-4 Polyester Mesh arenas (47.5 x 47.5 x 47.5 cm). This temperature was selected to lower the activity of adult flies, therefore easing insect handling, and reducing the number of host larvae required. Twenty adult females and ten males, together with fifty *G. platensis* fourth instar larvae on *E. globulus* bouquets, were placed in each arena. The 2:1 female:male ratio was chosen based on the observed sex ratio from laboratory colonies. Bouquets containing larvae for parasitization were replaced twice per week, for up to six weeks. The larvae exposed to parasitism were removed from the arena and placed in plastic containers (take-away type, 1 L) for a week, after which the larvae (no longer feeding) were incubated in Petri dishes until the detection of puparia. Subsequently, the puparia were individualized and incubated at 10 °C, 15 °C, or 20 °C, until the emergence of adults. These temperatures are within a range empirically found not to cause deleterious effects on the developing pupae and were used to synchronize the emergence of adults. Bouquets were prepared using five to ten *E. globulus* fresh shoots, approximately 20 cm in length, tied together with a sponge strip and placed in 250 ml Erlenmeyer flasks, previously filled with water. Finally, the sponge and the top of the Erlenmeyer flask was sealed with Parafilm® to prevent *G. platensis* larvae from drowning. Honey solution (50 % in water) was provided as food source inside the arenas in 4 ml plastic vials filled with cotton (1 cm height x 2.5 cm diameter) and paper strips.

For both *G. platensis* and *A. lasiophthalma* rearing, photoperiod was 14:10 L:D and relative humidity was 80 %, 75 %, and 70 % at 10 °C, 15 °C, and 20 °C, respectively.

2.3. *Species identification*

A subset of 31 adult tachinid flies that emerged from *G. platensis* in quarantine at the RAIZ Forest Protection laboratory were preserved dry on insect pins and labelled appropriately. They were sent to JL at the Australian National Insect Collection in Canberra (ANIC, part of the Commonwealth Scientific and Industrial Research Organization, CSIRO) for identification. The specimens were examined using a Leica M80 stereo microscope and identified to genus and species group using published keys. Several specimens of both sexes were selected for dissection of terminalia, as follows. The posterior portion of each abdomen, including the terminalia, was removed using cutting tweezers and relaxed overnight in 10 % KOH at 50 °C. The basic properties of the KOH were neutralized in 20 % acetic acid. Terminalia were moved to 100 % glycerol for further dissection and examination. Male terminalia were stained with a chlorazol black E (CBE) solution (made by mixing glycerol with a saturated solution CBE in 95 % EtOH in a ratio of 7:3 respectively) to allow the differentiation of over-cleared and unsclerotized structures. Stained terminalia were washed in storage solution (consisting of glycerol and 95 % EtOH in a ratio of 7:3 respectively) before being moved back to 100 % glycerol for examination. Terminalia were stored in polypropylene genitalia vials with fresh storage solution after they were studied and photographed. The vials were attached to the pins of their respective specimens.

Six of the RAIZ specimens (three males and three females) and their associated terminalia preparations were selected to be photographed, along with various *Anagonia* specimens from the ANIC, including Malloch's *Froggattimyia lasiophthalma* (=*Anagonia lasiophthalma*) female holotype and Colless' *Anagonia dayi* male holotype. Specimens were photographed at the ANIC using either a Leica M205 A or Visionary Digital BK and BK-plus CamLift systems. Focal stacks from all three imaging systems were processed in Capture One Pro 12.0.2 and then merged in Helicon focus 7.0.2 using the pyramid method; additional processing and annotations of images was performed in Adobe Photoshop CS5.

2.4. *Mating behavior*

According to Nakamura (1994) and Zhang et al. (2003), the ideal age for mating in some tachinids is when the males are 2–5 days old, and the females are newly emerged. Thus, to determine the pre-mating period of *A. lasiophthalma*, 114 newly emerged females from the established laboratory population (<24 h) were used. Females were grouped by emergence date, placed in custom made acrylic cages (40 x 40 x 40 cm) with older males (2–7 days) at a maximum ratio of 2 females:1 male, and fed with honey solution (50 % in water). Fifteen batches of three to fourteen same-aged females were used. The number of females used varied based on the availability for testing each day. Mating behavior was observed for 3-hour periods each day (between 2 pm and 6 pm). Whenever a male mounted a female, the couple was covered with an upside-down plastic vial (6,5 cm height x 3,8 cm diameter) and the duration of mating was recorded. Immediately after mating, both the female and the male were removed from the cage and placed in BugDorm-1 polypropylene cages (30 x 30 x 30 cm), to be used in subsequent fecundity and longevity studies. After the observation period, all males that had not mated were removed from the acrylic cage until the following observation period, leaving only the unmated females. This procedure was repeated daily until all the females had mated, or up to a maximum of ten days after emergence, after which the still unmated females were discarded. The tachinids were fed with honey solution (50 % in water). Each female or couple was considered a replicate for calculating the percentage of females that mated, the mean mating duration, and the pre-mating period over the 10-day experiment. Estimates were done in total and on each day during the 10-day experimental period.

2.5. *Female reproductive biology*

To determine the reproductive maturation of *A. lasiophthalma*, 11 unmated and 18 mated females were dissected and examined. For unmated females, dissections were made on adults aged 1-day (n = 1), 2-days (n = 2), 3-days (n = 2), 6-days (n = 1), 7-days (n = 1), 8-days (n = 2), 13-days (n = 1), and 14-days (n = 1). For mated females, dissections were made at 1-day (n = 6), 2-days (n = 3), 3-days (n = 1), 4-days (n = 1), 5-days (n = 1), 6-days (n = 2), 7-days (n = 2), and 8-days (n = 2) after copulation (female age at dissection ranged between one and eleven days). The abdomen of each female was removed with a scalpel and then opened using dissecting needles. After removing the internal organs, including the terminalia, an aqueous solution of 0.9 % NaCl was added. The ovaries and oviduct were examined and photographed using a Leica M125 stereomicroscope equipped with a Leica IC80 HD digital camera. The number of eggs and first instar larvae in the ovisac was counted. All females were fed with honey solution (50 % in water) from emergence until they were dissected.

2.6. *Lifetime fecundity and longevity*

Adult longevity of *A. lasiophthalma* was evaluated for both sexes in the presence and in the absence of hosts (females: n = 25/23; males: n = 14/8, respectively), to determine the effect of host availability on parasitoid longevity. The insects used for the trial were the same as those used in the mating behavior trial (refer to section 2.4). Therefore, the longevity data included the duration of the mating trial. Immediately after copulation, pairs of adults were individualized, placed in BugDorm-1 polypropylene cages (30 x 30 x 30 cm), and fed with honey solution (50 % in water). Insects were observed daily, except on weekends. Female realized fecundity, defined as the number of descendant pupae each female produced, was recorded simultaneously with longevity in the presence of the host. For this, five *G. platensis* larvae (fourth instar) were offered to each female for parasitization one day after mating and were replaced by new hosts every 48 h (72 h on weekends), until the parasitoid died. Larvae were placed in bouquets prepared as previously described. Hosts removed from the cages were examined daily, and, since *A. lasiophthalma* is a solitary parasitoid, the fate of each larva was recorded as: *A. lasiophthalma* puparium, *G. platensis* pupa (not parasitized), or unviable (larva died without producing parasitoid or host). The data were then used to determine progeny and parasitization rate for each female. After death, eleven females were randomly selected for abdomen dissections to count the number of eggs (immature and embryonated) that remained in the oviduct. This number was then used to calculate an approximation to the potential fecundity of each female by adding it to the number of pupae produced, i.e. the realized fecundity.

