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First record of an apparently rare fig wasp feeding strategy: obligate seed predation

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Abstract. 1. Fig trees require host-specific agaonid fig wasps for pollination, but their figs also support numerous non-pollinating fig wasps (NPFW) that gall fig tissues or develop as parasitoids.

2. *Ficus microcarpa* L. is widely naturalised outside its native range and the most invasive fig tree species. Seed predators are widely used for the biological control of invasive plants, but no obligate seed predatory (as opposed to ovule or fig wall galling) NPFW have been recorded previously from any fig trees.

3. *Philotrypesis* NPFW are usually regarded as parasitoids or 'inquilines' (parasitoids that also eat plant material) of pollinator fig wasps, but the present study provides evidence that *Philotrypesis taiwanensis*, a NPFW associated with *F. microcarpa*, is an obligate seed predator: (i) adults emerge from seeds of typical appearance, with a surrounding elaiosome; (ii) it shows no preference for figs occupied by fig wasp species, other than the pollinator; (iii) it only develops in figs that contain pollinated ovules, avoiding figs occupied by an agaonid that fails to pollinate; (iv) larvae are distributed in layers where seeds are concentrated and (v) it has a negative impact on seed but not pollinator offspring numbers.

4. *Philotrypesis* is a large genus, and further species are likely to be seed predators.

Key words. Agaonidae, biocontrol, *Ficus*, NPFW, *Philotrypesis*, seed predation.

Introduction

Ficus is a largely pantropical genus comprising more than 800 species of fig trees. The genus is characterised by the structure of its inflorescences (figs) and its obligate mutualism with pollinating fig wasps (Hymenoptera, Agaonidae) (Wiebes, 1979; Cook & Rasplus, 2003; Harrison, 2005). Each fig tree species is pollinated by one or a small number of host-specific agaonids (Cruaud *et al.*, 2012). Adult female agaonids enter figs in order to lay their eggs inside the ovules that line their inner surface. They also carry pollen into the figs and either actively or passively pollinate some of the ovules and gall the ovules where their eggs are laid. After several weeks, the next generation of females mate and then collect pollen before dispersing to receptive figs.

Fig trees are regarded as 'keystone' species in tropical forests because their figs are fed upon by more species of animals than any other tropical fruits (Shanahan *et al.*, 2001; Herre *et al.*, 2008), but after the introduction of their pollinators a small number of species have become naturalised outside their native ranges, where they can become invasive (McKey, 1989). Factors seen as favouring rapid alien fig tree establishment and spread include their abundant seed production, good seed viability, and extensive seed dispersal (Caughlin *et al.*, 2012; Miao *et al.*, 2012). The ability of fig trees to extend their distributions once pollinator populations become established should be favoured by a relative shortage of specialist fig seed-eating insects within the plants' introduced ranges, together with reduced predation of the pollinators by parasitoids – the 'enemy release hypothesis'

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(Keane & Crawley, 2002; Müller-Schärer & Schaffner, 2008; Pearson *et al.*, 2011). Figs support a wide taxonomic range of natural enemies that feed on the figs and their pollinators, including ants, mites, beetles, flies, and moths, some of which may have potential as biological control agents (Compton & Robertson, 1988; Herre, 1993; Miao *et al.*, 2012), but the most prominent and most species-rich group of natural enemies is non-pollinating fig wasps (NPFW).

Fig trees can support up to 30 or more largely host tree-specific NPFW (Compton & Hawkins, 1992). Like pollinator fig wasps, their larvae develop inside figs, with one larva developing per gall, but they reduce rather than enhance the reproductive success of their host plants. Most fig wasps utilise the ovules present inside figs for their larval development. Traditionally, the feeding biology of floral-feeding fig wasps (some also develop in the fig wall) has been placed in one of four categories: species that pollinate and gall the ovules, species that gall the ovules, parasitoids of other fig wasps, and 'inquilines' that develop in ovules galled by other species, killing and eating their larvae, but also feeding on plant tissue (Compton & van Noort, 1992). Direct investigations of larvae ecology in figs are difficult (Tzeng *et al.*, 2008), but it is increasingly apparent that this simplistic classification is inadequate and misleading and its use is often inconsistent with that used elsewhere (Chen *et al.*, 2013). NPFW that feed in ovules galled by other species can also modify and expand the galls (secondary gallers, Chen *et al.*, 2013), and there are also specialist hyper-parasitoids that utilise ovules that had been galled by one species and were then attacked by primary parasitoids (Compton *et al.*, 2009). Finally, there is also a species known to have larvae that are normally found in galled ovules, but small males can also complete their development inside fig seeds (Pereira *et al.*, 2007).

