

**The definitive version of this article is available at:**

<https://doi.org/10.1016/j.actao.2018.03.002>

## **Detecting the elusive cost of parasites on fig seed production**

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Running title: parasites reduce seed production of fig trees

## **Abstract**

Mutualisms provide essential ecosystem functions such as pollination and contribute considerably to global biodiversity. However, they are also exploited by parasites that remove resources and thus impose costs on one or both of the mutualistic partners. The fig/pollinator interaction is a classic obligate mutualism; it is pantropical and involves >750 *Ficus* species and their host-specific pollinating wasps (family Agaonidae). Figs also host parasites of the mutualism that should consume pollinators or seeds, depending on their larval ecology. We collected data from a large crop of figs on *Ficus glandifera* var. *brachysyce* in a Sulawesi rainforest with an unusually high number of *Eukoebelea* sp. parasites. We found that these parasites have a significant negative correlation with fig seed production as well as with pollinator offspring production. *Eukoebelea* wasps form the basal genus in subfamily Sycophaginae (Chalcidoidea) and their larval biology is considered unknown. Our analysis suggests that they feed as flower galls and impose direct costs on the fig tree, but a strategy including the consumption of pollinator larvae cannot be ruled out. We also present baseline data on the composition of the fig wasp community associated with *F. glandifera* var *brachysyce* and light trap catch data.

**Keywords:** conflict; costs; *Ficus*; mutualism; parasites; pollination

## Introduction

Mutualisms are ubiquitous and important biotic interactions that underpin some key ecosystem functions, such as pollination and nitrogen fixation (Foster and Wenseleers, 2006; Leigh Jr, 2010), and also drive the evolution of much biodiversity (Sachs and Simms, 2006; Wardle et al., 2004). However, they are also exploited by parasites, which impose immediate costs on at least one of the mutualists and influence the longer-term evolutionary trajectories of mutualisms (Yu, 2001). It is therefore crucial to understand the host specificity (Farache et al., this issue) and biology of these parasites and to conduct targeted studies to identify and quantify their costs.

The interaction between *Ficus* (Moraceae) and fig-pollinating wasps (Agaonidae) is a classic obligate mutualism, in which the fig and wasp require each other for reproduction and show very high reciprocal partner specificity (Janzen, 1979; Weiblen, 2002). Female pollinator wasps enter the enclosed *Ficus* inflorescences (figs) and pollinate the female flowers within. Each flower can either produce a seed or host the development of one pollinator or parasitic wasp. Flowering is asynchronous between fig trees in a population, which both sustains pollinator populations and provides an important year-round fruit supply for frugivores (Shanahan et al., 2001). This is of global significance since the >750 *Ficus* species are spread across all tropical continents (Berg and Corner, 2005).

Like other mutualisms, the fig/pollinator interaction is subject to parasitism. In particular, many species of non-pollinating fig wasps (NPFWs) also develop in fig inflorescences and, like the pollinators, are typically specialists on a single *Ficus* species (Cook and Segar, 2010). There are distinct guilds of NPFWs with different resource use strategies (Cook and Rasplus, 2003; Cruaud et al., 2011b; Kerdelhué et al., 2000; Segar et al., 2013; West et al., 1996). Small gall-inducers feed on fig flowers by galling them, having broadly negative effects on both seeds (through direct consumption) and pollinators (through competition). Seed eating appears to be an unusual strategy, but some inquiline will feed on seeds if

necessary (Pereira et al., 2007), hence only an effect on seeds is expected. In contrast, members of the small cleptoparasite guild exploit the galls of pollinators or small herbivores and kill them. Small cleptoparasites and pollinators should be negatively correlated, with no predicted relationship between small cleptoparasites and seeds.

Much larger wasp species also occur, and the large gall-inducers make large galls from flowers or fig wall tissue. Finally, the large wasps are also attacked by specialist large parasitoids or cleptoparasites that kill them (West et al., 1996). To date few manipulative experiments have been conducted on these large non-galling species and often their exact larval biology, whether they are parasitoids or cleptoparasites, is unknown. Indeed, trophic role in fig wasps is likely to be evolutionarily labile with endo- and exo-parasitoids existing in the same genus (Yadav and Borges, this issue), and this deserves to be studied in more detail. The typical pattern across fig species is that the large gall-inducers and their parasitoids/cleptoparasites are far less common than the smaller wasps (Segar et al., 2014, 2013). In addition, the large and small wasp faunas appear to operate as separate modules within the community (in at least some systems) with each set of gall-inducers having its own set of size-matched parasitoids/cleptoparasites (Compton et al., 1994; Segar et al., 2014, 2013).