2.7. Statistical analysis

Longevity of males and females in the presence or absence of hosts was compared with factorial ANOVA, after confirmation of data normality and homogeneity of variances, using insect sex and host presence as independent variables, followed by Tukey HSD *post-hoc* test, $p < 0.05$ (TIBCO Statistica, v.13.5.0.17). Pearson correlations between female longevity and fecundity were calculated using Microsoft Excel 2010 (V14.0.7268.5000). Mean values are shown as mean ± standard deviation, when applicable, unless otherwise stated.

3. Results

3.1. Survey of Gonipterus spp. Larvae in Tasmania

From the fourteen sampling sites, *Gonipterus* spp. larvae were collected in eleven locations (Table 1) and tachinids were found in ten of them (Table 2). A total of 141 larvae were found to be parasitized by *Anagonia* spp., resulting in a global parasitism rate of 6.9 % of the 2,043 larvae collected. Most parasitoids emerged from larvae collected in the North of Tasmania, in Ferny Hill Road and The Glen Road (Fig. 1), where parasitism rates were 12.2 % and 10.0 %, respectively. More than half of the puparia failed to produce adults, partly due to the presence of hyperparasitoids. At least two species were found, one unidentified Tetrastichinae (Hymenoptera: Eulophidae) and *Perilampus* sp. (Hymenoptera: Perilampidae), possibly *Perilampus tasmanicus* Cameron (J. Huber and C. Darling, personal communication). Based on morphological traits, multiple species of *Gonipterus* were present in the collected material including, at least, *G. platensis*, *G. pulverulentus* Lea, and *Gonipterus* sp. n. 1 (*sensu* Mapondera et al., 2012). An attempt was made to establish a link between *Gonipterus* species and the emerging parasitoids, based on molecular methods, but larval remains were too deteriorated after incubation and parasitoid emergence.

The 36 females and 31 males of *Anagonia* spp. that emerged were used to initiate laboratory populations. The Glen Road population was the only one to establish under laboratory conditions, whereas parasitoids reared from the Ferny Hill Road population and the mixed populations became extinct in the second generation. It is unclear whether multiple species of *Anagonia* were originally present in each batch, but *A. lasiophthalma* was the only species to have survived.

3.2. Species identification

Thirty-one adult tachinid flies were received at ANIC for identification by JL, consisting of 13 males and 18 females. These were identified first to *Anagonia* using the keys to Australian Tachinidae by Crosskey (1973), then to species using the revision of the *Froggattimyia-Anagonia* genus group by Colless (2012). Males were readily keyed to *A. lasiophthalma* in “Key to males of *Anagonia*” in Colless (2012: 184) by the joint possession of “eye profusely haired” (couplet 1) and “tergite 4 with 1 or more stout discal bristles” (couplet 2). Colless (2012) did not provide a key to females, but RAIZ females were unequivocally assigned to the *A. lasiophthalma* species group by the feature Colless (2012: 195) used to define the group: presence of a conspicuous piercer in the female terminalia. Four species were assigned to this group based on this characteristic and the piercers of each were illustrated in Figs. 84–87 (Colless 2012: 209–210). The piercer in RAIZ females matched most closely the longest and most curved of the piercers illustrated, that of *A. lasiophthalma* (Fig. 84, Colless, 2012: 209). *Anagonia lasiophthalma* is a known parasitoid of *Gonipterus scutellatus* (Colless, 2012: 196), currently known to be a complex of species (Mapondera et al., 2012), and the only member of the *A. lasiophthalma* species group known from Tasmania.

Our determination of the RAIZ species as *A. lasiophthalma* is not without some doubt. This is certainly the species circumscribed by Colless (2012) as *A. lasiophthalma*, but there is a degree of morphological variability in the ANIC specimens of *A. lasiophthalma* that could be either intraspecific or an indication of a second (cryptic and unrecognized) species. This second form differs from the RAIZ specimens (Fig. 2d–f) in possessing longer cerci in the male terminalia (Fig. 2c), and narrower gena and parafacial on the female head (Fig. 2a). For present purposes we treat the RAIZ species as *A. lasiophthalma* pending more conclusive evidence to the contrary.

3.3. Mating behavior

From a total of 114 *A. lasiophthalma* females analyzed, 57.9 % (n = 66) mated within the established 10-day period. Those females that mated did so soon after emerging, with 48.5 % mating within 24 h and 75.8 % within 48 h. No mating were observed between the seventh and the tenth day after emergence, stipulated in the study for discarding the females. On average, the pre-mating period for *A. lasiophthalma* females was 1.4 ± 2.2 days. However, we cannot sustain that mating cannot happen beyond the designated 10-day period or that females cannot mate multiple times throughout their lives. Successful mating lasted between 19 and 87 min, with an average of 41.2 ± 9.5 min. Three couples that mated for six minutes or less were excluded from the analysis, as these were found to be outliers and, in all cases, failed to produce progeny.

3.4. Female reproductive biology

One of the main features observed was the female terminalia, which

Table 2
Parasitism by *Anagonia* spp. in the sampling sites in Tasmania where *Gonipterus* spp. larvae were collected.

Sampling site	Number of *Gonipterus* spp. larvae		Number of emerging *Anagonia* spp.			Number of parasitized larvae that failed to produce *Anagonia* spp.		Parasitism rate (%)
	Collected	Parasitized by *Anagonia* spp.	Total	Female	Male	Failed to emerge from puparium	Hyperparasitized puparium	
Channel Highway	60	1	0	0	0	1	0	1.7
Cygnet	354	6	5	3	2	1	0	1.7
Deddington	286	14	9	6*	3	5	0	4.9
Ferny Hill Road	**567**	**69**	**28**	**14**	**14**	**36**	**5**	**12.2**
Grindelwald	142	1	0	0	0	1	0	0.7
Hobart	35	1	1	1	0	0	0	2.9
Leslie Road	58	2	2	0	2*	0	0	3.4
Missing Link Road	8	0	n.a.	n.a.	n.a.	n.a.	n.a.	0.0
Mount Nelson	40	1	0	0	0	1	0	2.5
Snug	33	1	0	0	0	1	0	3.0
The Glen Road	**448**	**45**	**21**	**11**	**10**	**21**	**3**	**10.0**
Total	2043	141	67	36	31	66	8	6.9

* One female from Deddington and one male from Leslie Road are suspected to be a second, unidentified *Anagonia* species.

