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## Parasitoids of the uniquely social colletid bee *Amphylaeus morosus* (Hymenoptera: Colletidae) in Victoria

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### Abstract

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Understanding how nest parasites contribute to brood mortality rates in host species is an important step towards uncovering the potential implications for host behaviour. This can be especially important for understanding the evolution of social living, where defence against parasites is often posited as a major benefit of cooperative nesting. Only two parasitoid species have previously been reported for the only known social colletid bee, *Amphylaeus morosus*: the gasteruptionid wasp, *Gasteruption primotarsale*, and the mutillid, *Ephutomorpha tyla*. Here we report six additional parasitoid species of *A. morosus*: the gasteruptionid wasps *G. atrinerve*, *G. globiceps*, *G. melanopoda* and *G. cinerescens*; the bombyliid fly *Anthrax maculatus*; and the mutillid wasp *Ephutomorpha* aff. *varipes*. The mechanisms of parasitism for these eight parasitoid species are described in combination with how they operate throughout the host brood rearing period and whether benefits of social nesting vary across the season.

### Keywords

Bombyliidae, Gasteruptionidae, host-association, Hylaeinae, Mutillidae, parasitism

### Introduction

Detailed host and parasite association data are important yet rarely explored for primitively social hosts, where defence against parasites is posited to be a driver of cooperative nesting. For insect parasitoids, the value of this information is often emphasised but is heavily weighted towards key taxon groups, such as Ichneumonidae, Braconidae, Chalcididae, Encyrtidae, Tachinidae and Bombyliidae, that are of economic importance (Heraty, 2017). Entomophagous insect associations with non-apid bees largely get overlooked but can have substantial consequences for brood mortality, behaviour and demography of host species (Segers et al., 2016; Smith et al., 2017). Additionally, parasite–host interactions in bees are seldom observed over their full life history, meaning the full series of parasites attacking a single host is often understated. In the small carpenter bees *Ceratina dupla* Say, 1837, and *C. calcarata* Robertson, 1900, Vickruck et al. (2010) reared a total of eight parasite species from brood cells after sampling nests across a six-month period. Weislo et al. (2004) similarly sampled nests of the halictine bee *Megalopta genalis* (Meade-Waldo, 1916) across one year and reported four brood parasites infiltrating nests.

However, Smith et al. (2017) noted that the full effect these brood parasites may have on the social nesting of *M. genalis* is hard to fully assess due to insufficient field data. For analogous species, the need for descriptive biological information combined with rigorous sampling across the colony life cycle is often needed to determine the true diversity and effect of brood parasitism.

The Australian hylaeine bee, *Amphylaeus morosus* (Smith, 1879) (Colletidae: Hylaeinae), is the only species in the hyper-diverse bee family Colletidae that is unambiguously known to exhibit social nesting. Social nesting in this species is very simple, with no known reproductive hierarchies or morphological differentiation between nestmates. *Amphylaeus morosus* has a semelparous and univoltine life cycle and may form social or solitary nests within the same population (Spessa et al., 2000). In the Dandenong Ranges, *A. morosus* uses naturally abscised dead fronds of the rough fern tree *Cyathea australis* to construct linear nests out of the pithy interior. Like many hylaeine bees, *A. morosus* mass provision cells with a mixture of pollen and nectar before ovipositing into the cells.

Spessa et al. (2000) first reported a species of *Gasteruption* wasp, later identified as *Gasteruption primotarsale* Pasteels, 1957 (Gasteruptionidae) (Parslow et al.,

2020), attacking nests of *A. morosus* in the Dandenong Ranges, Victoria, while a species of mutillid wasp, *Ephutomorpha tyla* Hearn, Williams & Parslow, 2019 (Mutillidae), was recently reported entering nests late in the brood rearing season (Hearn et al., 2019). Spessa et al. (2000) found that social nesting in *A. morosus* seemed to provide only minimal benefits in reducing parasitism by *G. primotarsale*. However, that study did not consider possible defence benefits against other parasites, and indeed no other parasites were identified in that study. *Gasteruption primotarsale* parasitises the earliest brood during nesting period and may therefore only explain potential benefits of social nesting during the early stages of brood provisioning. Additionally, observations of *E. tyla* adults in the nest towards the end of brood-rearing (Hearn et al., 2019) suggest that *E. tyla* may exploit the mature larvae of *A. morosus* towards the end of its nesting period. It is important to understand the full suite of parasites that attack *A. morosus* because if these parasites operate at other times in the nesting period, that information will further our understanding of why social nesting in *A. morosus* persists until brood maturation. In south-eastern Queensland, Houston (1969) reported a range of parasites and parasitoids attacking *A. morosus* nests, including the wasps *Agamerion pulchra* (Pteromalidae: Cleonyminae), *Coelopencyrtus* spp. (Encyrtidae), *Gasteruption* sp. (Gasteruptionidae) and an acarid mite. However, in the Dandenong Ranges, little is known about the full range of parasites that might be involved.