Surprisingly, there are no recorded examples of NPFW species that are obligate seed eaters (feeding only on un-galled, fertilised ovules that would have developed into viable seeds if they had remained un-eaten). The seeds of the Asian fig tree *F. microcarpa* L. are unusual in that they are surrounded by a fleshy outer layer that functions as an elaiosome, attracting ants that help disperse the seeds (Kaufmann *et al.*, 1991). One of us (L.B.) observed that adults of the NPFW *Philotrypesis taiwanensis* Chen (Pteromalidae) were consistently emerging from what appeared to be typical seeds of this species, rather than galled ovules, suggesting that it was a potential seed predator. To test this we addressed the following questions: (i) Is *P. taiwanensis* associated with any other species of fig wasps that might be potential hosts? (ii) What is its impact on seed and pollinator numbers? (iii) Is it absent from figs that could not have contained seeds? and (iv) Are *P. taiwanensis* larvae developing in the more peripheral layers within the figs, where most flowers develop as seeds?

Materials and methods

Ficus microcarpa *and its fig wasps*

Ficus microcarpa grows naturally as a strangler fig tree in tropical and subtropical forests of SE Asia and Australasia, but is also widely planted in tropical and warm temperate urban areas as an ornamental tree, where it has often become naturalised (Beardsley, 1998; Burrows & Burrows, 2003; Caughlin *et al.*, 2012). It produces crops of up to several thousand small figs in the leaf axils that reach about 10.1 ± 0.3 mm ($n = 20$ figs) in diameter when mature. As in other fig trees, its sexual reproduction depends on pollination by a host-specific agaonid fig wasp. The pollinator is recorded as *Eupristina verticillata* (Agaonidae), but this taxon represents a complex of several closely related and morphologically similar species (Sun *et al.*, 2011) including one species that is a 'cheater' (*Eupristina* sp.) that fails to pollinate the plant (J-Y Rasplus, Pers. Comm.) The cheater species is only recorded from Yunnan Province, China and has not been introduced elsewhere (R. Wang and S. G. Compton, unpublished), whereas pollinators of *F. microcarpa* have been introduced throughout most, but not all, of its introduced range (van Noort *et al.*, 2013). Expansion of *F. microcarpa* populations is linked to extensive seed dispersal by birds (Shanahan *et al.*, 2001). Seeds germinate on buildings and pavements in urban areas, where seedlings can cause architectural damage (Tan & Yeo, 2009; Caughlin *et al.*, 2012). Populations can also become established in natural areas and *F. microcarpa* has become invasive in Florida, Hawaii, and Bermuda (Kaufmann *et al.*, 1991; Nadel *et al.*, 1992; Beardsley, 1998; Starr *et al.*, 2003).

Reflecting the plant's broad natural distribution and monoecious breeding system (Compton & Hawkins, 1992), *F. microcarpa* figs are exploited by a large community of fig wasps comprising at least 30 NPFW species (Chen *et al.*, 1999; Feng & Huang, 2010; R. Wang and S. G. Compton, unpublished). Unlike agaonids, these NPFW do not enter the figs to lay their eggs. They have larvae that develop inside galled ovules, or are parasitoids of the gall-formers. They include approximately 14 species of putative gallers from subfamilies of Pteromalidae (Epichrysomallinae and Otitesellinae) and 16 species of putative parasitoids [Eurytomidae, Ormyridae, and Pteromalidae (Sycoryctinae)] (R. Wang and S. G. Compton, unpublished).

Females of *Philotrypesis* species (Sycoryctinae) are distinguished by having the two terminal segments of the gaster extended to partially enclose their long ovipositors (Bouček *et al.*, 1981). On the basis of colour they can be separated into species that are all black, or have combinations of yellow or orange and black (Jiang *et al.*, 2006). Males can be winged or flightless, and individuals often display dramatic variation within a single species (Murray, 1987; Jousselin *et al.*, 2004). Detailed early studies showed that the European *Philotrypesis caricae* L. is a parasitoid of the tree's pollinator that also consumes plant material (Joseph, 1957) and in the absence of further information this feeding behaviour has generally been assumed to be typical of the species-rich genus, as well as for sycorytines as a whole (Segar & Cook, 2012; Zhou *et al.*, 2012; Suleman *et al.*, 2013).

Philotrypesis taiwanensis has black females and exclusively flightless males. It was initially described by Chen *et al.* (1999) from Taiwan, and has a wide distribution that covers most of the natural range of *F. microcarpa*, including mainland China, the Philippines, and Thailand. It has also been introduced into Brazil, Florida, Puerto Rico, and Hawaii (Beardsley, 1998; Farache *et al.*, 2009; R. Wang and S. G. Compton, unpublished).

This species may also utilise another fig tree host, *Ficus benjamina* L. (Zhou *et al.*, 2012). Little is recorded about its biology, other than that females were observed to oviposit into figs that had been pollinated weeks earlier, which suggested that it may be a parasitoid, because gall-forming species usually oviposit earlier (Compton, 1993; Chen *et al.*, 1999).

Fig wasp faunal composition and impact of P. taiwanensis

From December 2010 to January 2013, mature *F. microcarpa* figs were collected from trees within the native range of the plant in Guangzhou (Guangdong Province, mainland China), Xishuangbanna (Yunnan Province, mainland China), Taipei (Taiwan), and Bangkok and Kanchanaburi (Thailand). Xishuangbanna, Bangkok, and Kanchanaburi have seasonal climates, with distinct wet and dry seasons, whereas Guangzhou and Taipei have less seasonal but more humid subtropical climates.