Most NPFW species belong to five subfamilies (Sycoryctinae, Sycophaginae, Otitesellinae, Sycoecinae, and Epichrysomallinae) of chalcid wasps associated exclusively with *Ficus*. Several studies have attempted to quantify the costs of parasites on various fig/pollinator mutualisms. Strikingly, when a significant cost has been found, it has usually involved reduced pollinator, not seed, production (Segar and Cook 2012). A simple negative effect on pollinator numbers provides evidence of pollinator predation, while flower galls may also reduce pollinator numbers through competition for galls, as well as gall numbers through direct consumption. It has been reported that several NPFW species across a range of higher taxa, including species of Sycoryctinae (Kerdelhué et al., 2000), Sycophaginae (West and

Herre, 1994), Otitesellinae (West et al., 1996) and Epichrysomallinae (Peng et al., 2010), can have a negative correlation with pollinator numbers. This cost technically falls on both mutualists, since the female pollinator wasps that die would also have contributed to fig male reproductive function by carrying pollen. However, there is less evidence that NPFWs consume flowers. This is not surprising for the most species-rich wasp subfamily (Sycoryctinae), since it is believed to consist mainly of parasitoids and cleptoparasites.

In contrast, larval ecology has diversified greatly in the large subfamily Sycophaginae (Cruaud et al., 2011b), resulting in geographic and taxonomic disparities in trophic role. Indeed, while some species are small cleptoparasites (Elias et al., 2012; Kerdelhué and Rasplus, 1996; Wang and Zheng, 2008) others (sometimes congenics, e.g. in *Idarnes* and *Sycophaga*) have also been shown to compete with pollinators for galls (Elias et al., 2012; Galil and Eisikowitch, 1969) or consume seeds (Pereira et al., 2007). Others still fill the niche of large gall-inducers (Cruaud et al., 2011b). We expect that this variation in larval ecology is an important factor determining the detection of flower galling by fig wasps in this subfamily. By using correlations between seeds and wasps it is possible to shed further light on larval ecology.

In this study we take advantage of a crop of figs that was infested with *Eukoebelea* sp. wasps to statistically examine correlations between these parasitic wasps, fig-pollinating wasps and seeds. A recent phylogeny reveals that *Eukoebelea* is the basal genus of the pantropical Sycophaginae subfamily (Cruaud et al., 2011a), but no *Eukoebelea* species has been studied in detail. Therefore insights into the ecology of this genus can help us to understand the diversification of feeding regime in the subfamily, the ancestral state is currently thought to be ovary galling (Elias et al., 2017). One reason is that these Australasian *Eukoebelea* wasps have low abundance and patchy occurrence in studies of figs from the section Malvanthera, which are their host plants. For example, there was a mean of only 0.7 wasps per fig

(across 8 crops and 255 figs) in *F. rubiginosa* (Segar et al., 2014) and of only 1.3 in *F. obliqua* (across 18 crops and 149 figs) (Segar and Cook, 2012), precluding informative statistical exploration of their effects. However, we sampled a single large crop of fruits in another malvantheran fig species, *F. glandifera* var. *brachysyce*, (Figure S1). We took advantage of the unprecedented numbers of *Eukoebelea* sp. wasps, and small numbers of those species which mask their effects, to identify their correlation with seed (female function) and pollinator numbers (male function), and to help clarify their controversial larval biology (Cruaud et al., 2011b). As outlined above, small gall-inducers are predicted to correlate negatively with both seeds and wasps, while small cleptoparasite numbers should correlate only with pollinator numbers. We also present data on the abundance of each species of non-pollinating fig wasp (NPFW) found in this, as yet undescribed, community of insects and provide supplementary data on the wasps collected at light in the study tree.

## Materials and Methods

The impact of parasitic wasps is often subtle, especially if numbers are low, and it can be masked by between-crop variation in resources available to individual fig-fruits (West et al., 1996). These differences in “productivity” of individual figs are manifested through differences in fig size and the numbers of flowers developing inside. We used a single large crop of figs from *Ficus glandifera* var. *brachysyce* (Figure S1). Importantly, the crop had high abundance of the focal parasite species, offering a strong signal:noise ratio when trying to infer its effects on the mutualism. We collected 52 late D-stage figs (just before wasp emergence) from within the canopy of a hemi-epiphytic rainforest tree on Buton Island (Sulawesi, Indonesia) and allowed the wasps to emerge into individual collecting pots. We then measured the diameter of each fig to the nearest 0.01 mm and dissected it under a microscope at 10-60x. Fig ovule contents were identified and recorded as: wasp (identified to genus and then morpho-species); seed; exited (with an emergence hole); or undeveloped. We also installed a light trap in the canopy of our

focal tree (see Supplementary Information) and ran it for 150 hours, which provided data on locally abundant pollinating and non-pollinating fig wasp species.

