

New challenges on the journey to control an old pest: can a tachinid come to the rescue?



Figure 1. *Gonipterus platensis* adults.

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Introduction

Eucalypts (*Eucalyptus* L'Hér., Myrtaceae) are among the most commonly introduced tree species in plantations worldwide (Wingfield *et al.* 2008). In Portugal, eucalypts were introduced in the mid 19th Century, but rapid expansion did not take place until the 1950s. Currently, the Tasmanian blue gum, *Eucalyptus globulus* Labill., is the most extensively planted forest species in the country, and it is the main source of raw material for the pulp and paper industry (Alves *et al.* 2007).

The Australian eucalypt snout beetles, *Gonipterus* spp. (Coleoptera: Curculionidae) are among the major pests of eucalypts wherever they have been introduced. *Gonipterus platensis* Marelli (Fig. 1) is the most widespread species found outside Australia, including New Zealand, eastern and western South America, southwestern North America, and southwestern Europe (Mapondera *et al.* 2012).

One of the curiosities regarding the management of snout beetles is that a single parasitoid species, *Anaphes nitens* (Girault) (Hymenoptera: Mymaridae) (Fig. 2), has been responsible for keeping these pests mostly under control in the majority of their current distribution. First released in South Africa almost a century ago, the success of this tiny egg parasitoid was so spectacular that a monument was erected to it in 1995 in that country (Londt 1996).



Figure 2. *Anaphes nitens* female parasitizing *Gonipterus platensis* egg capsule.

In southwestern Europe, *G. platensis* was detected in 1991 in Spain, from where it quickly dispersed to Portugal and became the main pest of eucalypts. Biological control with *A. nitens* quickly ensued and, like elsewhere in the world, satisfactory control was obtained in most of the country (Pérez Otero *et al.* 2003, Valente *et al.* 2004). However, in cooler regions of Portugal and Spain, *G. platensis* attacks remain high. In these regions, parasitism rates by *A. nitens* are low during peak oviposition periods of *G. platensis*, resulting in severe defoliation (Fig. 3) of eucalypt trees (Reis *et al.* 2012, Valente *et al.* 2018b).

For decades, the eucalypt snout beetle was considered to be a single species, *Gonipterus scutellatus* Gyllenhal, but it is in fact a complex of cryptic species (Mapondera *et al.* 2012). This realization led us to wonder if there was partial mismatch between the Tasmanian native *G. platensis* and *A. nitens*, which is native to southern mainland Australia. In addition, *E. globulus*, which is highly susceptible to *G. platensis*, is also originally from Tasmania. We then hypothesized that Tasmanian native parasitoids might be a better fit to control *G. platensis* or add to the control already exerted by *A. nitens*.



Figure 3. Severe defoliation by *Gonipterus platensis* in *Eucalyptus globulus* plantation, in Barcelos, Portugal.

Early surveys (2008–2012) – Egg parasitoids

The journey for alternative natural enemies of *G. platensis* in Portugal began in 2008, when one of us, Carlos Valente, travelled to Australia to collect and identify prospective species. He was aided by several people along the way, most notably Australian researchers David de Little, Jane Elek, Mamoru Matsuki, and Rolf Oberprieler. In Tasmania, Carlos surveyed 30 locations, collecting egg capsules and larvae of *Gonipterus* spp. From 800 egg capsules, 235 parasitoids emerged, of which 94% were *Anaphes tasmaniae* Huber & Prinsloo and 4% were *A. inexpectatus* Huber & Prinsloo (unpublished

data). This was a very exciting finding, as *A. tasmaniae* showed great promise, but we did not yet know if it was well adapted to *G. platensis*. It is important to note that, at this point, clarification on the identity of the species composing the *G. scutellatus* complex was not yet fully available.

In addition to the egg parasitoids, several *Gonipterus* larvae were found to be parasitized by tachinid flies. At the time, however, and given that very little was known about this group of insects, further research on the tachinids was placed on hold.