At least five *F. microcarpa* crops were sampled at each location, with each sample comprising at least 15 mature figs (late C or early D phases, *sensu* Galil & Eisikowitch, 1968). The total number of crops and figs sampled were 49 and 765, respectively (Table 1). The figs were initially stored in 70% ethanol. Each fig was sliced into quarters and soaked in water for more than 10 min to soften the galls before the contents of all the fig flowers were identified, using a binocular microscope. The flowers were allocated to the following categories: male flowers, seeds, unfertilised and undeveloped female flowers, galls containing wasps, and empty galls (‘bladders’). Fig wasps were identified primarily using Chen *et al.* (1999) and Feng and Huang (2010), and scored as additional morpho-species where necessary. After fig dissection, figs with *P. taiwanensis* were selected to record the impacts of *P. taiwanensis* on the male and female reproductive successes of each fig. Note that only crops with at least three figs containing *P. taiwanensis* were chosen.

Relationships with agaonids and seeds

Ficus microcarpa is one of a small number of fig trees that support a non-pollinating ‘cheater’ agaonid (*Eupristina* sp.) in addition to a typical agaonid pollinator. Males of the two species cannot currently be distinguished on the basis of their morphology. Figs entered exclusively by the former contain no seeds, and are therefore unsuitable for seed-eating species, but figs entered by both species contain galled ovules with agaonid larvae that are a potential resource for parasitoids or inquilines. *Eupristina* sp. was only present in the fig samples from Xishuangbanna, from where the sampled figs were divided according to which of the two agaonid species were present, or whether both species were present. The numbers of seeds, agaonids, and *P. taiwanensis* that had developed in the three groups of figs were compared.

Spatial stratification of ovules containing seeds and P. taiwanensis

Ovule pedicel lengths provide an indication of where inside a fig fig wasp larvae are developing, relative to the outer fig wall. Ovules with longer petioles are situated closer to the centre of a fig (Yu & Compton, 2012). In many figs, ovules that develop into seeds are concentrated closer to the fig wall than those that support agaonids and their parasitoids, because agaonids preferentially oviposit into shorter-styled flowers, which have longer pedicels (Compton & Nefdt, 1990; Dunn *et al.*, 2008). Figs from two *F. microcarpa* crops from Bangkok and Kanchanaburi contained high densities of *P. taiwanensis*. We recorded the pedicel lengths and contents of all female flowers that developed into either seeds or galls within a total of 26 figs from these two crops. The pedicel lengths were measured to the nearest 0.03 mm under a dissecting microscope using an eyepiece graticule. All the fig wasps inside the galls were then identified, and the female flowers were assigned into five categories: (i) seeds; (ii) occupied by pollinators; (iii) occupied by *P. taiwanensis*; (iv) occupied by other NPFW and (v) empty galls where no larvae completed development (‘bladders’).

Statistical analyses

All statistical analyses were carried out using R 2.14.2 (R Development Core Team, 2012). Response variables were square root or natural logarithm transformed where necessary. Likelihood ratio tests and multiple tests with Bonferroni’s correction were used to estimate the significance of fixed effects and pairwise comparisons, respectively.

The effects of *P. taiwanensis* on seed and agaonid numbers (using all native range samples) were tested using LMM (linear mixed models) in R package nlme version 3.1 (Pinheiro *et al.*, 2013), with crop identity as a random effect. This analysis was repeated after excluding data from Xishuangbanna, the only location where the non-pollinating agaonid *Eupristina* sp. was detected.

The effects of the presence/absence of seeds and agaonid identity (*Eupristina* sp. or *E. verticillata*) on the prevalence of *P. taiwanensis* (the proportion of figs where it was present) was tested using a generalised linear mixed model (GLMM) in R package lme4, version 1.0-5 (Bates *et al.*, 2013). Differences in the abundance of *P. taiwanensis* in figs were examined using LMM. Crop identity was a random effect in both analyses.

Differences in the pedicel lengths of flowers with different contents (other than bladders) were compared using LMM. We also used GLMM, assuming binomial distribution of residuals, to examine spatial stratification of flower categories by regressing the proportion of female flowers with different contents on pedicel lengths (to investigate the interaction between utilization type and pedicel length). Fig identity was set as a random effect in all analyses.

Results

At the five sites within the native range of *F. microcarpa*, *Eupristina* species were the most prevalent fig wasps (present in the highest proportion of figs) (Table 1). There were 28 NPFW species represented, including 10 galler species that belong to subfamilies Epichrysomallinae and Otitesellinae, and 18 putative parasitoid species in the families Eurytomidae,

Table 1. The contents of *Ficus microcarpa* figs collected within the native range of the plant and the sub-set of figs with *Philotrypesis taiwanensis* used in the analyses. *Eupristina verticillata* was the only *Eupristina* species except at Xishuangbanna, where the 'cheater' *Eupristina* sp. was also present. Figs that did not contain agaonids had been colonised by galling non-pollinating fig wasps (NPFW) that develop independently.