We performed two multiple-linear regression analyses with i) seed number and ii) pollinator numbers as the respective response variables and the two explanatory variables *Eukoebelea* number and fig diameter. We included fig size as a covariate as this is also correlated with the total number of wasps and seed produced ("productivity" - Cook and Power, 1996). We performed stepwise deletion of nonsignificant terms using F-tests. All analyses were conducted in R v 3.2.4 (R Development Core Team, 2016) and all models were fitted using type II sums of squares. We tested the null hypothesis that the estimated slopes of models (i) seeds=*Eukoebelea* and (ii) pollinators=*Eukoebelea* were not significantly different from each other by comparing the slopes of the two models with different response variables (Sokal and Rohlf, 1995). All models were checked for heteroscedasticity, normality, and influential observations both graphically and by using the Non-Constant Variance (NCV) Score test (homoscedasticity) as implemented in the R package 'car' (Fox and Weisberg, 2011) and the Shapiro Wilk tests (normality of residuals).

## **Results**

### **1.1 The Effect of Parasites on the Mutualism**

A single undescribed species of *Eukoebelea* represented 85% of all parasitic, or non-pollinating, fig wasps and had a significant negative effect on both seeds and pollinators (Table 1 and Figure 1). The relationship between *Eukoebelea* sp. parasites and both mutualists (fig seeds and pollinating wasps) was significantly negative, and therefore likely to have a biologically meaningful effect on overall fitness. The slopes of simplified models i) seeds=*Eukoebelea* and ii) pollinators=*Eukoebelea* were not statistically different ( $t=0.732$ ,  $p=0.466$ ). We found constant variance of the residuals when plotted against the fitted values and this hypothesis was not rejected by NCV Score tests, the distribution of the residuals of each

model was not significantly different from normal. All data are uploaded as ‘data in brief’ and can be downloaded from Mendeley Data ([dataset] Segar et al., 2018).

## 1.2 Fig Wasp Community Composition

The wider community of fig wasps associated with this single crop of *F. glandifera* var. *brachysyce*, was diverse, comprising 10 morpho-species in eight genera from six (sub)families (Table 2). All eight genera have other species that attack other figs in section Malvanthera (Table 2, note that *Dobunabaa* is not present in the two Australian species listed for comparison). This dataset shows that the community contains wasp genera from both the small (*Eukoebelea*, *Sycoscapter* and *Dobunabaa*) and large wasp (*Meselatus*, *Herodotia*, *Sycophila* and *Megastigmus*) community modules that have been reported in other malvantheran fig species (Table 2). It is likely that molecular analysis will reveal further diversity within some genera. *F. glandifera* var. *brachysyce* is pollinated by both *Pleistodontes mandibularis* (black cuticle) and *P. rennellensis* (yellow cuticle). While both species have three ocelli the eyes of *Pleistodontes mandibularis* are slightly smaller, even when considering its shorter head (Figure S2).

## Discussion

To our knowledge, this is the first time that *Eukoebelea* has been shown to reduce seed (and wasp) production in a fig species and one of only a few studies to show this for a sycophagine wasp (see Galil and Eisikowitch, 1969; Pereira et al., 2007 for examples from *Idarnes* and *Sycophaga*). Recent studies have also demonstrated a mechanism for galling in some *Idarnes* species (Elias et al., 2012; Jansen-González et al., 2014). Our results are significant since Sycophaginae is the only higher taxon of wasps attacking fig fruits that is truly pantropical, and while its feeding biology is poorly understood it is clearly diverse, even within some genera. It is perhaps surprising that such a result has not been found more often before. We think that several factors contribute to this. First, the numbers of sycophagine wasps can be



low, especially in Old World fig species, making it difficult to detect statistical effects when there are other uncontrolled variables, often including multiple interacting wasp species (Segar et al., 2014). Indeed, studies analysing several crops in parallel tend to find the largest effects of NPFWs when their prevalence is highest (Conchou et al., 2014; Cook and Power, 1996). We have been fortunate to encounter and analyse a case where a single species of sycophagine is dominant and therefore its effects are more easily detected. This allows us to attribute the effects that we find without ambiguity. Second, it is harder to detect the effect of NPFW on seeds than pollinators, because only gall-inducers and seed-eaters are expected to have an effect on seeds while almost all guilds (including these) will influence pollinator numbers. Third, larval ecology varies greatly within Sycophaginae, necessitating focused species level studies.