The following year, we obtained authorization to import parasitized egg capsules and larvae of *Gonipterus* spp. and to study the emerging parasitoids under confinement, in Portugal. Between 2009 and 2012, more than 5100 egg capsules and 1250 larvae were collected in thirteen locations in Tasmania (Fig. 4) and imported into quarantine. Unsurprisingly, the majority of emerging egg parasitoids were *A. tasmaniae*, followed by *A. inexpectatus*. Three other parasitoids were recovered from egg capsules: *Centrodora damoni* (Girault) (Hymenoptera: Aphelinidae), *Cirrospilus* sp. (Hymenoptera: Eulophidae), and *Euderus* sp. (Hymenoptera: Eulophidae), but were rare. In 2012, we were surprised to find a few specimens of *A. nitens* in Tasmania, which seems to be a recent invasion. In addition, at least three parasitoid species were recovered from the larvae, namely *Entedon magnificus* (Girault & Dodd) (Hymenoptera: Eulophidae), *Anagonia* cf. *lasiophthalma* (Malloch) (Diptera: Tachinidae), and *Oxyserphus* sp. (Hymenoptera: Proctotrupidae) (Valente *et al.*, 2017b).

While *A. tasmaniae* was initially the most promising candidate for a biological control program against the snout beetle, it was *A. inexpectatus* that most readily accepted *G. platensis* eggs and became established under laboratory conditions. After several unsuccessful attempts to rear *A. tasmaniae*, we therefore focused on *A. inexpectatus*. Following a set of studies on its biology and risk assessment (Valente *et al.*, 2017a, b), *A. inexpectatus* has now been widely released in Portugal. Seven years after the initial releases, this parasitoid seems to have established, but field monitoring indicates it is still rare (unpublished data). Competition studies (Valente *et al.* 2018a) suggest that *A. inexpectatus* and *A. nitens* should be able to coexist, but whether the former species will eventually help to reduce pest levels in a significant way is still uncertain.

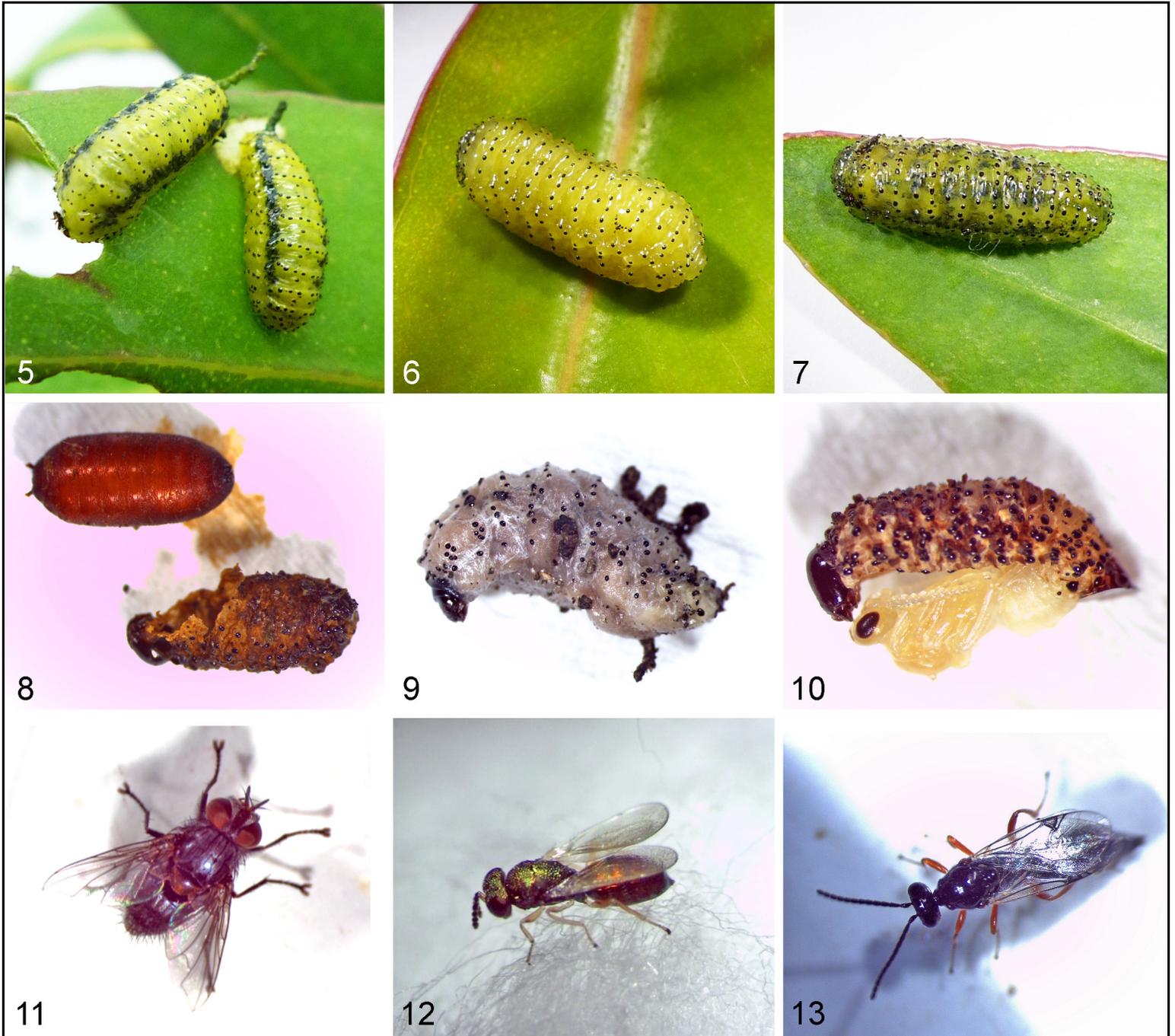
Recent surveys (2016–2017) – Larval parasitoids