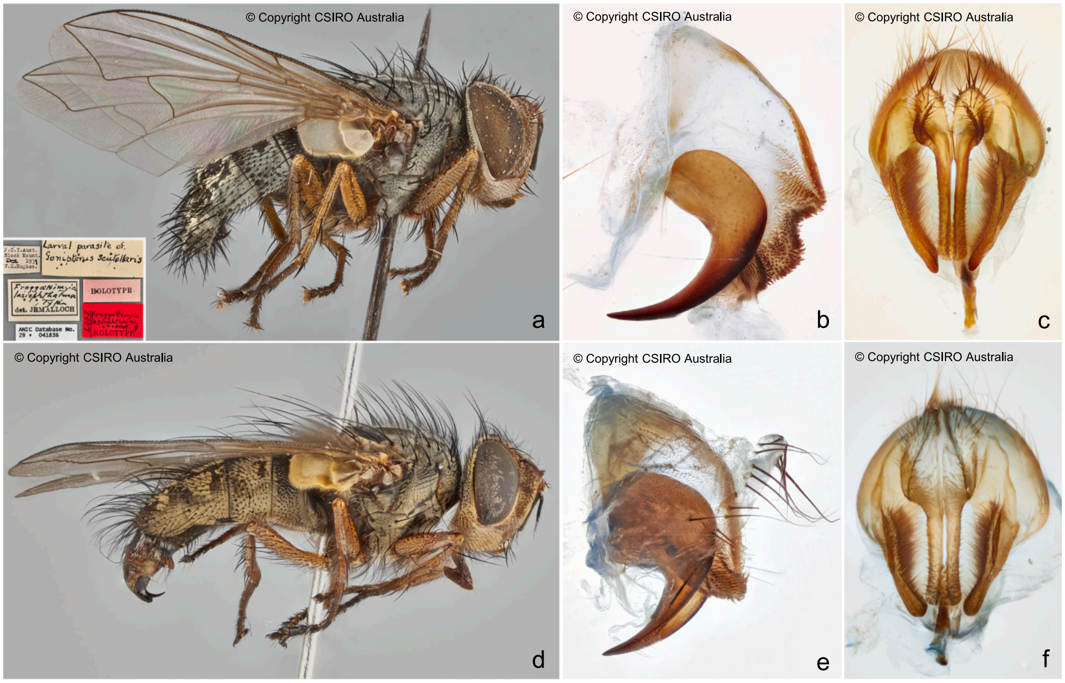

Fig. 2. Comparison between the specimens reared in Portugal and *Anagonia lasiophthalma* specimens at the Australian National Insect Collection (ANIC): (a) holotype ANIC female; (b) terminalia of ANIC female; (c) terminalia of ANIC male; (d) Portuguese female; (e) terminalia of Portuguese female with conspicuous, sharp-pointed, thorn-like piercer; (f) terminalia of Portuguese male.

is equipped with a conspicuous, sharp-pointed, curved piercer used to penetrate the host (Fig. 2e). In newly emerged unmated females, dissections revealed two ovaries containing hundreds of oocytes and undeveloped eggs (Fig. 3a), three spermathecae attached near the base of the lateral oviducts, two accessory glands, and a small and wrinkled ovisac (Fig. 3a and 3b). In ten of the eleven unmated females, no eggs were discharged from the ovaries to the ovisac. In mated females, the first eggs could be seen in the ovisac within 24 h after copulation and the ovisac extended visibly as the fertilized eggs descended towards the ovipositor. The eggs gradually accumulated along the ovisac, arranged next to each other and uniformly oriented in a single transverse row. The eggs were translucent and seemed to be fertilized prior to entering the ovisac. The first embryonated eggs, i.e., mature eggs with first instar larvae visible though the chorion, were observed on the fifth/sixth day after copulation (Fig. 3d). On the seventh/eighth day after copulation, the ovisac was mostly filled with embryonated eggs, with translucent eggs at an early development stage only at the proximal parts of the oviduct (Fig. 3e and 3f). The ovisac progressively increased in size over time, while the ovaries still had hundreds of eggs inside. Due to time constraints, a small number of females was dissected, therefore the duration of embryogenesis presented here is an approximation.

3.5. *Lifetime fecundity and longevity*

The average time required for females to initiate parasitism after emergence was 9.8 ± 2.4 days, ranging between seven and fifteen days. The average period between mating and parasitism initiation was 8.8 ± 2.4 days. Throughout their life, each female produced an average progeny of 34.6 ± 19.7 puparia, with a maximum of 67 pupae by a single female (realized fecundity). Oviposition varied with female age, with a peak between the 15th and 35th day (3rd and 5th week, respectively) after the emergence of the females (Fig. 4). During the peak, each female produced, on average, roughly six offspring per week. After this period, offspring production steadily declined and, after the tenth week, no parasitism was observed. *Post-mortem* dissections of eleven females revealed that they still contained, on average, 32 ± 27 eggs in varying stages of embryonic development in the ovisac (minimum of 11; maximum of 106), and numerous undeveloped eggs in the ovaries (not counted). The potential fecundity, i.e., number of confirmed cases of parasitism plus eggs retained in the ovisac *post-mortem*, per female, was 73 ± 21 (minimum of 32; maximum of 109). This value does not fully reflect the potential fecundity of females, as instances of superparasitism were not accounted for. As *A. lasiophthalma* is a solitary parasitoid, any surplus eggs laid in a host were not recorded. Furthermore, parasitized larvae that failed to produce puparia were not considered. The proportion of host larvae that did not successfully develop into a parasitoid or host pupa (i.e., unviable), remained relatively constant at 40.9 ± 26.2 % (mean proportion of unviable hosts per week).

The longevity of *A. lasiophthalma* female and male adults was determined in the presence and absence of hosts (Fig. 5). Longevity was found to be higher in the absence of hosts ($F_{1,66} = 23.6$, $p < 0.001$), and *post-hoc* analysis indicated significant differences only for females ($p < 0.001$). No significant differences were found between female and male longevity ($F_{1,66} = 2.4$, $p = 0.12$). The longevity of females was 84.4 ± 19.1 and 59.0 ± 21.2 days in the absence and in the presence of hosts, respectively. A similar pattern was observed for males, with a longevity of 74.4 ± 7.9 and 54.6 ± 9.7 days in the absence and in the presence of hosts, respectively. Furthermore, a weak, non-significant, direct correlation was found between female longevity and the number of offspring produced ($R^2 = 0.104$).

4. Discussion

Tachinids constitute the largest lineage of endoparasitoid insects outside the Hymenoptera, with the vast majority of hosts being phytophagous insects, including many economically significant pest species (Forbes et al., 2018; Stireman et al., 2019). Yet, despite more than a century of research, the use of tachinids as biocontrol agents has seen mixed success, with a lack of specific knowledge regarding the agent's biology, behavior and ecology often being the main reason behind the failure (and occasionally deleterious consequences) of such