We found that seed and pollinator numbers decreased as *Eukoebelea* sp. numbers increased, suggesting that this species has a negative impact on male and female reproductive function of its host fig. However, there is no large difference in the explanatory power of the two models and the slopes are not statistically different. While not significant, the difference in slope fits the relative proportions of pollinators and seeds and this does not contradict an indifferent use of both long and short styled flowers by *Eukoebelea* sp. in this infested crop, hence a cost to both seeds and wasps. Detailed dissections of figs with unhatched wasps are needed to test this hypothesis. If *Eukoebelea* sp. were a parasitoid we would expect to find a much greater impact on pollinator abundance in comparison to other putative parasitoids given its unusually high abundance (e.g. see Segar and Cook, 2012). Its significant but smaller impact on pollinator numbers could also be explained as a consequence of competition for the same pool of flowers that pollinators typically use. Further, we have observed other *Eukoebelea* species from *F. rubiginosa*, *F. obliqua* and *F. macrophylla* ovipositing at exactly the same time as pollinators (Segar et al., 2014), again suggesting that wasps in this genus are more likely to be gallers than parasitoids (Elias et al., 2008; Kerdelhué and Rasplus,

1996). However, even this observation does not preclude cleptoparasitism, because *Philotrypesis caricae* (Joseph, 1959), which also oviposits alongside pollinators, is a cleptoparasite.

We cannot rule out the possibility that *Eukoebelea* as a genus has a mixed feeding strategy and may in some cases may be an inquiline, and as such we suggest that further experimental (Elias et al., 2012; Wang and Zheng, 2008) and anatomical evidence (Ghara et al., 2011; Ghara and Borges, 2010) should be collected to clarify its trophic role. For example, multiple transitions of feeding regime have occurred in the genera *Sycophaga* (Cruaud et al., 2011b) and *Idarnes* (Elias et al., 2012) and more studies of separate *Eukoebelea* species are clearly required. A correlative effect similar to that found in this study could also result from cleptoparasitism of figs with disproportionately high numbers of pollinators. In addition, we found no pollinator free figs. Indeed, resolving the larval ecology of *Eukoebelea* is crucial for further studies of its evolutionary ecology. For example, Cruaud et al. (2011b) classify *Eukoebelea* as a “late galler”, but consider it more likely to be a cleptoparasite, while little evidence exists either way. We agree with these authors that further studies are needed, preferably across multiple species.

More generally, the community of fig wasps associated with *F. glandifera* var. *brachysyce* was taxonomically similar (Table 2) to the communities of other better studied malvantheran *Ficus* species such as *F. rubiginosa*, *F. obliqua* and *F. hesperidiiformes* (Cook and Power 1996; Segar and Cook 2012; Segar et al. 2013; Segar et al. unpublished data), despite our limited sampling opportunities. This suggests considerable conservation of taxonomic community structure at the *Ficus* section level. This is especially likely given that all four of these *Ficus* species are in different series representing separate radiations, mostly in similar habitats (Rønsted et al., 2008). Furthermore, the community of wasps associated with *F. glandifera* var. *brachysyce* comprises the same two small and large wasp trophic modules and has an overall structure that is reminiscent of better described African (Compton et al., 1994) and Australian communities (Segar et al., 2014) and again suggesting conservation of community structure. Interestingly, we recorded two species of *Pleistodontes* associated with this *Ficus* species: one black and one yellow.

This mirrors the case of *F. rubiginosa*, a malvantheran *Ficus* species endemic to Australia, which has coexisting black and yellow *Pleistodontes* species in the Townsville region of Queensland (Darwell et al., 2014). More generally, black and yellow co-pollinators are also associated with *F. septica* (Rodriguez et al., 2017).

To our knowledge this study represents the first ecological study of any *Eukoebelea* species; certainly, it is the first to present evidence that *Eukoebelea* wasps can have a negative impact on both mutualistic partners. Whilst *Eukoebelea* wasps can reduce both pollinator and seed production, they appear to have coexisted with malvantheran *Ficus* species and their pollinators for about 45 MY (Cruaud et al., 2011a). Our previous studies have found that members of this genus are often present in low densities, and this may contribute to the long-term co-existence of *Eukoebelea* and their host trees.