After the biological control programs with *A. nitens* and *A. inexpectatus*, our focus shifted to larval parasitoids, as this life stage of the snout beetle is currently free of natural enemies outside its native range. Our results, which have been partly published in Valente *et al.* (2017b) and Garcia *et al.* (2019), can be summarized here as follows. In 2016 and 2017,



Figure 4. Collection sites for egg capsules and larvae of *Gonipterus* species in Tasmania in 2012.

over 3500 *Gonipterus* spp. larvae were imported from Tasmania. At least four *Gonipterus* species were present in the imported material: *G. notographus* Boisduval, *G. platensis*, *G. pulverulentus* Lea, and *Gonipterus* sp. n. 1 (*sensu* Mapondera *et al.* 2012, Garcia *et al.* 2019) (Figs. 5–7). The same three larval parasitoids that were identified in 2012 emerged from this material; i.e., *E. magnificus*, *A. cf. lasiophthalma* and *Oxyserphus* sp. (Figs. 8–13). Larval parasitism rates were 12.0% in 2016 and 14.5% in 2017 (Fig. 14). It is worth noting that more species may have emerged, as these three parasitoid groups may in fact be cryptic species complexes.



Figures 5–13. 5. Larvae of *Gonipterus platensis*. 6. Larva of *Gonipterus pulverulentus*. 7. Larva of *Gonipterus* sp. n. 1. 8–13. Parasitoid species that emerged from the *Gonipterus* larvae imported from Tasmania. 8. Puparium of *Anagonia cf. lasiophthalma* and host remains. 9. Larva of *Gonipterus* sp. parasitized by *Entedon magnificus*. 10. Larva of *Gonipterus* sp. parasitized by *Oxyserphus* sp. 11. *Anagonia cf. lasiophthalma*. 12. *Entedon magnificus*. 13. *Oxyserphus* sp.

The next step was establishing laboratory populations of the larval parasitoids. Although *E. magnificus* females accepted *G. platensis* larvae, residual numbers of female progeny emerged. In 2016, the parental generation started with more than 750 adults (40% females) that successfully parasitized 91 *G. platensis* hosts and originated 427 progeny. However, only 3% were female, and the population went extinct in the second laboratory generation. To this day, we have not been able to understand what conditions were missing to trigger female production. A similar situation occurred with *Oxyserphus* sp. A total of 39 individuals (60% females) were offered *G. platensis* larvae. Thirty-three larvae were parasitized, from which 13 male and no female *Oxyserphus* sp. emerged. After an initially failed attempt to rear the tachinid *A. cf. lasiophthalma*, in 2017 this species reproduced successfully and has been continuously reared for six generations.