	Total figs (% figs)				Selected figs (mean ± SE)							
Study site	Crops	Figs	*Eupristina* species prevalence (% figs)	*P. taiwanensis* prevalence	Crops	Figs	Female flowers	Seeds	*Eupristina* species	*P. taiwanensis*	Galler NPFW	Parasitoid NPFW
Guangzhou	12	169	75.1	35.5	5	52	136.3 ± 5.2	17.2 ± 2.1	28.6 ± 3.8	6.8 ± 0.8	2.7 ± 0.4	2.6 ± 0.5
Xishuangbanna	16	273	97.4	21.2	9	53	188.2 ± 7.0	28.1 ± 2.6	60.1 ± 6.8	7.2 ± 0.9	3.9 ± 0.7	3.1 ± 0.6
Taipei	11	220	45.5	20.9	3	34	150.1 ± 7.3	21.5 ± 2.3	21.2 ± 2.3	13.7 ± 2.6	3.7 ± 0.8	11.1 ± 2.0
Bangkok	4	40	45.0	17.5	2	7	169.4 ± 28.2	22.3 ± 3.9	30.7 ± 3.2	14.3 ± 6.0	1.3 ± 0.6	0
Kanchanaburi	6	63	68.3	34.9	1	21	203.6 ± 10.1	29.5 ± 3.6	43.6 ± 4.8	16.5 ± 2.4	2.0 ± 0.6	2.1 ± 0.9
Total	49	765	72.4	25.2	20	167	165.4 ± 3.9	23.3 ± 2.3	39.3 ± 5.1	9.8 ± 1.4	3.1 ± 0.6	4.3 ± 1.0

Ormyridae and Pteromalidae, subfamilies Sycoryctinae and Pireninae. *Philotrypesis taiwanensis* was also common and widespread, and was recorded from 25% of the figs.

A total of 167 figs containing *P. taiwanensis* were analysed from 20 crops where this species was present in three or more figs (Table S1). The average numbers of female flowers in the figs varied from less than 140 to over 200, depending on the location (Table 1). *Eupristina* individuals were found in each fig and were the most numerous species of fig wasp, with *P. taiwanensis* the most abundant NPFW (57% of total NPFW) (Table 1). The assemblage of NPFW associated with *P. taiwanensis* was not consistent. *Odontofroggatia galili* Wiebes (156 individuals, 5.4%) and *Sycoscapter gajimaru* (Ishii) (343 individuals, 11.9%) were the most common galler and parasitoid species but were only present in 35 (21.0% of the 167 figs) and 40 (24.0%) of the figs, respectively. We also recorded six galler species including two *Walkerella*, one *Micranisa*, and three *Odontofroggatia* species and eight putative parasitoid species comprising two *Philotrypesis*, two *Sycoryctes*, one *Sycoscapter*, and three *Sycophila* species, but their prevalence and abundance were low (Table S1). Eleven additional NPFW species (one *Acophila*, one *Meselatus*, one *Walkerella*, one *Ormyrus*, four *Philotrypesis*, one *Sirovena*, and two *Sycophila* species) were present in the same crops of figs, but absent from figs that contained *P. taiwanensis*.

Impact of P. taiwanensis

Among the 167 figs containing *P. taiwanensis,* there was a significant negative relationship between the numbers of this species and the numbers of seeds, but no significant relationship between the numbers of *P. taiwanensis* and agaonids (LMM: seed numbers vs. *P. taiwanensis*: slope $= -0.067 \pm 0.013$, df $= 1$, LR $= 26.61$, $P < 0.001$; agaonids vs. *P. taiwanensis*: slope $= 0.027 \pm 0.014$, d.f. $= 1$, LR $= 3.59$, $P = 0.058$; Fig. 1a,b). Similar results were obtained when figs from Xishuangbanna (where cheater agaonids were present) were removed from the analysis (LMM: seed production vs. *P. taiwanensis*: slope $= -0.079 \pm 0.012$, d.f. $= 1$, LR $= 38.03$, $P < 0.001$; *E. verticillata* vs. *P. taiwanensis*: slope $= 0.023 \pm 0.014$, d.f. $= 1$, LR $= 2.75$, $P = 0.098$; Fig. 1c,d).

Relationships with agaonids and seeds

Agaonid offspring were present in 266 of the figs collected from Xishuangbanna, of which 74, 86, and 106 contained offspring of only pollinators (*E. verticillata*), only cheaters (*Eupristina* sp.) or both species, respectively. Unexpectedly, seeds were recorded in 16 figs (18.6%) that contained no pollinator offspring, although at most only five seeds were present in any one fig – far fewer than in figs entered by the typical pollinator (Table 2). Figs where offspring of both agaonid species were present also contained fewer seeds than figs where pollinator offspring were the only agaonids recorded. Total numbers of agaonid offspring were nonetheless higher in figs where both agaonids were present (Table 2).

Philotrypesis taiwanensis emerged from four figs (6.5%) that lacked seeds, a prevalence that was significantly lower than that recorded from figs that also contained seeds (26.5%, $n = 54$; GLMM: d.f. $= 1$, LR $= 7.16$, $P = 0.007$). Three out of the four figs that contained *P. taiwanensis* offspring but no seeds were occupied by cheater offspring, but no pollinator offspring and the other was occupied by both agaonid species. The abundance of *P. taiwanensis* offspring was also far lower in figs that contained only cheaters, compared with the other two groups of figs (Tables 2 and 3), but there was no significant difference in *P. taiwanensis* offspring abundance between figs containing only pollinator offspring and those that contained offspring of both agaonids (Tables 2 and 3).