Here, we identify eight different parasitoid species that attack *A. morosus*, including novel host records for the *Gasteruption* wasps *G. atrinerve* (Kieffer, 1911), *G. globiceps* Pasteels, 1957, *G. melanopoda* Pasteels, 1957, and *G. cinerescens* Schletterer, 1885, the bombyliid fly *Anthrax maculatus* Macquart, 1846 (Diptera: Bombyliidae), and the mutillid wasp *Ephutomorpha* aff. *varipes* (André, 1895). We examine the specific mechanisms of parasitism and eclosion timings of these parasitoids, which have been poorly understood. It is important that the respective host associations are well documented to fully recognise how these multiple parasitoid species may influence the nesting behaviour of the only known social bee in the colletid family.

## Materials and methods

### Nest collections

Nests of *Amphylaeus morosus* were collected from naturally abscised fronds that haphazardly fall around the base of the rough tree fern *Cyathea australis* R.Br. Domin. These nests were collected in the Gembrook, Cockatoo and Marysville regions of the Dandenong Ranges, Victoria, Australia. Nests were sampled over five collection periods: 4–6 December 2017, 6–8 December 2018, 21–22 November 2019, 11 January 2020 and 27 February 2020. The collection region primarily consists of cool temperate montane habitat dominated by tall canopies of *Eucalyptus regnans* F. Muell and *E. viminalis* Labill, with a mixture of ferny, shrubby and grassy understories. Nests were collected early in the morning, late in the afternoon or during periods of light

rain when bees were inactive to ensure all colony members were present. Nest entrances were sealed upon collection and all nests were stored in cool insulated boxes and transported to the laboratory where they were stored at 10°C until processed.

### Parasitism data

Nests were dissected lengthwise and the contents, including adult female *A. morosus*, immatures and nest parasitoids, were recorded. Where possible, parasitised cells were carefully extracted from the nests and left to develop in Petri dishes at ambient room temperature. Petri dishes were kept moist by occasionally spraying filter paper rectangles inside each dish with water. Once the specimens had reached adulthood, defined as fully mature and mobile, they were placed in 99% ethanol for preservation and later identification. For parasitoids, where the pupal stages form cocoons (e.g. Mutillidae), at least one pupal case from each nest was dissected in coordination with the nest being opened to determine the developmental stage of each immature.

Not all the *Gasteruption* specimens were successfully reared to pupae or adulthood because developmental rates for *Gasteruption* species lagged significantly behind host maturation rates; due to this, the specific parasitisation rates for each *Gasteruption* species were unable to be determined. Further, the scarcity of larval descriptions based on morphology for the genus *Gasteruption* made it impossible to discriminate species at the larval stage (Bogusch et al., 2018). Therefore, the parasitisation rates for each species are combined and treated at the genus level *Gasteruption*.

### Specimen identification

Specimens were examined using a Nikon SMZ1000 stereomicroscope at the South Australian Museum, Adelaide, Australia. Images were taken using a Visionary Digital LK imaging system (Dun, Inc.) with a Canon EOS 5DsR camera at Flinders University, Adelaide, Australia. Images were produced using Zerene Stacker (Zerene Systems LLC) software and cropped and resized in Photoshop CS5.

Material examined in this study were initially identified using the following keys: *Amphylaeus morosus* specimens were identified using Houston's (1975) key to Australian hylaeine bees; *Gasteruption atrinerve*, *G. globiceps*, *G. melanopoda*, *G. primotarsale* and *G. cinerescens* were identified by B.A. Parslow (South Australian Museum) using Pasteel's (1957) key to the Australian *Gasteruption* and comparison with type material; *Ephutomorpha tyla* specimens were identified by L.R. Hearn by comparing specimens with type material held at the South Australian Museum; and *Anthrax maculatus* was identified by X. Li (Florida Museum). Voucher specimens for the examined species have been deposited at the Melbourne Museum (Table S1).

DNA sequencing for the eight parasitoids of *A. morosus* was performed by the Canadian Centre for DNA Barcoding at the Biodiversity Institute of Ontario using standard protocols (Ivanova et al., 2006; Table S1).

Table 1. Prevalence of parasitoid species in host nests and brood cells at each collection period. Mean parasitised cells calculated from parasitised nests only\*.

Nest collection			Parasitised nests				Parasitised cells (total/mean)*			
Year	Total nests	Total cells	<i>Gasteruption</i>	<i>Anthrax maculatus</i>	<i>Ephutomorpha tyla</i>	<i>Ephutomorpha aff. varipes</i>	<i>Gasteruption</i>	<i>Anthrax maculatus</i>	<i>Ephutomorpha tyla</i>	<i>Ephutomorpha aff. varipes</i>
Early summer 2017	85	400	16	0	9	0	22/1.38	0/0	37/4.11	0/0
Early summer 2018	6	29	0	2	0	0	0/0	7/3.5	0/0	0/0
Late spring 2019	27	124	7	0	0	0	9/1.29	0/0	0/0	0/0
Mid-summer 2020	26	109	1	2	2	1	1/1	15/7.5	4/2	8/8
Late summer 2020	16	78	3	0	4	0	3/1	0/0	26/6.5	0/0
<b>Total</b>	<b>160</b>	<b>740</b>	<b>27</b>	<b>4</b>	<b>15</b>	<b>1</b>	<b>35/1.29</b>	<b>22/5.5</b>	<b>67/4.47</b>	<b>8/8</b>