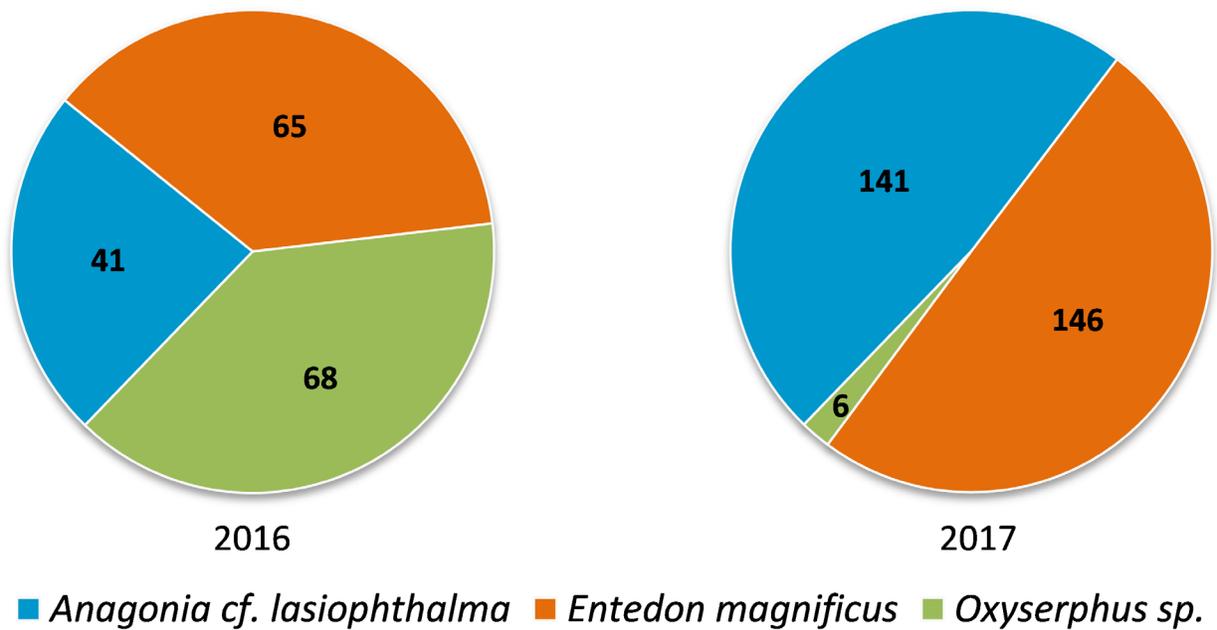


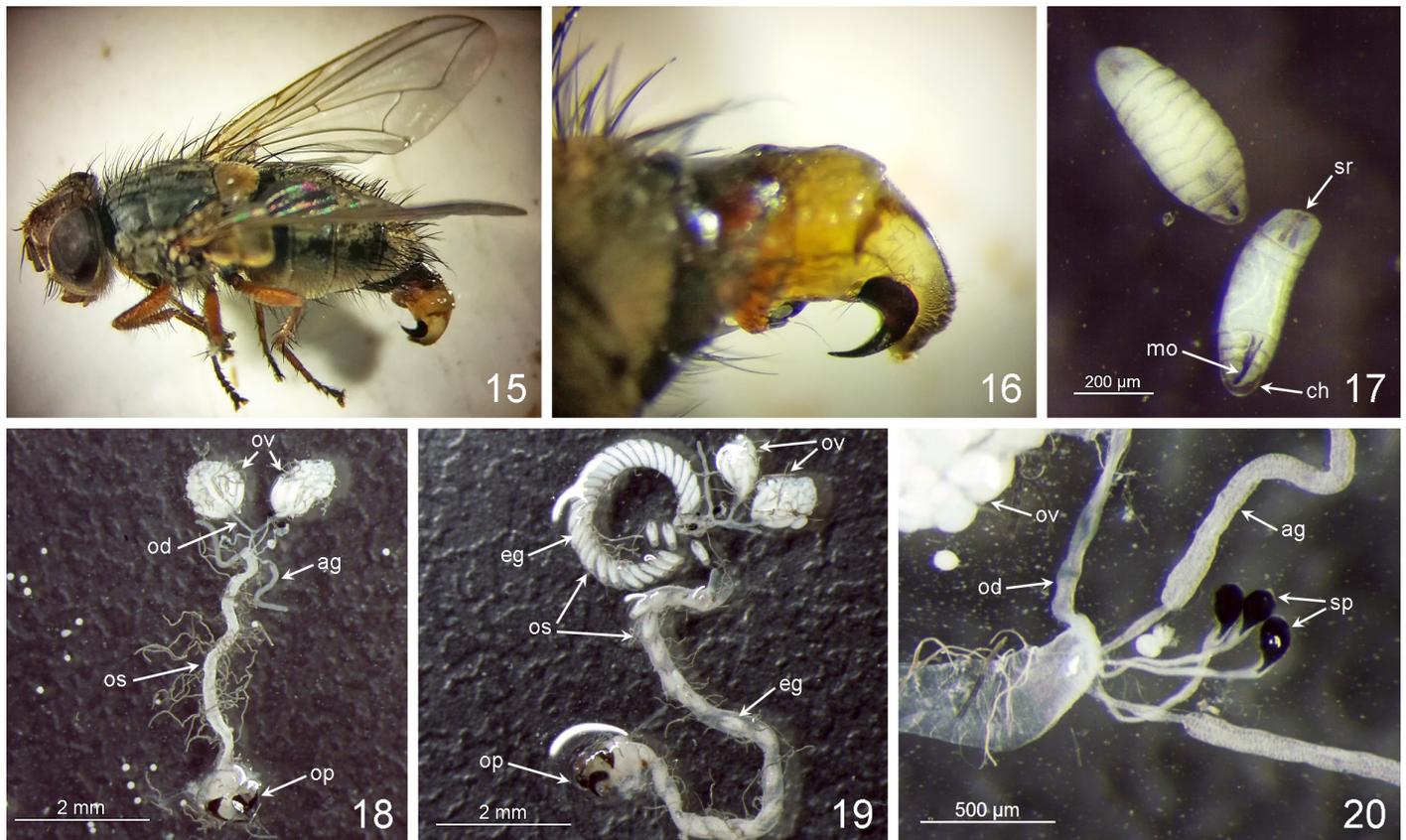
Figure 14. Number of imported larvae parasitized by each parasitoid species, in 2016 and 2017.