Spatial stratification of ovules containing seeds and P. taiwanensis

The lengths of the pedicels of 1863 female flowers were measured ($n = 26$ figs). Galls containing offspring of *E. verticillata, P. taiwanensis*, *Odontofroggatia ishii* Wiebes and *S. gajimaru* were present in these figs. Flowers containing pollinator offspring had pedicels 0.39 ± 0.01 mm in length (mean ± SE, $n = 778$) that were significantly longer than those of flowers that developed seeds (0.31 ± 0.01 mm, $n = 506$). The pedicels of flowers containing *P. taiwanensis* (0.19 ± 0.01 mm, $n = 230$) were significantly shorter than even those containing seeds, but not

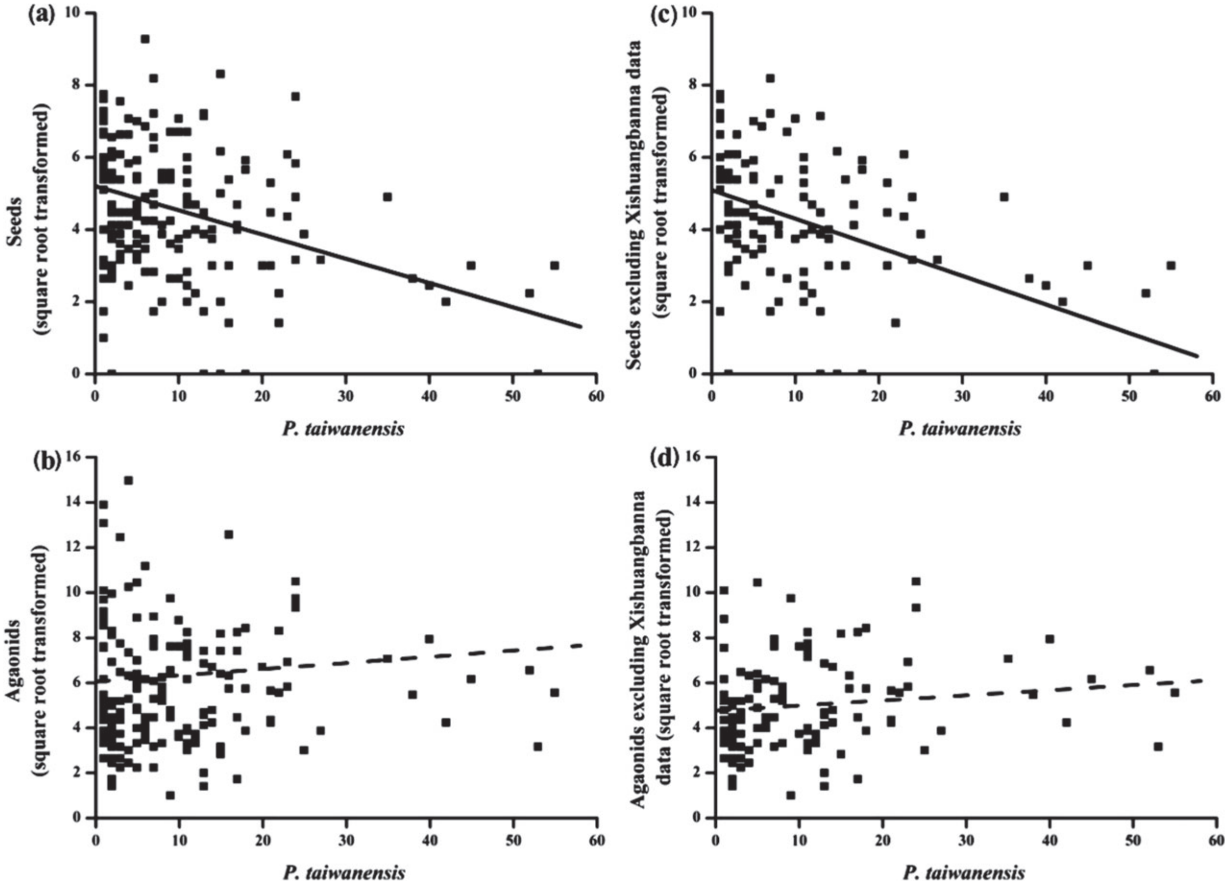

Fig. 1. The relationship between numbers of *Philotrypesis taiwanensis* and seed and agaonid numbers in shared *Ficus microcarpa* figs. All figs (a, b) or after excluding figs from Xishuangbanna, where the cheater agaonid is present (c, d). Note that the linear relationships between abundance of agaonids and *P. taiwanensis* (dashed lines) were not significant [LMMs (linear mixed models)].

Table 2. The presence of *Philotrypesis taiwanensis* in *Ficus microcarpa* figs from Xishuangbanna that had either been entered by the pollinator *Eupristina verticillata*, by the 'cheater' *Eupristina* sp., or by both species. Fig wasp abundance was calculated only in the figs where that particular species was present.