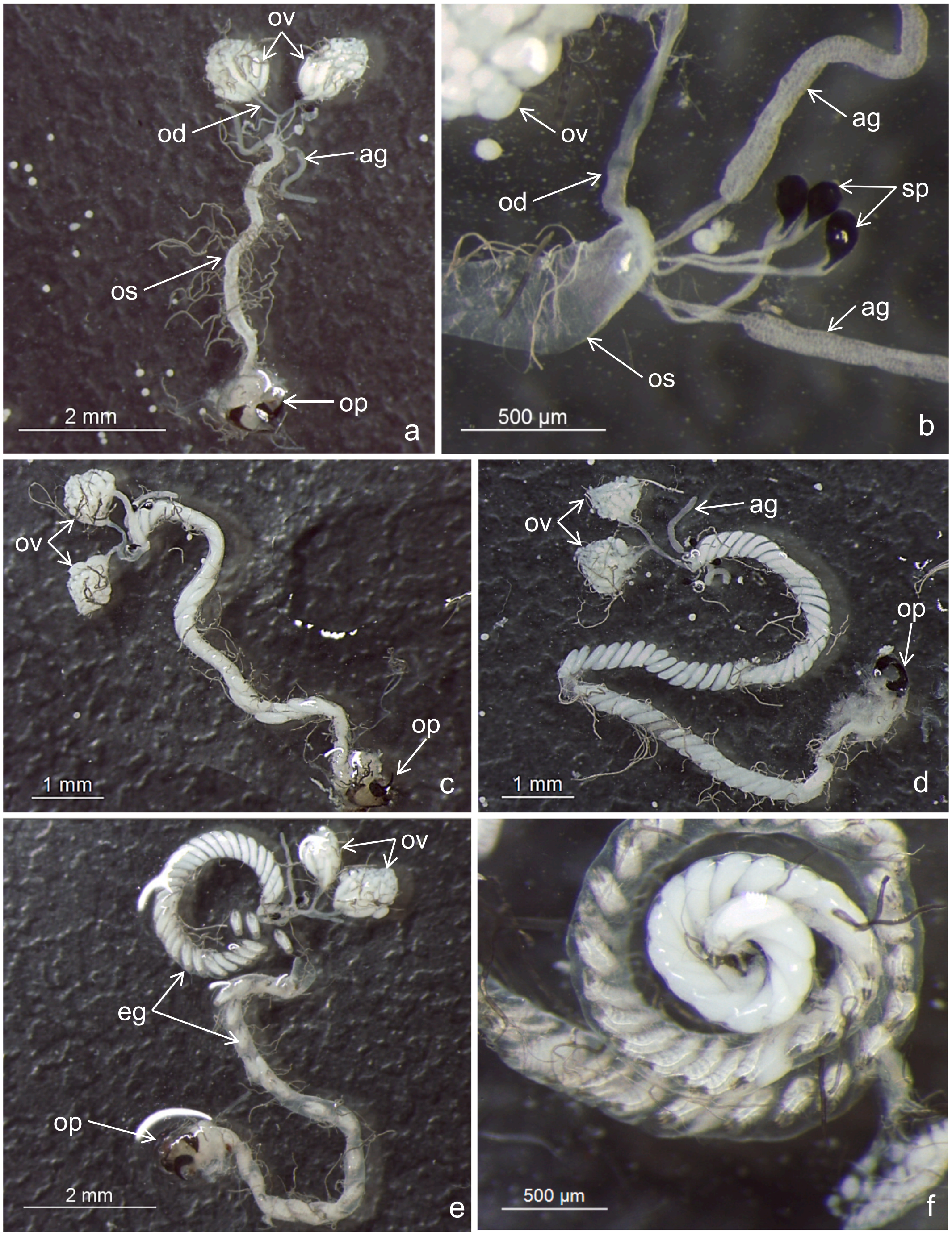

Fig. 3. Reproductive system of: (a) unmated female; (b) mated female (detail, eggs removed from ovisac); (c) mated female, one day after copulation; (d) mated female, six days after copulation, with first embryonated eggs; (e) mated female, eight days after copulation; (f) mated female, twelve days after copulation, with visible fully developed larvae (first instars) in ovisac and eggs at a very early stage of development near the oviducts. Abbreviations: ag, accessory gland; eg, egg; od, oviduct; op, ovipositor; os, ovisac; ov, ovary; sp, spermatheca. Photos a, b and e have been previously used in Gonçalves et al. (2020).

programs (Grenier, 1988; Dindo and Grenier, 2023). In this context, the biology of *A. lasiophthalma* was studied because of its potential use as a biological control agent against a key pest of eucalypts, *G. platensis*. *Anagonia lasiophthalma* is virtually unknown outside of the taxonomic literature, and the work presented here suggests that additional investigations may even be warranted in that domain, particularly with respect to the possibility that a second cryptic species may be currently treated as *A. lasiophthalma*. While a taxonomic revision was outside the scope of this study, further refinements of species boundaries and the elucidation of potential complexes could be gained through future work integrating the available morphological, molecular, and biological evidence. This would aim to test whether both observed forms of *A. lasiophthalma* are conspecific and should (ideally) include a COI sequence from Malloch's *Froggattimyia lasiophthalma* (=*Anagonia lasiophthalma*) female holotype (if recoverable).

A biological control program with a larval parasitoid of *G. platensis* is promising, as currently only egg parasitoids are available as classical biological control agents for this pest (Mayorga et al., 2013; Branco et al., 2016). Recent introductions of egg parasitoids against *G. platensis* have had limited success, most likely due to competitive advantage by

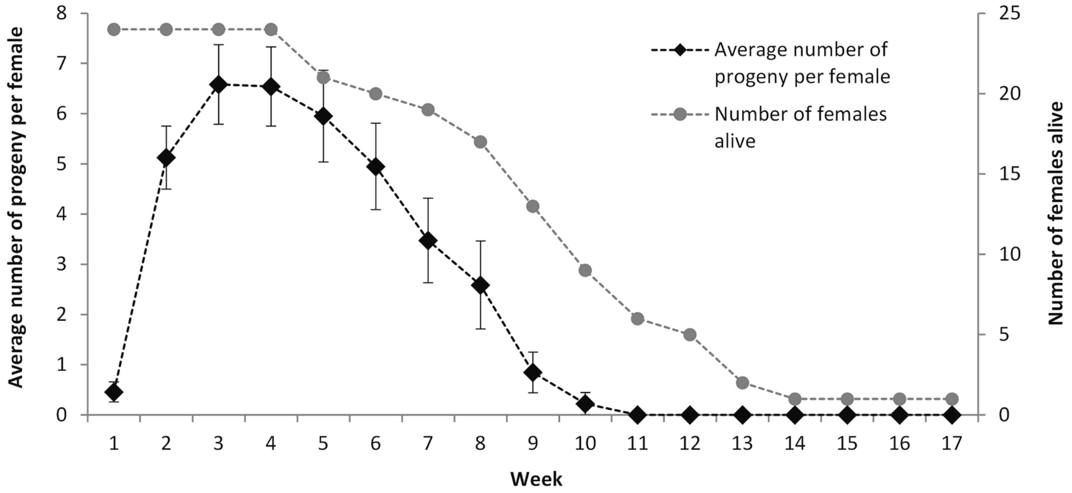

Fig. 4. Progeny (number of puparia) produced per *Anagonia lasiophthalma* female throughout the oviposition period (weeks after female emergence). Bars denote standard error.

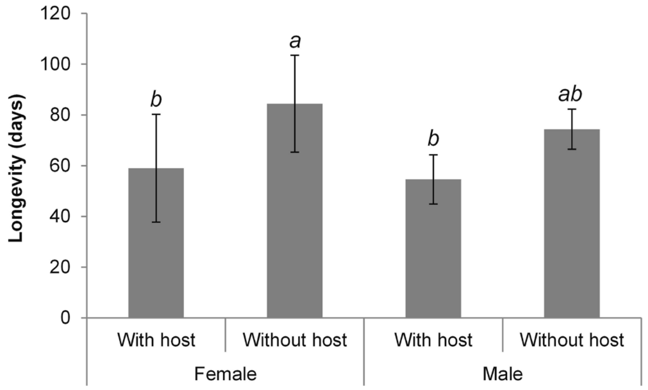

Fig. 5. Longevity of *Anagonia lasiophthalma* female and male adults, in the presence or absence of hosts (bars indicate standard deviation; different letters indicate significant differences, factorial ANOVA and *post-hoc* Tukey HSD test, $p < 0.05$).

the incumbent *A. nitens* (Valente et al., 2019). Because no larval parasitoids of *G. platensis* are known to occur outside Australia, *A. lasiophthalma* would face no competition and improved pest control may be achieved with the introduction of this species. The field survey in Tasmania suggests modest global parasitism rates by *Anagonia* spp., of 6.9 % of the *Gonipterus* spp. larvae, even though in the locations where the tachinid was most abundant, parasitism reached 10 % and 12 % (in The Glen Road and Ferny Hill Road, respectively). Previous surveys in Tasmania reported lower global parasitism rates by *Anagonia* spp. on *Gonipterus* spp., (Valente et al., 2017b). Other quantifications of attack by tachinids on *Gonipterus* spp. are included in a report by Loch (2008), describing larval parasitism of less than 5 % in Western Australia. Also, a recent study in Queensland (Souza et al., 2021) indicates a 17 % parasitism rate of *Gonipterus* spp. larvae by tachinids, although the authors identify the use of a small sample size as a caveat. There is currently no information on parasitoid-host associations between *Anagonia* species and *Gonipterus* species. While *A. lasiophthalma* can parasitize *G. platensis* effectively, it remains uncertain whether G. platensis is the preferred host compared to other *Gonipterus* species. This has implications for the specific parasitism rates on *G. platensis*.