Besides the lack of appropriate stimuli under laboratory conditions, as was likely the case in our *E. magnificus* rearing attempt, parasitoid-host mismatch may also explain why some parasitoids were successfully reared on *G. platensis* while others were not. It was more often than not difficult to assign a larval type to the emerging parasitoid. As larvae develop, their exterior characteristics change. For instance, the characteristic lateral stripes of *G. platensis* only become visible on third instar larvae. However, as larvae begin to show evidence of being parasitized, their distinguishing features fade, often before positive identification of the host larva is made. Molecular studies were used to provide insight into the association between *Gonipterus* spp. and their egg parasitoids (Garcia *et al.* 2019), but such methods have not yet been successfully used for larval parasitoids. Because our aim has been to retrieve as many parasitoids as possible, DNA analyses of the hosts were attempted only on larval remains, after parasitoids had emerged. It is possible that fresh tissue from parasitized larvae, prior to parasitoid emergence, would have to be used. However, our records indicate that most *A. cf. lasiophthalma* emerged from the *G. platensis* morphotype (as illustrated in Fig. 5), suggesting that this species may very well be a preferred host in the wild.

Anagonia cf. lasiophthalma as a biological control agent

In our efforts to initiate a biological control program with *A. cf. lasiophthalma*, we started by devising a rearing protocol in the laboratory. The initial steps were empirical, as we did not know the reproductive strategy of this poorly studied genus, which led us to study its basic biology. In addition to the pragmatic need to maintain *A. cf. lasiophthalma* populations under confinement, understanding the life history of this potential biological control agent is paramount for a sound risk/benefit analysis. It is widely accepted that the ideal natural enemy to be used in classical biological control (*sensu* Eilenberg *et al.* 2001¹) should be host specific, well adapted to the climatic conditions of the target habitat and to the pests' life cycle, and have good host searching ability and mobility, among other traits.

While biological studies are still underway and unpublished, we have clarified some characteristics. The first major trait of the species is that the females insert their eggs into the host larvae, a mechanism that is common to some members of the Blondeliini (Stireman *et al.* 2006). The female terminalia are equipped with a conspicuous, sharp-pointed, curved piercer (Figs. 15, 16), which it uses to penetrate the host. We have determined that females will mostly mate within 48 hours after emergence and require an additional week (at 20°C) before they start parasitizing hosts. Dissections revealed that the species is ovolarviparous; i.e., after mating, the eggs are incubated in the uterus, with the distal portions of the ovisac containing fully embryonated eggs (Figs. 17–20). What we have gathered so far was valuable to developing a rearing protocol for *A. cf. lasiophthalma*, and populations have thrived (Figs. 21, 22–30).