## Results

### *Nest architecture and composition*

In the Dandenong Ranges, females of *A. morosus* construct their nests in dead abscised fronds of the rough fern tree *Cyathea australis* by excavating the pithy interiors of the stems to create an unbranched linear tunnel. Nests were on average  $212.63 \pm 59.39$  mm long (mean  $\pm$  standard deviation;  $N = 156$ ) with nest entrance diameters of  $5.65 \pm 0.61$  mm ( $N = 52$ ). In other regions, nests also occur in the dead flower scapes of *Xanthorrhoea* spp. (L. Hearn, personal observation). The tunnel is lined with a cellophane-like material, a characteristic common to hylaeine bees, that is thought to act as a waterproofing and antimicrobial agent (Almeida, 2008). Provisioning of brood cells generally begins in mid-spring, and cells are laid sequentially starting from the distal end of the fern frond towards the proximal end. Brood production can last until mid-summer across the Dandenong Ranges populations, allowing different parasitoid species to attack at different brood development stages (fig. 1).

Nest architecture in *A. morosus* can be broadly categorised into nests with brood cells interspersed with vestibules and nests with brood cells laid sequentially (fig. 2). Vestibules are empty spaces containing no provisions or brood and are common in many stem-nesting Hymenoptera (Danks, 1971). In *A. morosus*, vestibular cells are frequently found in solitary nests, but nest burrows are fully excavated before the first cell is provisioned. This limitation to nest length once cell provisioning has begun suggests that there may be a trade-off between the number of potential brood cells and constructing vestibular cells to deter nest parasitoids.

### *Host associations*

***Gasteruption* Latreille, 1796 (fig. 3a).** *Gasteruption* is the most abundant parasitoid of *A. morosus* in terms of nests parasitised, parasitising 16.9% (27/160) nests across all collection periods (Table 1). Of the nests parasitised by *Gasteruption*, only the first three brood cells were found to contain parasitoid immatures. *Gasteruption* parasitised cells were often followed by vestibular cells (11/35 parasitised cells),

detritus (9/35) or provisioned cells that failed to develop (4/35).

The larvae of *Gasteruption* are predator-inquilines in the nests of *A. morosus* where they kill the host egg or developing host larva before consuming the entire host provisions (Bogusch et al., 2018; Parslow et al., 2020). They then construct, using anal secretions, dark semi-cocoons above and below the larvae separating the parasitised cell from the rest of the nest (Eardley and Daly, 2007; Malyshev, 1968; Westrich, 2018). Although some species of *Gasteruption* have been reported to consume the contents of multiple adjacent cells (Donovan, 2007; Malyshev, 1968; Westrich, 2018), *Gasteruption* immatures were only observed to occupy a single host cell in our nests of *A. morosus*.

Adult eclosion of *Gasteruption* occurred far later in the season compared with *A. morosus* (fig. 4). In most cases, *Gasteruption* brood were still at a late larval stage at the time *A. morosus* cells were reaching adult eclosion. Five species of *Gasteruption* were reared from host nests. One of these, *Gasteruption primotarsale* Pasteels, 1957, has been previously recorded from nests of *A. morosus* (Spessa et al., 2000). The novel host association for *G. atrinerve* (Kieffer, 1911); *G. globiceps* Pasteels, 1957; *G. melanopoda* Pasteels, 1957; and *G. cinerescens* Schletterer, 1885, were determined using adults reared from host nests.

***Anthrax maculatus* Macquart, 1846 (fig. 3d).** Four nests were found to be parasitised by the bombyliid fly *Anthrax maculatus* across two separate sampling periods. *Anthrax maculatus* was never directly observed ovipositing into or hovering around the nests of *A. morosus*. Yet, developing *A. maculatus* immatures were found occupying both single and consecutive *A. morosus* brood cells, accounting for 24.1% (7/29) and 13.8% (15/109) of the total brood cells across the early summer (December 2018) and mid-summer (January 2020) collection periods, respectively. The pupae of *A. maculatus* were primarily observed occupying cells in the middle of the nests, but in one instance, every cell in the nest contained parasitised immatures. All brood cells parasitised by *A. maculatus* contained late-stage pupae when the nest was opened, and in nests containing both *A. maculatus*