Field parasitism rates by Blondeliini species on Coleoptera reported in the literature are highly variable in space and time, ranging from residual levels to parasitism peaks of up to 90 % (Elliott, 1978; Murray, 1982; Messina, 1983; Tanton and Epila, 1984; de Little et al., 1990; Fay et al., 1993; Lipa et al., 1998; Rice, 2005; Nahrung et al., 2008). The survey described here and previous ones (Valente et al., 2017b) focused on periods of high abundance of *Gonipterus* spp., and therefore do not capture the ecological variations of tachinid populations in time. Nevertheless, they likely underestimate the potential efficacy of *A. lasiophthalma* in a new environment for two main reasons. Firstly, in its native habitat, *Anagonia* spp. are likely to compete with other larval parasitoids, such as *E. magnificus* and *Oxyserphus* sp., which will not be the case in a novel habitat. Secondly, in Tasmania, *Anagonia* spp. are hyperparasitized by several species, which they will not encounter in a new environment, so long as proper triage is performed prior to field releases. In the present study, *Perilampus* sp. and an unidentified Tetrastichinae were observed in 5.7 % of field collected hosts, but higher hyperparasitism rates have been reported for related species. Tanton and Epila (1984) studied the parasitoid complex of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) in Australian Capital Territory, which includes the tachinids *Anagonia anguliventris* (Malloch), *Anagonia tillyardi* (Malloch), and *Paropsivora* spp. The authors found that *P. tasmanicus* and *Mesochorus* sp. (Hymenoptera: Ichneumonidae) attacked 68 % and 35 % of tachinid larvae, respectively, with multiparasitism by both species in 24 % of parasitized tachinids. In Queensland, Nahrung et al. (2008) reported that *Perilampus* sp. emerged from 17 % of tachinid pupae attacking *P. atomaria*. On the other hand, there is the possibility that native hyperparasitoids and/or predators, in Portugal or elsewhere, may affect *A. lasiophthalma*, as has been reported for other tachinid species, such as *Cyzenis albicans* Fallén (Broadley et al., 2018). The efficacy of *A. lasiophthalma* to provide additional control of *G. platensis* in a novel location can therefore only be adequately assessed under field conditions. Furthermore, factors like local environmental conditions (e.g., climate, altitude), the phenology of the host tree, or population dynamics of *G. platensis* will determine the parasitoid's ability to establish successful populations and ultimately contribute to pest control. Another aspect worth noting is that, in previous surveys (unpublished data), *Anagonia* spp. had been mostly recovered from *Gonipterus* spp. larvae collected in the southern areas around Hobart, but these specimens were never identified to the species level nor were attempts made to rear them. It is therefore possible that other *Anagonia* species may also be well adapted to *G. platensis*, but further surveys would be required to clarify this.

The success of a classical biological control program relies on the possibility of rearing the natural enemies in enough numbers to perform all the necessary studies, including efficacy and risk assessment, and ultimately field releases. Mating success is recognized as a crucial factor for tachinids, with reports of mating rates in literature varying between

21 % and 74 %, depending on female and male age, temperature, and light intensity (Quednau, 1993; Nakamura, 1994; Lauzière et al., 2001; Zhang et al., 2003; Ho et al., 2011). In this study, 58 % of the females mated within the established 10-day period after emergence, 76 % of which within 48 h after emergence, resulting in a mean pre-mating period of 1.4 days. Such age-dependent mating pattern is common among tachinids, including several Exoristinae, such as *Celatoria compressa* (Wulp) (Zhang et al., 2003), *Exorista japonica* (Townsend) (Nakamura, 1994), *Lixadmontia franki* Wood and Cave (Suazo et al., 2008), and *Zenillia dolosa* (Meigen) (Ho et al., 2011). Abdominal dissections performed in virgin and mated females revealed that *A. lasiophthalma* females emerge with an egg complement in their ovaries, which they release into the ovisac after copulation. Among unmated females, one specimen released eggs into the ovisac, a rare occurrence that was previously documented in *Lydella jalisco* Woodley by Lauzière et al. (2001). However, embryonic development never occurred in virgin females. In mated females, the observed pre-oviposition period of 8.8 days was comparable to that observed in *L. franki* at a similar temperature (21 °C) (Suazo et al., 2008).

It is widely acknowledged that tachinids that lay their eggs directly in or on their hosts tend to have high success rates but low fecundity, while species that oviposit close to a host or indiscriminately on the hosts' food plant have lower success and generally produce hundreds to thousands of eggs (O'Hara, 1985, 2008; Stireman et al., 2006). *Anagonia lasiophthalma* fits this pattern as, in the present study, fecundity was relatively low, on average 35 offspring per female throughout their lifetime. Among the Blondeliini, species that lay embryonated eggs in or on the host have comparable fecundities, such as *Balde striatum* Rice*, C. compressa*, or *Ceranthia samarensis* (Villeneuve), with average fecundities ranging between 30 and 135 (Quednau, 1993; Zhang et al., 2003, 2004; Rice, 2005). The oviposition pattern of females revealed a clear peak around the third to fifth week after emergence. While roughly half of the females lived for nine weeks or more, parasitism was residual after this date, yet *post-mortem* dissections of females revealed the presence of dozens to hundreds of eggs in the ovaries (not counted) and in the ovisac (on average, 32 eggs). This suggests that neither longevity nor fecundity should be limiting factors for *A. lasiophthalma*. In the present study, the mean longevity of females and males was 59 and 55 days, respectively, in the presence of hosts. Studies on other Exoristinae species tested at comparable temperatures (c.a. 20 °C) report longevities ranging from 14 to 23 days (females) and 12 to 20 days (males) (Lauzière et al., 2001; Zhang et al., 2004; Rice, 2005). Moreover, *A. lasiophthalma* lived significantly longer in the absence of hosts (on average, 84 days for females and 74 days for males), possibly due to lower energy costs associated with parasitism and reproductive activities, as suggested for other Exoristinae (Lauzière et al., 2001; Kalyebi and Nakamura, 2006). This has implications for the biological control of *G. platensis*, which typically has one or two population peaks per year, in spring and autumn (Cordero-Rivera et al., 1999; Ceia et al., 2021), alternating with periods of low density that can last for several months. The longevity of *A. lasiophthalma* increased in the absence of the host, which improves the probability of survival during periods of host scarcity. Specifically, some laboratory-reared insects survived for over three months at 20 °C, when honey-fed. Under field conditions, food sources may be provided by eucalypt and understory vegetation flowers, namely nectar and pollen, which are known to improve both longevity and fecundity in tachinids (Quednau, 1993; Dindo et al., 2019; Dindo and Grenier, 2023), as well as honeydew from psyllids and other sap-sucking insects. It is also possible that *A. lasiophthalma* may have developed ways to synchronize its life cycle with *Gonipterus* spp., bypassing periods of host shortage. Many tachinids display physiological integration with their host, such as *Gonia cinerascens* Rondani, in which first instar larvae will molt only at the larval-pupal transformation of their host (Baronio and Sehnal, 1980; Dindo and Grenier, 2023). In *Pseudoperichaeta nigrolineata* (Walker), parasitoid development requires hormonal signals from the host, including suspension when the host larva enters diapause (Ramadhane et al., 1987, 1988; Dindo and Grenier, 2023). In *A. lasiophthalma*, first instar larvae form respiratory funnels shortly after parasitism and quickly kill the host larvae, which suggests that physiological integration with the host is unlikely, but whether or not such mechanisms exist would require further studies. In addition, no information is currently available on other possible synchronization strategies, such as diapause or aestivation.