Figures 15-20. 15. Female *Anagonia cf. lasiophthalma*. 16. Terminalia of female *A. cf. lasiophthalma* with conspicuous, sharp-pointed, thorn-like piercer. 17. Embryonated eggs. 18. Reproductive system of unmated female. 19. Reproductive system of mated female, 12 days after copulation. 20. Detail of the reproductive tract of mated female, 6 days after copulation (eggs removed from ovisac). Abbreviations: ag, accessory gland; ch, chorion; mo, mouthparts; od, oviduct; op, ovipositor; os, ovisac; ov, ovary; sp, spermatheca; sr, spiracle.

¹"The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control".

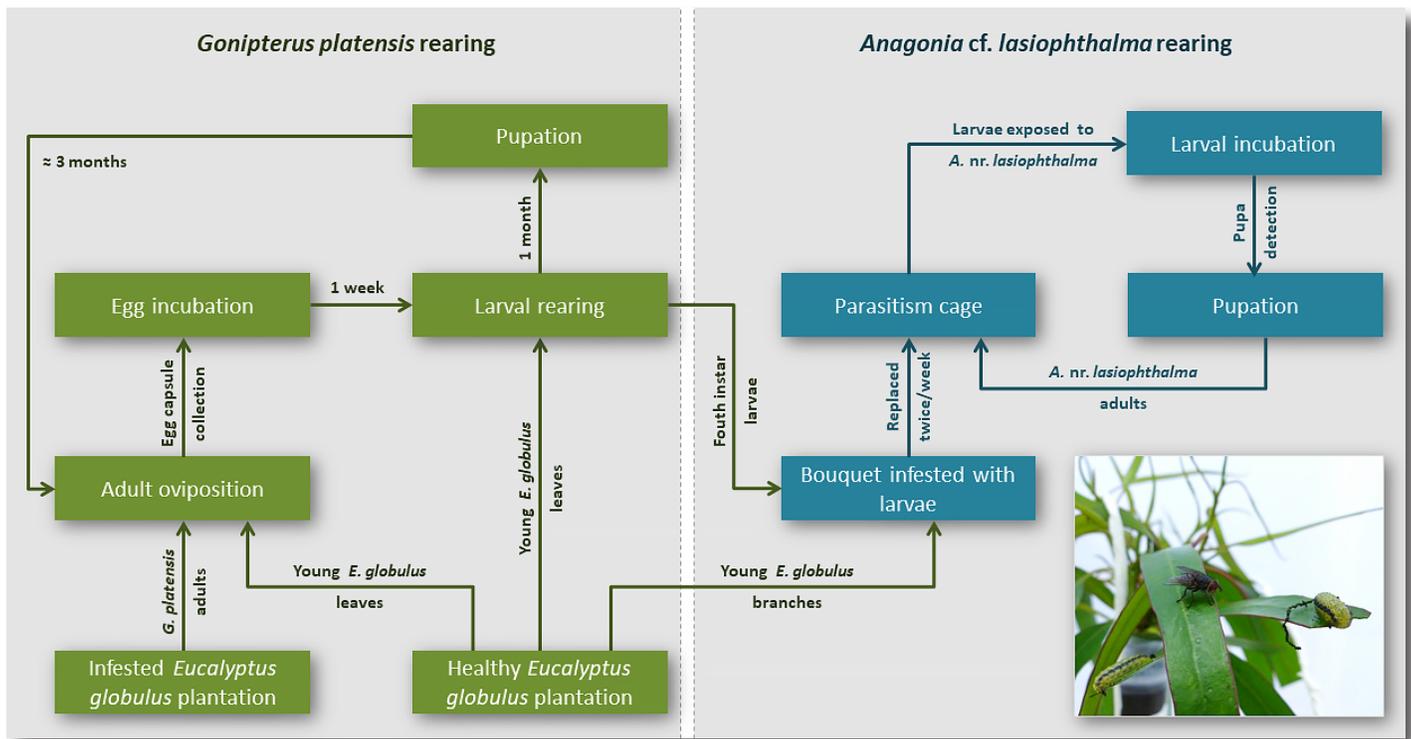


Figure 21. Rearing scheme of *Anagonia cf. lasiophthalma* and its host *Gonipterus platensis*.