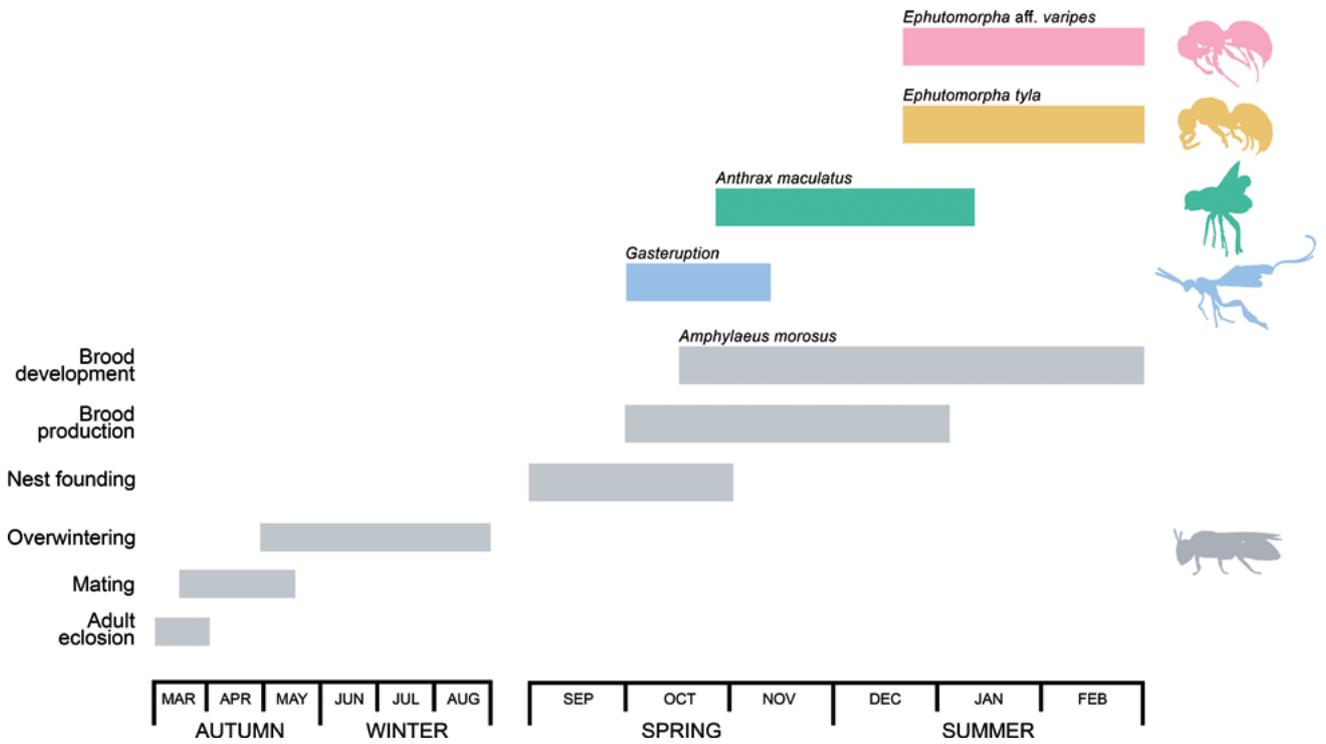


Figure 1. Annual cycle of *Amphylaeus morosus* across the full univoltine, semelparous life cycle in the Dandenong Ranges, Victoria (grey). The timings of nest parasite attacks (coloured) were estimated from rates of occurrence in nests across the brood development period.