The use of *A. lasiophthalma* against *G. platensis* outside Australia will depend heavily on its host-specificity, i.e., the risk it may pose to native fauna in the new habitat, versus its potential benefit. There is a common perception of tachinids as a polyphagous group that is likely skewed by a small subset of highly polyphagous species (Stireman and Singer, 2003), with *Compsilura concinnata* (Meigen) being the most extreme example, with at least 180 host species of Lepidoptera and Hymenoptera (Symphyta) in North America, where it was introduced (Elkinton et al., 2006). In contrast to *C. concinnata*, the tachinid *Istocheta aldrichi* (Mesnil) was introduced into North America for control of the invasive Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), about 100 years ago, and has not spread to other hosts (Arnaud, 1978). Furthermore, studies of hosts used by some tachinids in Costa Rica have revealed that presumed generalists are actually species complexes that consist of species ranging from specialists to generalists (Smith et al., 2006, 2007). The ability of a tachinid to attack multiple host species is often associated with its ability to escape the host immune response. Many species avoid encapsulation by the host by forming respiratory funnels, as was observed in *A. lasiophthalma*, whereas some species are known to move to specific body parts or form a'cloak' formed by cells from the host (Yamashita et al., 2019; Dindo and Grenier, 2023). Several other factors may play a role in the host range of individual tachinid species, such as visual and olfactory cues for oviposition, reproductive strategy, host plant, and synchrony with the host. The ability of *A. lasiophthalma* to parasitize or otherwise affect non-target organisms will therefore need to be tested prior to its introduction.

CRediT authorship contribution statement

Cátia Martins: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Catarina Afonso:** Conceptualization, Investigation, Writing – review & editing. **Carlos Valente:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Ana Raquel Reis:** Conceptualization, Funding acquisition, Writing – review & editing. **James O'Hara:** Validation, Writing – original draft, Writing – review & editing. **James Lumbers:** Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Manuela Branco:** Conceptualization, Supervision, Writing – review & editing. **Catarina I. Gonçalves:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Arnaud Jr., P.H., 1978. A host-parasite catalog of North American Tachinidae (Diptera). United States Department of Agriculture. Miscellaneous Publication 1319, 1–860.

Baronio, P., Sehnal, F., 1980. Dependence of the parasitoid *Gonia cinerascens* on the hormones of its lepidopterous hosts. J. Insect Physiol. 26, 619–626. https://doi.org/10.1016/0022-1910(80)90031-1.

Belshaw, R., 1993. Tachinid flies. Diptera: Tachinidae. Handbooks for the Identification of British Insects Vol. 10, part 4a(i). Royal Entomological Society of London, London.

Branco, M., Battisti, A., Mendel, Z., 2016. Foliage feeding invasive insects: Defoliators and. In: Makers, G., in: Paine, T.D., Lieutier, F., . (Eds.), Insects and Diseases of Mediterranean Forest Systems. Springer International Publishing, Cham, pp. 211–238. https://doi.org/10.1007/978-3-319-24744-1_8.

Broadley, H.J., Kelly, E.A., Elkinton, J.S., Kula, R.R., Boettner, G.H., 2018. Identification and impact of hyperparasitoids and predators affecting Cyzenis albicans (Tachinidae), a recently introduced biological control agent of winter moth (Operophtera brumata L.) in the northeastern U.S.A. Biol. Control. 121, 99–108. https://doi.org/10.1016/j.biocontrol.2018.01.011.

Campinhos, E., 1999. Sustainable plantations of high-yield shape *Eucalyptus* trees for production of fiber: the Aracruz case. New For. 17, 129–143. https://doi.org/10.1023/A:1006562225915.

Cantrell, B.K., 1986. An updated host catalogue for the Australian Tachinidae (Diptera). Aust. J. Entomol. 25, 255–265. https://doi.org/10.1111/j.1440-6055.1986.tb01112.x.

Ceia, R.S., Faria, N., Lopes, P.B., Alves, J., Silva, A.A., Valente, C., Gonçalves, C.I., Mata, V.A., Santos, S.A.P., Azevedo-Pereira, H.M.V.S., Sousa, J.P., Silva, L.P., 2021. Local and landscape effects on the occurrence and abundance of the Eucalyptus weevil Gonipterus platensis (Coleoptera: Curculionidae). For. Ecol. Manag. 500, 119618 https://doi.org/10.1016/j.foreco.2021.119618.

Colless, D.H., 2012. The Froggattimyia-Anagonia genus group (Diptera: Tachinidae). Rec. Aust. Mus. 64, 167–211. https://doi.org/10.3853/j.0067-1975.64.2012.1590.

Cordero-Rivera, A., Santolamazza-Carbone, S., Andrés, J.A., 1999. Life cycle and biological control of the Eucalyptus snout beetle (Coleoptera, Curculionidae) by Anaphes nitens (Hymenoptera, Mymaridae) in north-west Spain. Agric. for. Entomol. 1, 103–109. https://doi.org/10.1046/j.1461-9563.1999.00016.x.

Costa e Silva J., Borralho, N.M.G., Araújo, J.A., Vaillancourt, R.E., Potts, B.M.,, 2009. Genetic parameters for growth, wood density and pulp yield in *Eucalyptus globulus*. Tree Genet. Genomes 5, 291–305. https://doi.org/10.1007/s11295-008-0174-9.

Crosskey, R.W., 1973. A conspectus of the Tachinidae (Diptera) of Australia, including keys to the supraspecific taxa and taxonomic and host catalogues. Bull. Br. Mus. Nat. Hist. Entomol. Supp. 19, 1–167.

de Little, D.W., Elliott, H.J., Madden, J.L., Bashford, R., 1990. Stage-specific mortality in two field populations of immature Chrysophtharta bimaculata (Olivier) (Coleoptera: Chrysomelidae). Aus. J. Entomol. 29, 51–55. https://doi.org/10.1111/j.1440-6055.1990.tb00314.x.

Dindo, M.L., Grenier, S., 2023. Production of dipteran parasitoids. In: Morales-Ramos, J. A., Rojas, M.G., Shapiro-Ilan, D.I. (Eds.), Mass Production of Beneficial Insects. Academic Press, San Diego, pp. 71–100. https://doi.org/10.1016/B978-0-12-822106-8.00003-8.

Dindo, M.L., Rezaei, M., De Clercq, P., 2019. Improvements in the rearing of the tachinid parasitoid Exorista larvarum (Diptera: Tachinidae): influence of adult food on female longevity and reproduction capacity. J. Insect Sci. 19, 1–6. https://doi.org/10.1093/jisesa/iey122.

Elkinton, J.S., Parry, D., Boettner, G.H., 2006. Implicating an introduced generalist parasitoid in the invasive browntail moth's enigmatic demise. Ecology 87, 2664–2672. https://doi.org/10.1890/0012-9658(2006)87[2664:IAIGPI]2.0.CO;2.

Elliott, H.J., 1978. Studies on the fireblight beetle, Pyrgoides orphana (Erichsen) (Coleoptera: Chrysomelidae) and its effect on the growth of silver wattle in Tasmania. Aust. for. 41, 160–166. https://doi.org/10.1080/00049158.1978.10674187.

Fay, H.A.C., Storey, R.I., Defaveri, S.G., Brown, J.D., 1993. Suppression of reproductive development and longevity in the redshouldered leaf beetle Monolepta australis (Col.: Chrysomelidae), by the tachinid, Monoleptophaga caldwelli (Dipt.). Entomophaga 38, 335–342. https://doi.org/10.1007/BF02374450.