		Eupristina species	Seeds	*Philotrypesis taiwanensis*	
Contents	Total figs	(Mean ± SE)	(Mean ± SE)	Prevalence (% figs)	(Mean ± SE)
Only pollinators	74	50.1 ± 3.7	43.6 ± 3.9	36.0	7.6 ± 1.0
Only cheaters	86	85.3 ± 4.2	0.5 ± 0.1	4.1	1.3 ± 0.3
Both species	106	83.8 ± 3.5	12.4 ± 1.3	22.6	6.5 ± 1.5
Total	266	73.3 ± 2.4	19.2 ± 1.7	21.8	6.8 ± 0.8

significantly shorter than those containing all other NPFW combined (0.26 ± 0.03 mm, $n = 74$). The other NPFW occupied flowers with a wide range of pedicel lengths, reflecting between-species diversity, with some species preferring more central flowers and other species preferring more peripheral flowers (Table 4 and Fig. 2a).

With increasing pedicel lengths, an increasing proportion of female flowers were occupied by *E. verticillata* rather *P. taiwanensis*, other NPFW or seeds, leading to a significant interaction between pedicel lengths and utilisation. Agaonid offspring were therefore concentrated in the centre of the figs, with seeds and *P. taiwanensis* concentrated towards the outer wall of the figs (Table 4 and Fig. 2b). The proportions of female flowers occupied by other NPFW and seeds was independent of pedicel lengths (GLMM: for other NPFW: $z = -1.85$, $P = 0.065$; for seeds: $z = -0.61$, $P = 0.542$).

Discussion

Confirmation of the larval feeding behaviour of fig wasps is difficult, but several forms of evidence consistently suggest that

Table 3. The effects of agaonid species (*Eupristina verticillata* pollinators, *Eupristina* sp. cheaters or a mix of both species) on the prevalence and abundance of *Philotrypesis taiwanensis* in shared figs. Note that *P. taiwanensis* abundance was calculated only in the figs where it was recorded.

P. taiwanensis	Explanatory variable	Model	d.f.	Likelihood ratio	Pairwise comparison	df	t/z value
Prevalence	Agaonid species	GLMM	1	14.40***	Only pollinators vs. mix	–	-1.437^{NS}
					Only pollinators vs. only cheaters	–	−3.183**
					Mix vs. only cheaters	–	−2.588*
Abundance	Agaonid species	LMM	1	6.15*	Only pollinators vs. mix	44	-1.59^{NS}
					Only pollinators vs. only cheaters	44	−2.63*
					Mix vs. only cheaters	44	-2.00^{NS}

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
NS, not significant; GLMM, generalised linear models; LMMs, linear mixed models.

Table 4. Comparisons of the pedicel lengths of flowers using linear mixed models (LMM) or generalised linear models (GLMM) testing the differences in pedicel lengths (PL) of female flowers with different contents [UT, other galling non-pollinating fig wasps (NPFW), pollinators, *Philotrypesis taiwanensis*, and seeds), and slopes of the proportions of female flowers being utilised (PFFU) by different utilisation types (UT) with increasing pedicel lengths.

Region	Response variable	Explanatory variable	Model	d.f.	Likelihood ratio	Pairwise comparison	df	t/z value
Thailand	PL	UT	LMM	3	180.37**	Other NPFW vs. pollinators	1559	−8.90 **
						Other NPFW vs. *P. taiwanensis*	1559	-1.66^{NS}
						Other NPFW vs. seeds	1559	−6.84 **
						Pollinator vs. *P. taiwanensis*	1559	11.70 **
						Pollinator vs. seeds	1559	4.04 **
						P. taiwanensis vs. seeds	1559	−7.98 **
Thailand	PFFU	PL × UT	GLMM	3	203.03**	Other NPFW vs. pollinators (along with PL)	–	−5.27 **
						Other NPFW vs. *P. taiwanensis* (along with PL)	–	3.63 *
						Other NPFW vs. seeds (along with PL)	–	-1.52^{NS}
						Pollinator vs. *P. taiwanensis* (along with PL)	–	11.30 **
						Pollinator vs. seeds (along with PL)	–	7.64 **
						P. taiwanensis vs. seeds (along with PL)	–	−6.94 **

*$P < 0.01$; **$P < 0.001$.
NS, not significant.

P. taiwanensis has larvae that feed on developing seeds. Adults emerge from ovules that are surrounded by an elaiosome, a feature absent from galled ovules. They come from figs that contain several different species of fig wasps, but display a preference only for figs that contain pollinators, suggesting that they are not parasitoids of NPFW, but are associated with agaonids. Furthermore, they avoided figs containing an agaonid species that fails to pollinate, suggesting that it is the presence of seeds, not agaonids *per se*, that is required. This does not rule out the possibility that *P. taiwanensis* is a highly specific parasitoid of *E. verticillata*, that avoids other *Eupristina* species, but *E. verticillata* galls do not develop an elaiosome around them. *Philotrypesis taiwanensis* larvae were also distributed towards the periphery of the figs, where seeds (and other NPFW) were abundant, and not towards the centre of the figs, where *E. verticillata* galls are concentrated. Finally, *P. taiwanensis* had a negative impact on seed numbers, but not on pollinator offspring numbers.