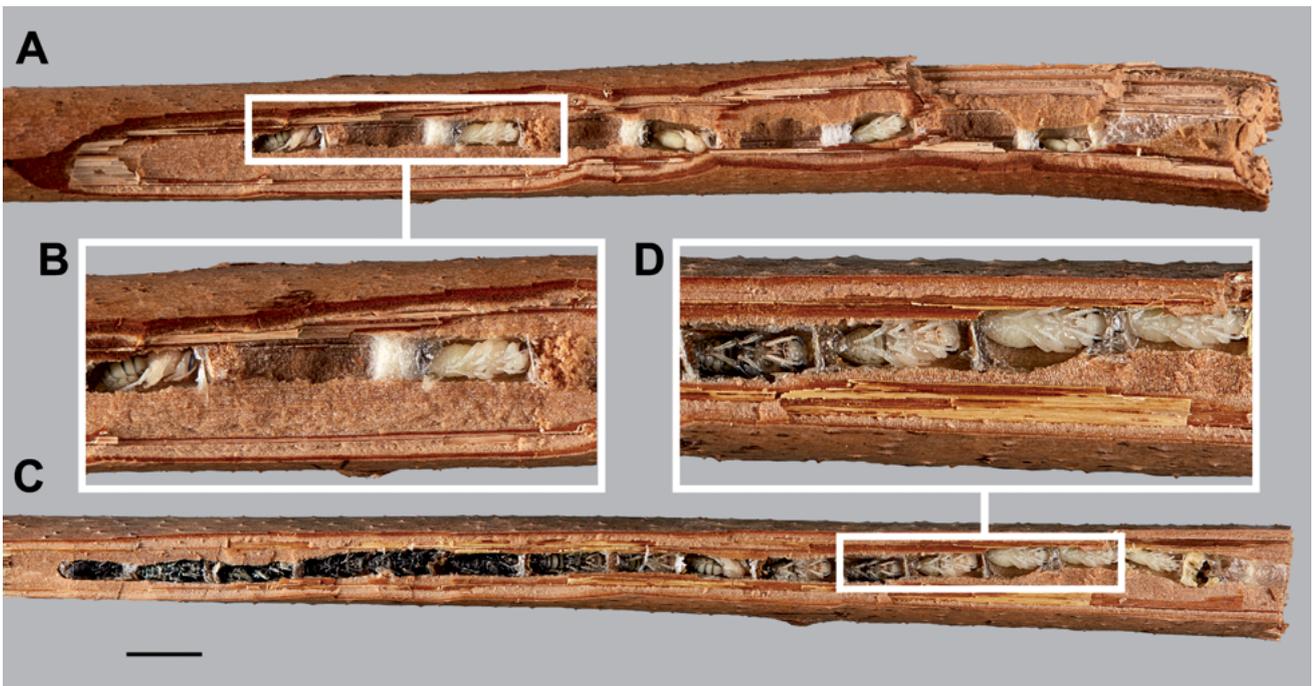


Figure 2. Nest dissections showing the two typical nesting structures for *Amphylaeus morosus* in the Dandenong Ranges: a, typical structure of a solitary nest; b, close-up of brood cells directly followed by a pithy cell plug, interspersed with vestibular cells; c, typical structure of a social nest; d, close-up of brood cells laid sequentially. Scale bar = 10 mm.

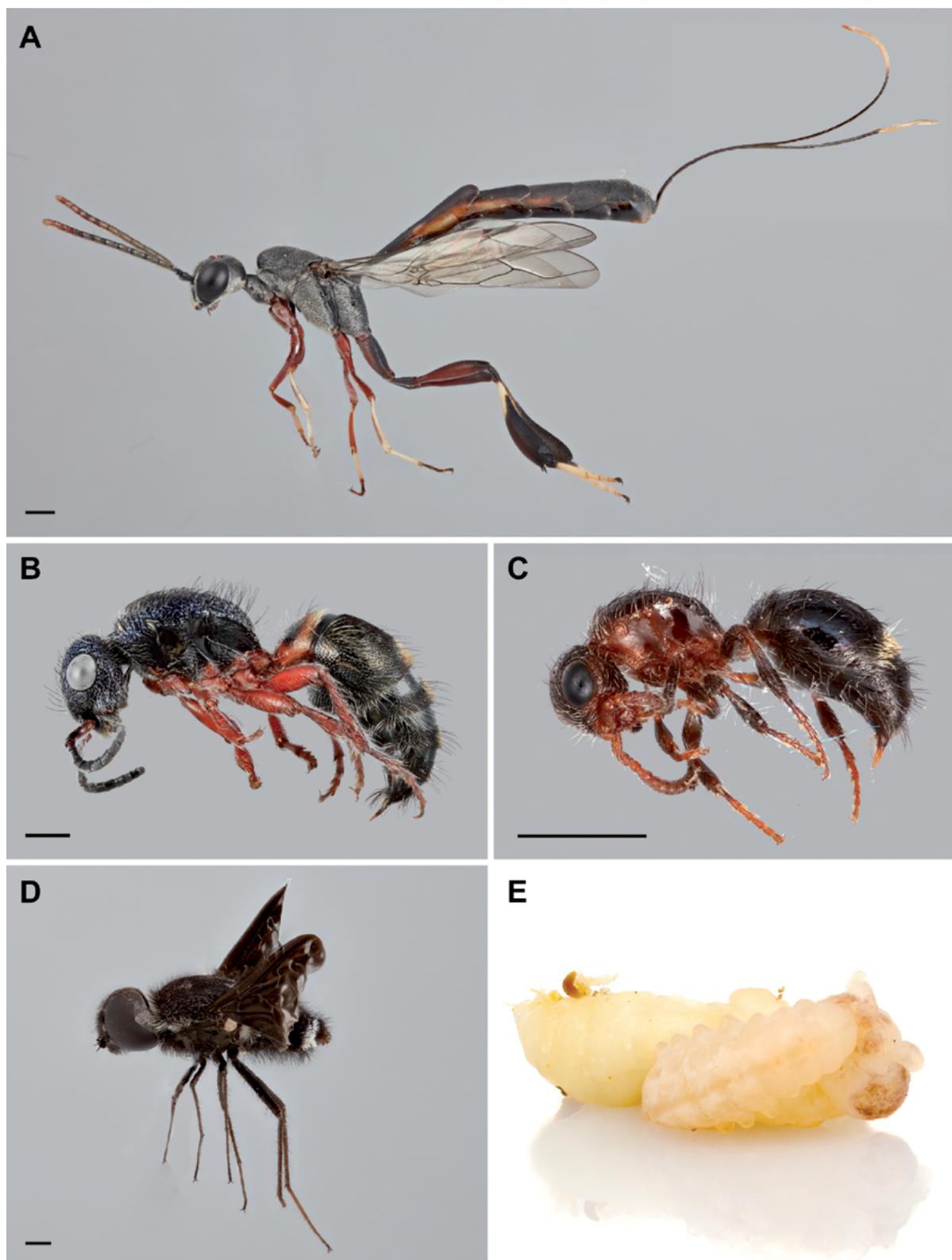


Figure 3. Images of the parasitoids reared from *Amphylaeus morosus* nests: a, lateral habitus of *Gasteruption globiceps*; b, lateral habitus of *Ephutomorpha tyla*; c, lateral habitus of *Ephutomorpha* aff. *Varipes*; d, lateral habitus of *Anthrax maculatus* e, *Ephutomorpha* aff. *varipes* larvae feeding on *A. morosus* pupae (photo credit James Dorey, 2020). Scale bar = 1.0 mm.

immatures and unparasitised host immatures, the host brood cells contained mid- to late-stage pupae. The pupae of *A. maculatus* were packed into the host brood cells and eclosed to adults within minutes of being extracted from the brood cell in the nest. The emergence of adult *A. maculatus* therefore appeared to be stimulated by disturbance but still synchronised with host adult eclosion (fig. 4).