### **Acknowledgements**

We would like to thank Vicki Tough, James Clarke and the residents of Labundobundo village for assistance in collecting the syconia and also Jack Whitham and Catherine Jones for help in the laboratory. We are grateful to Derek Dunn and Lizzie Jones for comments on the manuscript and Jean-Yves Rasplus for identifying the pollinators caught at light and providing photos of the two *Pleistodontes* species associated with *Ficus glandifera* var *brachysyce*. We thank NERC, BBSRC, Operation Wallacea and the University of Reading for supporting our research on fig wasps. STS is also funded by the Grant Agency of the Czech Republic (grant number 15-24571S). We thank the Indonesian State Ministry for Research and Technology (RISTEK) for granting research permits to STS and PW.

### **Author Contributions**

STS conceived the study, conducted much of the field and laboratory work, analysed the data and wrote

the first draft of the manuscript. AM, PMW and JMC helped to write the manuscript, PW assisted in the field and JMC helped to conceive the study and write the first draft of the manuscript. All authors commented on a first and revised drafts of the manuscript and contributed substantially to the text.

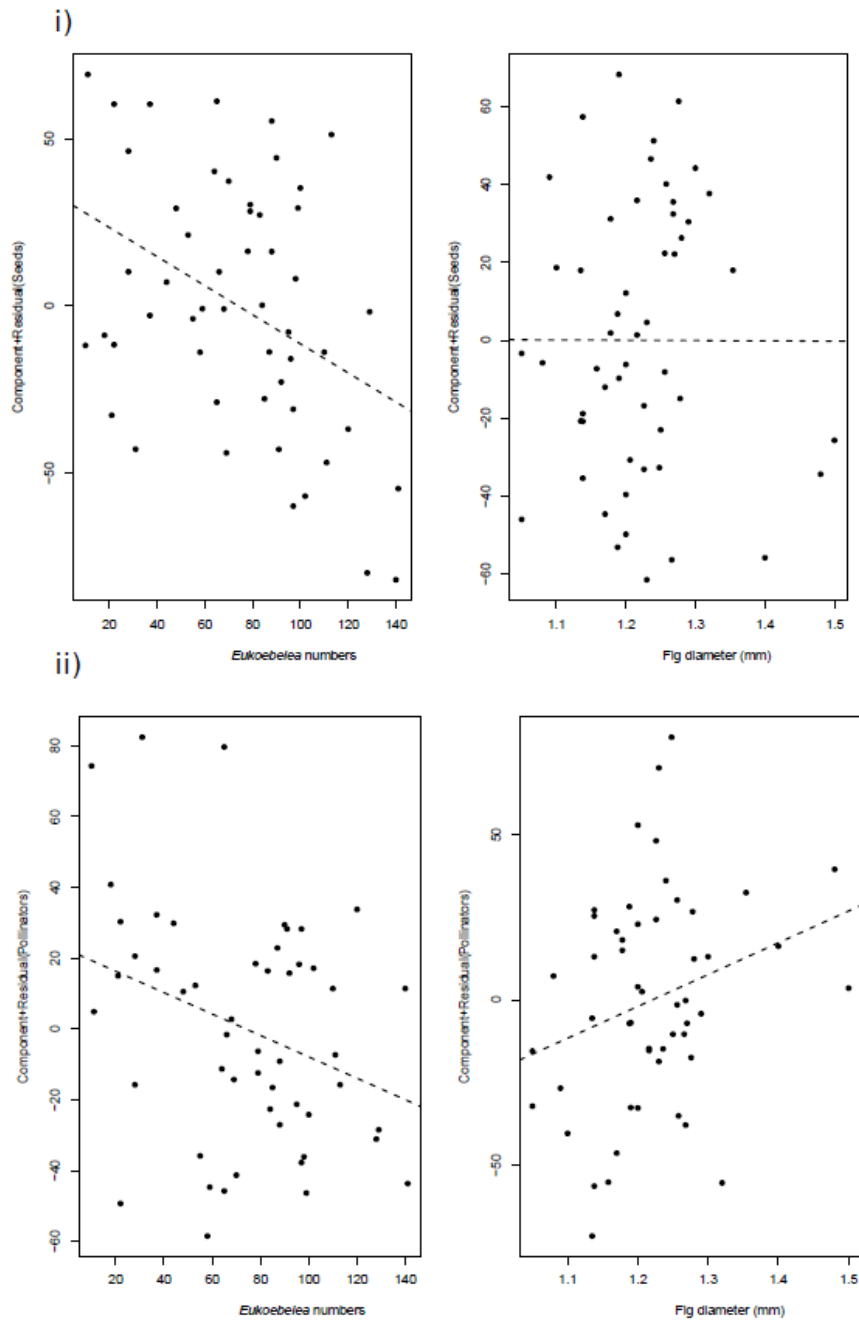
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**Figure 1.** Component plus residual plots of the maximal models: pollinators (i) or seeds (ii) against the number of *Eukoebelea* sp. per syconium for *Ficus glandifera* var. *brachysyce* and fig diameter (mm). See Table 1 for details of relationships.