A few figs that provided no evidence of pollinator entry nonetheless contained small numbers of seeds. It seems likely that foundresses of *Eupristina* sp., the cheater agaonid, occasionally carry a few pollen grains on their bodies when they enter figs, as was recorded with a cheater agaonid associated with a different species of fig tree (Compton *et al.*, 1991). Alternatively, *E. verticillata* foundresses may also have entered the figs but failed to reproduce, or only unmated *E. verticillata* foundresses had entered them (unmated female foundresses are only able to produce sons, which we could not distinguish, West *et al.*, 1997).

From the host tree's perspective, its female, but not male, reproductive output is reduced by *P. taiwanensis*, which is contrary to the more widespread situation, where NPFW reduce the male reproductive success of figs more than they reduce female reproductive success (Segar & Cook, 2012; Suleman *et al.*, 2013). The negative effects of *P. taiwanensis* on seed numbers were substantial, with figs containing 20 or more offspring of *P. taiwanensis* containing less than half the number of seeds than figs from the same crops where it was absent (and pollinator offspring were present). Experimental addition of *P. taiwanensis* to figs is nevertheless required to accurately assess the impact of this species on seed production. Black *Philotrypesis* species do not form a monophyletic group (Jiang *et al.*, 2006), hence there is reason to assume that other species with this colouration will have a similar biology.

The maximum abundance of *P. taiwanensis* in any one fig was noticeably higher than that achieved by the other *Philotrypesis* species (and other parasitoid NPFW), but similar to that achieved by the more common of the galling

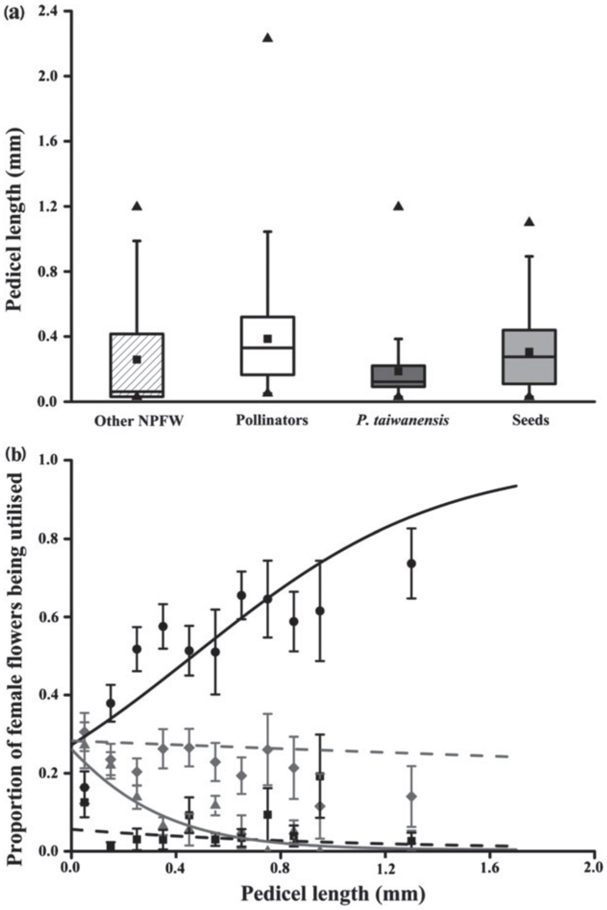

Fig. 2. (a) Box-plot for pedicel lengths of female flowers occupied by 'other' non-pollinating fig wasps (NPFW), pollinators, *Philotrypesis taiwanensis* or seeds. Line, box, whiskers, black squares, and black triangles represent the median, the range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum, and maximum values of pedicel lengths in each utilisation type. (b) The relationship between ovule pedicel lengths and the proportions of the ovules containing other NPFW (black dashed line), agaonids (black line), *P. taiwanensis* (grey line), and seeds (grey dashed line). Data (mean ± SE) are shown for every 0.1 mm in pedicel length except the longest, which includes all data with pedicel lengths longer than 1.00 mm (mean pedicel length for this interval was *c*. 1.3 mm).

species. Host-specific seed predators have had notable success as bio-control agents against invasive plant species (Garren & Strauss, 2009; Baraibar *et al.*, 2011), if they can attain high densities (Knochel *et al.*, 2010). *Philotrypesis taiwanensis* may therefore have a potential value as an agent for use against *F. microcarpa* outside its native range. However, we have not identified any parasitoid NPFW that target *P. taiwanensis* (possibly because seed tissue is likely to be chemically distinct from that present in galls, and most parasitoid NPFW may also feed on some plant material). This suggests that *P. taiwanensis* will not benefit from an absence of its own natural enemies outside its native range (Keane & Crawley, 2002; Müller-Schärer & Schaffner, 2008; Pearson *et al.*, 2011), and so is less likely to reach higher densities there. Consistent with this, *P. taiwanensis* has already been accidentally introduced with no notable impact into Brazil, Hawaii, and Florida, where *F. microcarpa* has been planted (Beardsley, 1998; Farache *et al.*, 2009). Nonetheless, in combination with other NPFW such as *Sycoscapter gajimaru* that reduce pollinator numbers and other NPFW that gall ovules, *P. taiwanensis* still has the potential to help reduce the rate of spread of its host plant.