***Ephutomorpha tyla* Hearn, Williams, and Parslow, 2019 (fig. 3b).** The host association for *Ephutomorpha tyla* has already been described by Hearn et al. (2019). Here, we present additional details based on new data. In two nests, a single adult female *E. tyla* was observed trying to break through the most proximal cell in the nest, while in a further 21 instances, adult females were observed waiting in the nest entrance. *Ephutomorpha tyla* does not appear to specifically target certain nest types, attacking nests with up to 14 brood cells or nests with only four brood cells interspersed with vestibules. A total of 29 nests contained either parasitised immatures, an adult female *E. tyla* in the entrance, or both. No adult female *A. morosus* were present in 69% (20/29) of these nests.

Parasitised brood cells consisted of brown papery cocoons that occupied the entirety of the brood cell. As the *E. tyla* immatures developed, the brood cells became darker and increasingly difficult to open. In nine affected nests, all brood cells were parasitised by *E. tyla*, with damaged partitions between cells. The body sizes of male and female *E. tyla* were generally consistent. However, in some cases there were marked differences in body size within each sex.

***Ephutomorpha* aff. *varipes* (André, 1895) (fig. 3c).** Larvae of *Ephutomorpha* aff. *varipes* were observed as external parasitoids of the mature larvae, prepupae and pupae of *A. morosus* and were observed feeding on young pupae (fig. 3e). Larvae of *E. aff. varipes* remained on their host until mature before producing a light-brown silk-like cocoon and eclosing as an adult. The ectoparasitoid larva of *E. aff. varipes* were observed on multiple consecutive *A. morosus* immatures in one nest. The first three brood cells of the parasitised nest contained fully healthy *A. morosus* immatures, whereas the next eight brood cells were occupied by parasitised larvae, all of which eclosed as males. *Ephutomorpha* aff. *varipes* was observed