Forbes, A.A., Bagley, R.K., Beer, M.A., Hippee, A.C., Widmayer, H.A., 2018. Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. BMC Ecol. 18, 1–11. https://doi.org/10.1186/s12898-018-0176-x.

Garcia, A., Allen, G.R., Oberprieler, R.G., Ramos, A.P., Valente, C., Reis, A., Franco, J.C., Branco, M., 2019. Biological control of *Gonipterus*: Uncovering the associations between eucalypts, weevils and parasitoids in their native range. For. Ecol. Manag. 443, 106–116. https://doi.org/10.1016/j.foreco.2019.04.004.

Gonçalves, C.I., Valente, C., Afonso, C., Martins, C., Reis, A.R., Garcia, A., Branco, M., 2020. New challenges on the journey to control an old pest: Can a tachinid come to the rescue? The Tachinid times 33, 4–13.

González-García, S., Hospido, A., Moreira, M.T., Romero, J., Feijoo, G., 2009. Environmental impact assessment of total chlorine free pulp from *Eucalyptus globulus* in Spain. J. Clean. Prod. 17, 1010–1016. https://doi.org/10.1016/j.jclepro.2009.02.017.

Grenier, S., 1988. Applied biological control with tachinid flies (Diptera, Tachinidae): a review. Anz. Schädlingskde. Pflanzenschutz, Umweltschutz 61, 49–56. https://doi.org/10.1007/BF01906254.

Hanks, L.M., Millar, J.G., Paine, T.D., Campbell, C.D., 2000. Classical biological control of the Australian weevil Gonipterus scutellatus (Coleoptera: Curculionidae) in California. Environ. Entomol. 29, 369–375. https://doi.org/10.1603/0046-225X(2000)029[0369:CBCOTA]2.0.CO;2.

Ho, G.T.T., Ichiki, R.T., Nakamura, S., 2011. Reproductive biology of the microtype tachinid fly Zenillia dolosa (Meigen) (Diptera: Tachinidae). Entomol. Sci. 14, 210–215. https://doi.org/10.1111/j.1479-8298.2010.00441.x.

Huber, J.T., Prinsloo, G.L., 1990. Redescription of Anaphes nitens (Girault) and description of two new species of Anaphes Halliday (Hymenoptera: Mymaridae), parasite of Gonipterus scutellatus Gyllenhal (Coleoptera: Curculionidae) in Tasmania. Aust. J. Entomol. Soc. 29, 333–341. https://doi.org/10.1111/j.1440-6055.1990.tb00373.x.

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. Biol. Invasions 18, 921–933. https://doi.org/10.1007/s10530-016-1081-x.

ICNF, 2019. 6.° Inventário Florestal Nacional: 2015 - Relatório Final. https://www.icnf.pt/api/file/doc/c8cc40b3b7ec8541 (accessed 23 August 2022).

Kalyebi, A., Nakamura, S., 2006. The biology of the parasitoid fly Drino inconspicuoides (Diptera: Tachinidae) in the host Mythimna separata (Lepidoptera: Noctuidae). Appl. Entomol. Zool. 41, 365–370. https://doi.org/10.9734/JSRR/2014/8522.

Kenis, M., Branco, M., 2010. Impact of alien terrestrial arthropods in Europe, in: Roques, A., Kenis, M., Lees, D., Lopez-Vaamonde, C., Rabitsch, W., Rasplus, J.-Y., Roy, D. (Eds.), Alien terrestrial arthropods of Europe. BioRisk 4, 51-71.

Lauzière, I., Legaspi, J.C., Legaspi, B.C., Smith, J.W., Jones, W.A., 2001. Life-history studies of Lydella jalisco (Diptera: Tachinidae), a parasitoid of Eoreuma loftini (Lepidoptera: Pyralidae). BioControl 46, 71–90. https://doi.org/10.1023/A:1009972316870.

Lipa, J.J., Iriarte, J., Tschorsnig, P.H., Caballero, P., 1998. Incidencia de Meigenia mutabilis (Meigen) (Diptera: Tachinidae) en poblaciones de Colaspidema atrum (Oliv.) (Coleoptera: Chrysomelidae). Bol. San. Veg. Plagas 24, 347–352.

Loch, A., 2006. Phenology of Eucalyptus weevil, Gonipterus scutellatus Gyllenhal (Coleoptera: Curculionidae), and chrysomelid beetles in Eucalyptus globulus plantations in south-western Australia. Agric. for. Entomol. 8, 155–165. https://doi.org/10.1111/j.1461-9563.2006.00294.x.

Loch, A.D., 2008. Parasitism of the *Eucalyptus* weevil, *Gonipterus scutellatus* Gyllenhal, by the egg parasitoid, *Anaphes nitens* Girault, in *Eucalyptus globulus* plantations in southwestern Australia. Biol. Control 47, 1–7. https://doi.org/10.1016/j.biocontrol.2008.07.015.

Loch, A.D., Floyd, R.B., 2001. Insect pests of Tasmanian blue gum, *Eucalyptus globulus globulus*, in south-western Australia: history, current perspectives and future prospects. Austral Ecol. 26, 458–466. https://doi.org/10.1046/j.1442-9993.2001.01145.x.

Mapondera, T.S., Burgess, T., Matsuki, M., Oberprieler, R.G., 2012. Identification and molecular phylogenetics of the cryptic species of the Gonipterus scutellatus complex (Coleoptera: Curculionidae: Gonipterini). Aust. J. Entomol. 51, 175–188. https://doi.org/10.1111/j.1440-6055.2011.00853.x.

Mayorga, S.I., Jaques, L., Peragallo, M., 2013. *Anaphes tasmaniae*, parasitoid of *Gonipterus platensis* (Marelli, 1926) (Coleoptera: Curculionidae) introduced in Chile. 4th International Symposium on Biological Control of Arthropods, Pucón, Chile.

Messina, F.J., 1983. Parasitism of two goldenrod beetles (Coleoptera: Chrysomelidae) by Aplomyiopsis xylota (Diptera: Tachinidae). Environ. Entomol. 12, 807–809. https://doi.org/10.1093/ee/12.3.807.

Murray, D.A.H., 1982. Life history of Monolepta australis (Jacoby) (Coleoptera: Chrysomelidae). Aust. J. Entomol. 21, 119–122. https://doi.org/10.1111/j.1440-6055.1982.tb01777.x.

Nahrung, H.F., Duffy, M.P., Lawson, S.A., Clarke, A.R., 2008. Natural enemies of Paropsis atomaria Olivier (Coleoptera: Chrysomelidae) in south-eastern Queensland eucalypt plantations. Aust. J. Entomol. 47, 188–194. https://doi.org/10.1111/j.1440-6055.2008.00656.x.

Nakamura, S., 1994. Parasitization and life history parameters of Exorista japonica (Diptera: Tachinidae) using the common armyworm Pseudaletia separata (Lepidoptera: Noctuidae) as host. Appl. Entomol. Zool. 29, 133–140. https://doi.org/10.1303/aez.29.133.

O'Hara, J.E., 1985. Oviposition strategies in the Tachinidae, a family of beneficial parasitic flies. Univ. Alta. Agric. for. Bull. 8 (2), 31–34.

O'Hara, J.E., 2008. Tachinid flies (Diptera: Tachinidae). In: Capinera, J.L. (Ed.), Encyclopedia of Entomology. Springer Science & Business Media, Dordrecht.

O'Hara, J.E., Henderson, S.J., Wood, D.M., 2020. Preliminary checklist of the Tachinidae of the world. accessed 23 August 2022 Version 2, 1. http://www.nadsdiptera.org/Tach/WorldTachs/Checklist/Worldchecklist.html.