There is still much information to be gathered on *A. cf. lasiophthalma* and its possible use as a biological control agent against *G. platensis*. Because many tachinid species have a wide range of hosts, the major deal-breaker will likely be potential non-target effects, particularly host specificity. The suitability of non-target organisms for the development of *A. cf. lasiophthalma* offspring will need to be tested. Furthermore, we will need to ascertain if females may kill unsuitable non-target species when attempting to parasitize them. In fact, we have observed that the largest fourth (and final) instar larvae of *G. platensis* are more likely to produce viable offspring, while smaller third or fourth instar larvae will often die before the *Anagonia* larva is mature.

At this point, it is impossible to predict how host-specific *A. cf. lasiophthalma* may be. If testing under confinement reveals that it is not strictly monophagous, risk analysis may take into account mitigating factors. For instance, we suspect that *A. cf. lasiophthalma* females respond to stimuli from the environment to find and parasitize their hosts. While it is not yet clear which cues may be relevant, such as chemical stimuli from the host plants or the hosts themselves, or visual cues, this may impact the probability of *A. cf. lasiophthalma* dispersing outside of the target environment (i.e., eucalypt plantations) and physically encountering putative non-target organisms.

The aim of a classical biological control program is for natural enemies to establish and sustain permanent populations, but whether *A. cf. lasiophthalma* populations will (or will not) adapt to the target environment is unknown at this point. Notably, *G. platensis* populations typically have one or two population peaks each year, in spring and fall, alternating with periods of low populations that can last several months. How *A. cf. lasiophthalma* will survive such fluctuations is unclear, but there are several possibilities. Specifically, some laboratory-reared insects have been found to live for over three months at 20°C, as long as they are honey-fed. In the wild, food sources may be provided by both eucalypt flowers and understory vegetation. It is also possible that *A. cf. lasiophthalma* may have developed ways to synchronize its life cycle with that of its host through such mechanisms as diapause or aestivation.



Figures 22–30. 22. Arrival of *Eucalyptus globulus* branches to the laboratory. 23. *Gonipterus platensis* adults and egg capsules in rearing cages. 24. Larvae maintenance. 25. *Anagonia* sp. maintenance. 26. Eucalypt bouquet with *G. platensis* larvae. 27. *Anagonia* cf. *lasiophthalma* female parasitizing *G. platensis* larva. 28. Unparasitized *G. platensis* larvae. 29. Parasitized *G. platensis* larvae. 30. *Anagonia* cf. *lasiophthalma* puparium. Pictured are RAIZ employees Rui Gomes (Fig. 22), Sofia Simões (Fig. 24) and Cátia Martins (Fig. 25).

The identity of *Anagonia* cf. *lasiophthalma* (Malloch)

As we unraveled the life history characteristics of *A. cf. lasiophthalma* that suggest it may indeed be a good biological control agent against the snout beetle, we began working towards giving it a name. In fact, until a few weeks ago, we were referring to this tachinid as *Anagonia* sp. We are indebted to Bryan Cantrell for providing a genus identification in an earlier stage, and to he and Jim O’Hara for narrowing the possibilities to *A. lasiophthalma*, *A. dayi* Colless, or perhaps an unidentified species. The late Donald H. Colless’ review of the *Froggattimyia*-*Anagonia* genus group (Colless 2012) is the best available identification guide to members of this group, but a more reliable identification would involve direct comparisons between specimens of our species and specimens identified by Donald Colless (J. O’Hara, pers. comm.). We are

currently pursuing this with the help of the Australian National Insect Collection (ANIC) team in Canberra (Australia). To date, a morphological comparison by James Lumbers, a Ph.D. student at ANIC studying Tachinidae, has revealed a close similarity between our specimens and *A. lasiophthalma*. However, our specimens are not an exact match and therefore could be either the same or a different species. James suggested we use the name *A. cf. lasiophthalma* for now and should use molecular data to resolve this species problem. ANIC has recently provided us with reliably identified specimens of *A. lasiophthalma* that we can use for DNA extraction and comparison with our specimens. This molecular work is currently being performed at the Research Center in Biodiversity and Genetic Resources (CIBIO) in Porto (Portugal) by Vanessa Mata.

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