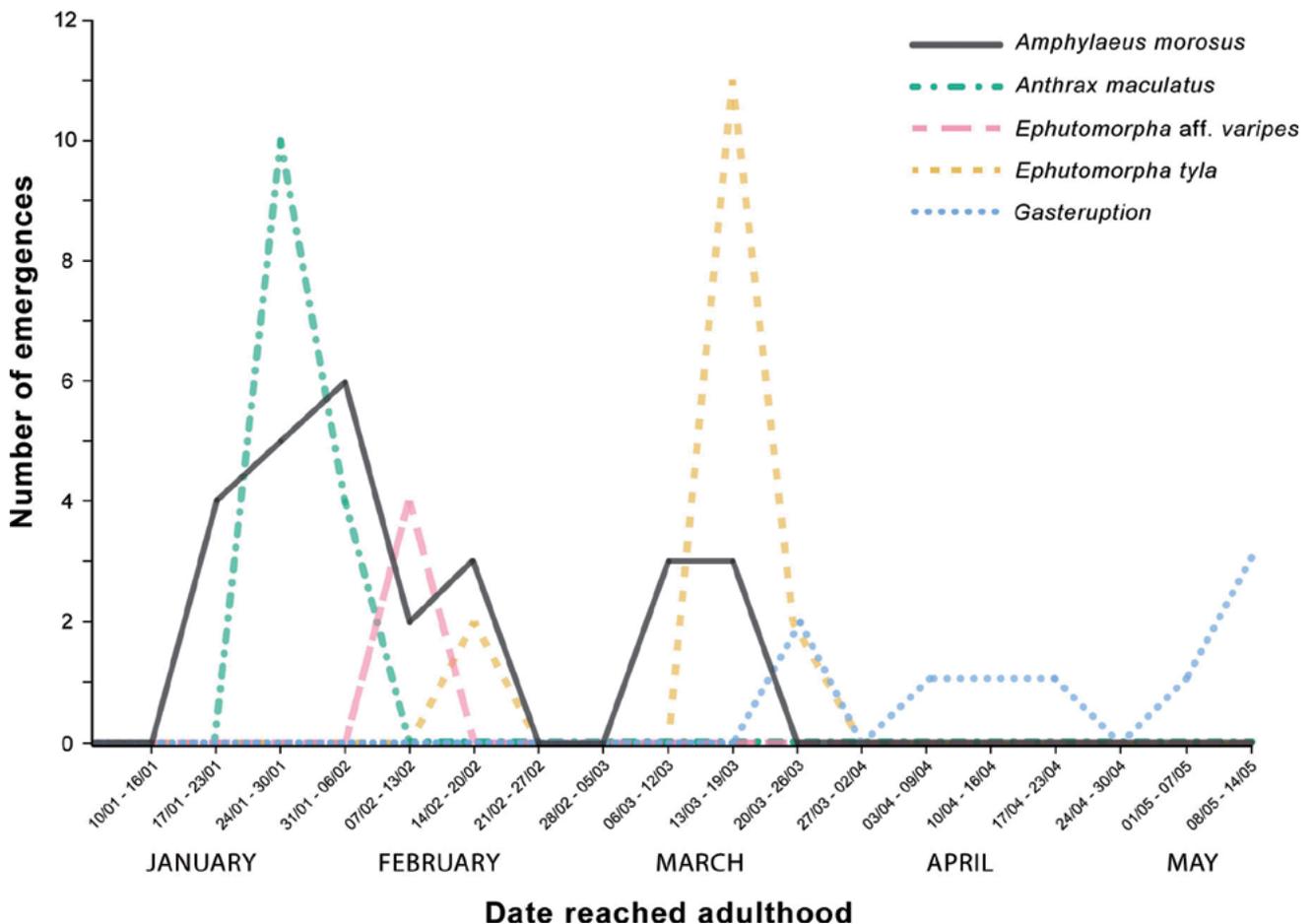


Figure 4. Eclosion phenologies of *Amphylaeus morosus* and its nest parasites. Emergence observations are only reported for the provisioning and rearing phase during 2019. Host emergences are only reported for nests containing parasitised brood.

parasitising several species that occupy excised *Cyathea australis* fronds, including another mass provisioning bee, *Hylaeus* sp., and a crabronid wasp, *Pison* sp. While, on numerous occasions, females were observed in the nest entrance of the allodapine bee, *Exoneura robusta* Cockrell, 1922, no parasitic association has been confirmed.

#### Other enemies

Of the 243 potentially viable *A. morosus* nests collected, 34.2% (83/243) contained no *A. morosus* adults or brood cells and had been superseded or temporarily occupied by other insect species. Twenty-four of these nests were occupied by *Pison* sp. (Hymenoptera: Crabronidae), two nests were occupied by the pteromalid wasp of the genus *Eupelmophotismus* (Hymenoptera: Pteromalidae), while one nest contained recently eclosed encyrtid wasps from the genus *Coelopencyrtus* (Hymenoptera: Encyrtidae). Five nests contained clerid beetle larvae (Coleoptera: Cleridae), including two instances of nests with *A. morosus* immatures and adults that contained beetle larvae in the nest entrance.

#### Discussion

Nest parasitoids are known to have detrimental effects on the survival and productivity of bees (Vickruck et al., 2010; Weislo et al., 1994). Of the parasitoid species that attack *A. morosus*, the predator-inquilines of the genus *Gasteruption* were the most prevalent, but least detrimental, only parasitising a mean of 1.29 cells per infiltrated nest, with 16.9% of nests being infiltrated. We never observed *Gasteruption* entering nests of *A. morosus*, possibly because our nest collections were restricted to cool weather conditions when insect activity was low; it is likely that *Gasteruption* wait for the adult bee to leave before infiltrating the nest and ovipositing in an open brood cell (Macedo et al., 2012; Parslow et al., 2020). Parasitisation by *Gasteruption* was only observed in single female nests, which suggests that multifemale nests are guarded by one female while other individuals collect provisions, restricting the opportunity for wasps to oviposit (Parslow et al., 2020). The extended development time of *Gasteruption* in *A. morosus* nests has been similarly reported in *Gasteruption brachychaetum* Schrottky, 1906a, larvae in nests of *Hylaeus* aff. *guaraniticus* (Schrottky, 1906b) (Macedo et al., 2012). The resulting asynchrony in adult emergence between *Gasteruption* immatures and host could be attributed to delaying their eclosion until hosts have started establishing new nests, which may explain observations of *Gasteruption* pupae in overwintering *A. morosus* nests (L. Hearn, personal observation). For *Gasteruption*, parasitising the distal nest cells could be a factor of this extended development period and avoiding being disturbed by emerging bees and other emerging parasitoids. There is limited information on *Gasteruption* host specialisation, with a large proportion of records based on single observations (Parslow et al. 2020). It is unlikely that *A. morosus* is the only host for *G. atrinerve*, *G. globiceps*, *G. melanopoda* and *G. cinerescens* because their distributions extend beyond the distribution of *A. morosus* (Atlas of Living Australia 2021; Parslow, 2020; Pasteels, 1957).