Quednau, F.W., 1993. Reproductive biology and laboratory rearing of Ceranthia samarensis (Villeneuve) (Diptera: Tachinidae), a parasitoid of the gypsy moth, Lymantria dispar (L.). Can. Entomol. 125, 749–759. https://doi.org/10.4039/Ent125749-4.

Ramadhane, A., Grenier, S., Plantevin, G., 1987. Physiological interactions and development synchronisations between nondiapausing *Ostrinia nubilalis* larvae and the tachinid parasitoid *Pseudoperichaeta nigrolineata*. Entomol. Exp. Appl. 45, 157–165. https://doi.org/10.1111/j.1570-7458.1987.tb01076.x.

Ramadhane, A., Grenier, S., Plantevin, G., 1988. Photoperiod, temperature and ecdysteroid influences on physiological interactions between diapausing *Ostrinia* *nubilalis* larvae and the tachinid parasitoid *Pseudoperichaeta nigrolineata*. Entomol. Exp. Appl. 48, 275–282. https://doi.org/10.1111/j.1570-7458.1988.tb01174.x.

Reis, A.R., Ferreira, L., Tomé, M., Araújo, C., Branco, M., 2012. Efficiency of biological control of Gonipterus platensis (Coleoptera: Curculionidae) by Anaphes nitens (Hymenoptera: Mymaridae) in cold areas of the Iberian Peninsula: implications for defoliation and wood production in Eucalyptus globulus. For. Ecol. Manag. 270, 216–222. https://doi.org/10.1016/j.foreco.2012.01.038.

Rice, A.D., 2005. The larval parasitoid guild of Chrysophtharta agricola (Coleoptera: Chrysomelidae): Host-parasitoid ecological and developmental interactions. University of Tasmania. Doctoral dissertation.

SAG (2014) Informativo Fitosanitario Forestal, Nr. 9,, November 2014. Servicio Agrícola y Ganadero. Santiago, Chile.

Secf, 2010. Situación de los bosques y del sector forestal en España. Informe 2010. Sociedad Española de Ciencias Florestales, Palencia.

Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W., Hebert, P.D.N., 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). PNAS 103, 3657–3662. https://doi.org/10.1073/pnas.0511318103.

Smith, M.A., Wood, D.M., Janzen, D.H., Hallwachs, W., Hebert, P.D.N., 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. PNAS 104, 4967–4972. https://doi.org/10.1073/pnas.0700050104.

Souza, N.M., Lawson, S.A., Nahrung, H.F., 2021. Gonipterus (Coleoptera: Curculionidae) in subtropical Australia: host associations and natural enemies. Austral Entomol. 60, 588–597. https://doi.org/10.1111/aen.12552.

Stape, J.L., Binkley, D., Ryan, M.G., 2004. *Eucalyptus* production and the supply, use and efficiency of use of water, light and nitrogen across a geographic gradient in Brazil. For. Ecol. Manag. 193, 17–31. https://doi.org/10.1016/j.foreco.2004.01.020.

Stireman III, J.O., O'Hara, J.E., Wood, D.M., 2006. Tachinidae: evolution, behavior, and ecology. Annu. Rev. Entomol. 51, 525–555. https://doi.org/10.1146/annurev.ento.51.110104.151133.

Stireman III, J.O., Cerretti, P., O'Hara, J.E., Blaschke, J.D., Moulton, J.K., 2019. Molecular phylogeny and evolution of world Tachinidae (Diptera). Mol. Phylogenet. Evol. 139, 106358 https://doi.org/10.1016/j.ympev.2018.12.002.

Stireman III, J.O., Singer, M.S., 2003. What determines host range in parasitoids? An analysis of a tachinid parasitoid community. Oecologia 135, 629–638. https://doi.org/10.1007/s00442-003-1235-2.

Suazo, A., Cave, R.D., Frank, J.H., 2008. Reproductive biology and development of Lixadmontia franki (Diptera: Tachinidae), a parasitoid of bromeliad-eating weevils. Fla. Entomol. 91 (3), 453–459. https://doi.org/10.1653/0015-4040(2008)91[453:RBADOL]2.0.CO;2.

Tanton, M.T., Epila, J.S.O., 1984. Parasitization of larvae of Paropsis atomaria Ol. (Coleoptera: Chrysomelidae) in the Australian Capital Territory. Aust. J. Zool. 32, 251–329. https://doi.org/10.1071/ZO9840251.

Tooke, F.G.C., 1995. The *eucalyptus* snout-beetle, *Gonipterus scutellatus* Gyll. A study of its ecology and control by biological means. Entomology Memoirs, Department of Agriculture and Forestry, Pretoria.

Valente, C., Afonso, C., Gonçalves, C.I., Alonso-Zarazaga, M.A., Reis, A.R., Branco, M., 2017a. Environmental risk assessment of the egg parasitoid *Anaphes inexpectatus* for classical biological control of the *Eucalyptus* snout beetle, *Gonipterus platensis*. BioControl 62, 457–468. https://doi.org/10.1007/s10526-017-9809-9.

Valente, C., Gonçalves, C.I., Reis, A.R., Branco, M., 2017b. Pre-selection and biological potential of the egg parasitoid *Anaphes inexpectatus* for the control of the *Eucalyptus* snout beetle, *Gonipterus Platensis*. J. Pest Sci. 90, 911–923. https://doi.org/10.1007/s10340-017-0839-y.

Valente, C., Gonçalves, C.I., Monteiro, F., Gaspar, J., Silva, M., Sottomayor, M., Paiva, M. R., Branco, M., 2018. Economic outcome of classical biological control: a case study on the *Eucalyptus* snout beetle, *Gonipterus platensis*, and the parasitoid *Anaphes nitens*. Ecol. Econ. 149, 40–47. https://doi.org/10.1016/j.ecolecon.2018.03.001.

Valente, C., Afonso, C., Gonçalves, C.I., Branco, M., 2019. Assessing the competitive interactions between two egg parasitoids of the Eucalyptus snout beetle, *Gonipterus platensis*, and their implications for biological control. Biol. Control 130, 80–87. https://doi.org/10.1016/j.biocontrol.2018.10.002.

Wingfield, M.J., Slippers, B., Hurley, B.P., Coutinho, T., Wingfield, B.D., Roux, J., 2008. Eucalypt pests and diseases: growing threats to plantation productivity. South. for. 70, 139–144. https://doi.org/10.2989/SOUTH.FOR.2008.70.2.9.537.

Wood, D.M., 1985. A taxonomic conspectus of the Blondeliini of North and Central America and the West Indies (Diptera: Tachinidae). Mem. Entomol. Soc. Can. 117, 3–130. https://doi.org/10.4039/entm117132fv.

Yamashita, K., Zhang, K., Ichiki, R.T., Nakamura, S., Furukawa, S., 2019. Novel host immune evasion strategy of the endoparasitoid *Drino inconspicuoides*. Bull. Entomol. Res. 109, 643–648. https://doi.org/10.1017/S0007485318001049.

Zhang, F., Toepfer, S., Kuhlmann, U., 2003. Basic biology and small-scale rearing of Celatoria compressa (Diptera: Tachinidae), a parasitoid of Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). Bull. Entomol. Res. 93, 569–575. https://doi.org/10.1079/BER2003264.

Zhang, F., Toepfer, S., Riley, K., Kuhlmann, U., 2004. Reproductive biology of Celatoria compressa (Diptera: Tachinidae), a parasitoid of Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). Biocontrol Sci. Technol. 14, 5–16. https://doi.org/10.1080/09583150310001606552.