**Table 1.** Results of linear models for *F. glandifera* var *brachysyce* testing for correlations between i) seed numbers and ii) pollinator numbers with *Eukoebelea* and fig diameter (mm). In each case the results of both a full model and the simplest model are given. Model fit ( $R^2$ ) is given for each response variable separately as well as for the complete models (calculated using type II sums of squares). The test statistics and significance of tests for homoscedasticity (Chi-square) and normality of residuals ( $w$ ) are given in the last two columns.

Response	Explanatory	DF	Estimate	SE	F	<i>p</i>	$R^2$	Chi-square	<i>p</i>	<i>w</i>	<i>p</i>
Model: seeds= <i>Eukoebelea</i> +Fig Diameter	<i>Eukoebelea</i>	49,2	-0.434	0.146	8.850	0.005	0.153				
	Fig Diameter	49,2	-0.742	55.278	0.000	0.989	0.000	0.053	0.818	0.971	0.225
							0.153				
Model: seeds= <i>Eukoebelea</i>	<i>Eukoebelea</i>	50,1	-0.434	0.144	9.057	0.004	0.153	0.051	0.822	0.971	0.228
Model: pollinators= <i>Eukoebelea</i> +Fig Diameter	<i>Eukoebelea</i>	49,2	-0.302	0.132	5.271	0.026	0.091				
	Fig Diameter	49,2	96.374	49.865	3.735	0.059	0.064	0.988	0.320	0.987	0.844
							0.149				
Model: pollinators= <i>Eukoebelea</i>	<i>Eukoebelea</i>	50,1	-0.290	0.135	4.600	0.037	0.084	3.627	0.057	0.991	0.961



**Table 2.** The mean ( $\pm 1$  s.e.m.) contents of one crop of *F. glandifera* var *brachysyce* sampled on Buton Island Sulawesi. Wasps are classified to genus level. For comparison the number of morpho-species known per genus is also given in brackets for *F. rubiginosa* (n=255) (Segar et al., 2014) and *F. obliqua* (n=149) (Segar and Cook, 2012). For the last column NA's represent cases where that genus was not found in *F. glandifera* var *brachysyce*.

(Sub)Family	Genus	Number of Species	Mean Individuals per Syconium
Agaonidae	<i>Pleistodontes</i>	2 (2,2)	83.7 $\pm$ 4.7
Pteromalidae	<i>Sycoscapter</i>	2 (2,1)	8.3 $\pm$ 1.2
Pteromalidae	<i>Dobunabaa</i>	1 (0,0)	0.9 $\pm$ 0.2
Pteromalidae	<i>Watshamiella</i>	0 (2,1)	NA
Pteromalidae	<i>Philotrypesis</i>	0 (2,1)	NA
Sycophaginae	<i>Eukoebelea</i>	1 (1,1)	74.0 $\pm$ 4.7
Sycophaginae	<i>Pseudidarnes</i>	0 (1,1)	NA
Epichrysomallinae	<i>Meselatus</i>	1 (1,0)	0.06 $\pm$ 0.03
Epichrysomallinae	<i>Herodotia</i>	1 (1,1)	0.4 $\pm$ 0.1
Eurytomidae	<i>Sycophila</i>	1 (2,1)	0.2 $\pm$ 0.08
Torymidae	<i>Megastigmus</i>	1 (1,1)	0.4 $\pm$ 0.1
Ormyridae	<i>Ormyrus</i>	0 (1,0)	NA
Seeds	NA	NA	119.8 $\pm$ 5.2
Undeveloped Ovules	NA	NA	11.7 $\pm$ 2.3