In contrast, parasitisation by the dipteran *Anthrax maculatus* had a greater effect when invading *A. morosus* nests, parasitising 5.5 brood cells on average across *A. maculatus* infiltrated nests. Previous studies have suggested that adult bombyliid females identify a host nest and oviposit directly into the nest entrance (Bohart et al., 1960). However, there are also reports of adults ovipositing haphazardly onto the ground, leaving the planidium to search for a host nest (Yeates and Greathead, 2008). This latter scenario could be likely in the Dandenong Ranges given the high density of *A. morosus* nests that can occur. It was unknown if the larvae of *A. maculatus* feed on both the immatures and provisions in a cell. Gerling and Hermann (1976) reported early instar larvae of the bombyliid fly, *Xenox tigrinus* (Evenhuis, 1984) (previously *Anthrax tigrinus*), feeding on the pollen bread in *Xylocopa virginica* (Linnaeus, 1771) nests. However, bombyliid fly immatures are also known to feed on the mature larvae of their hosts (Felicoli et al., 2017; Minckley, 1989). The delay in adult eclosion by *A. maculatus* compared with its host is consistent with observations by Minckley (1989) of *Anthrax xylocopae* (Marston, 1970) delaying emergence to synchronise with its host. This is because adults are not able to break through the cell partitions and therefore must wait for hosts to emerge (Gerling and Hermann, 1976). This could explain why *A. maculatus* puparium in our current study appeared to be stimulated by disturbance when removed from their cells.

In our study, *Ephutomorpha tyla* was the most abundant and destructive nest parasitoid in terms of both the number of brood cells parasitised and (potentially) adult hosts killed. *Ephutomorpha tyla* was rarely observed in nests with an adult host present, supporting similar reports of mutillids forcefully removing or killing host guards before ovipositing (Brothers et al., 2000). Adult *E. tyla* were generally observed in the nest entrances and on some occasions attempting to break through the cell closest to the entrance, suggesting that *E. tyla* may open the brood cell closest to the entrance to check for host brood before ovipositing. The size differences between reared adults of *E. tyla* are likely due to differences in the consumed host immatures. For instance, in *A. morosus*, the males are generally smaller than the females (Spessa, 1999). Additionally, the amount of provisions in each brood cell can vary dramatically, which can influence the size of the host immatures and subsequently the ectoparasitoid feeding on it (Brothers, 1989). Comparatively, the size differences observed in adults of *E. aff. varipes* support the notion that it is likely a parasitoid of multiple insect hosts; this accords with reports by Mickel (1928) that variation in body sizes within mutillid species is linked to them exploiting a range of hosts of varying size. *Ephutomorpha* aff. *varipes* was observed in nests of numerous different host species including the allodapine bee, *Exoneura robusta* Cockerell, 1922. However, given Brothers' Rule (Brothers et al., 2000), which states mutillids will only attack fully enclosed immatures, it is unlikely that such a condition is universal given that *E. robusta* progressively rears its brood in open linear nests. These observations suggest that *E. aff. varipes* is a generalist parasitoid of hosts using *Cyathea australis* as a nesting substrate, rather than any specific hymenopteran host, which accords with the notion that some mutillid species are adapted to specific situations rather than hosts (Brothers, 1989).

Attack by wingless parasitoids such as mutillid wasps is uncommon in stem-nesting bees (Ronchetti and Polidori, 2020). Michener (1985) suggested that this may be because predators need to search in a three-dimensional space looking for sparsely distributed nesting sites, whereas ground-nesting hymenopteran entrances can be found using a two-dimensional search pattern. In *A. morosus*, the high rates of parasitism might be attributable to the high density of their nesting substrate around mature tree ferns (Groulx and Forrest, 2018). This high density of nesting sites may act in a similar system to the ground nesting aggregations of halictid bees, where nesting sites are abundant, and parasitism can be prevalent (Wcislo, 1996).

The mechanisms of parasitism and bionomy of parasitoids has generally been poorly understood. Our study finds that parasitoids of *A. morosus* have adapted to host nesting behaviour and suggests variation in the oviposition chronology of each parasitoid species, with *Gasteruption* wasps ovipositing at the beginning of brood provisioning, *A. maculatus* ovipositing across the middle stages of brood development and the two mutillid species ovipositing in nests at the end of the season. This variation in parasitoid pressure could have implications for understanding the factors driving social nesting in the only known social bee in the family Colletidae.

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## Supplementary material

Table S1. Table showing BOLD sequencing reference number and voucher number for deposited material. All material deposited at Melbourne Museum.

Family	Species	Melbourne Museum Voucher number	BOLD reference number
Gasteruptionidae	<i>Gasteruption primotarsale</i>	HYM-97074	OZBOL2593-21
Gasteruptionidae	<i>Gasteruption globiceps</i>	HYM-97075	OZBOL2607-21
Gasteruptionidae	<i>Gasteruption atrinerve</i>	HYM-97076	OZBOL2606-21
Gasteruptionidae	<i>Gasteruption cinerescens</i>	HYM-97077	OZBOL2605-21
Gasteruptionidae	<i>Gasteruption melanopoda</i>	HYM-97078	N/A
Bombyliidae	<i>Anthrax maculatus</i>	DIP-2590	OZBOL2594-21
Mutillidae	<i>Ephutomorpha tyla</i>	HYM-97079	OZBOL2596-21
Mutillidae	<i>Ephutomorpha</i> aff. <i>varipes</i>	HYM-97080	OZBOL2599-21