An explanation for the rarity of typical seed predators among fig wasps is related to the constraints acting on fig trees as a result of their mutualistic association with pollinator fig wasps. The male component of fig tree reproduction (pollen donation) depends on the successful development of agaonid larvae inside ovules that the pollinators have galled (and often pollinated). This limits the extent to which the plant can develop chemical defences to protect its ovules, because any increase in chemical defences is likely to harm the agaonid larvae, as well as other insects that are damaging the seeds. This constraint mean that ovules inside figs are likely to be poorly defended, compared with those of other plants. After pollination, developing seeds are under no such constraints, and we hypothesise that levels of defensive compounds rise rapidly at this time, making them a less attractive resource than the tissues present in galled ovules. Constraints are absent throughout ovule development in about half of all fig tree species, because they have two functionally discrete sexes of trees, that specialise in either seed or pollinator production (Greeff & Compton, 2002). Female (seed-producing) trees are not constrained by the need to avoid harming pollinator larvae and their ovules are utilised by very few species of NPFW, presumably because they are better defended than ovules in male figs.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12122

Table S1. Prevalence (proportion of figs occupied) and total abundance of each fig wasp species in the 167 figs that contained *Philotrypesis taiwanensis*. The full generic name was given only when it was first mentioned and abbreviation is used for other species in the same genus.

References

Baraibar, B., Carrión, E., Recasens, J. & Westerman, P.R. (2011) Unravelling the process of weed seed predation: developing options for better weed control. *Biological Control*, **56**, 85–90.

Bates, D., Maechler, M. & Bolker, B. (2013) *lme4: Linear Mixed-Effects Models Using S4 Classes*. R package version 0.999999-2. [WWW document]. URL http://CRAN.R-project.org/package=lme4 [accessed on 20 October 2013].

Beardsley, W.J. (1998) Chalcid wasps (Hymenoptera: Chalcidoidea) associated with fruit of *Ficus microcarpa* in Hawaii. *Proceedings of the Hawaiian Entomological Society*, **33**, 19–34.

Bouček, Z., Watsham, A. & Wiebes, J.T. (1981) The fig wasp fauna of the receptacles of *Ficus thonningii* (Hymenoptera, Chalcidoidea). *Tijdschrift Voor Entomologie*, **124**, 149–233.

Burrows, J. & Burrows, S. (2003) *Figs of Southern & South-central Africa*. Umdaus Press, Hatfield, U.K.

Caughlin, T., Wheeler, J.H., Jankowski, J. & Lichstein, J.W. (2012) Urbanized landscapes favored by fig-eating birds increase invasive but not native juvenile strangler fig abundance. *Ecology*, **93**, 1571–1580.

Chen, Y.-R., Chuang, W.-C. & Wu, W.-J. (1999) Chalcid wasps on *Ficus microcarpa* L. in Taiwan (Hymenoptera: Chalcidoidea). *Journal of Taiwan Museum*, **52**, 39–79.

Chen, H.-H., Yang, D.-R., Gu, D., Compton, S.G. & Peng, Y.-Q. (2013) Secondary galling: a novel feeding strategy among 'non-pollinating' fig wasps from *Ficus curtipes*. *Ecological Entomology*, **38**, 381–389.

Compton, S.G. (1993) One way to be a fig. *African Entomology*, **1**, 151–158.

Compton, S.G. & Hawkins, B.A. (1992) Determinants of species richness in southern African fig wasp assemblages. *Oecologia*, **91**, 68–74.

Compton, S.G. & Nefdt, R.J.C. (1990) The figs and fig wasps of *Ficus burtt-davyi*. *Mitteilungen aus dem Institut für Allgemeine Botanik Hamburg*, **23a**, 441–450.

Compton, S.G. & van Noort, S. (1992) Southern African fig wasp assemblages: host relationships and resource utilization. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen*, **95**, 423–435.

Compton, S.G. & Robertson, H.G. (1988) Complex interactions between mutualisms: ants tending Homopterans protect fig seeds and pollinators. *Ecology*, **69**, 1302–1305.

Compton, S.G., Holton, K.C., Rashbrook, V.K., van Noort, S., Vincent, S.L. & Ware, A.B. (1991) Studies of *Ceratosolen galili*, a non-pollinating agaonid fig wasp. *Biotropica*, **23**, 188–194.

Compton, S.G., van Noort, S., McLeish, M., Deeble, M. & Stone, V. (2009) Sneaky African wasps that oviposit through holes drilled by other species. *African Natural History*, **9**, 9–15.

Cook, J.M. & Rasplus, J.-Y. (2003) Mutualism with attitude: coevolving fig wasps and figs. *Trends in Ecology & Evolution*, **18**, 241–248.

Cruaud, A., Ronsted, N., Chantarasuwan, B., Chou, L.-S., Clement, W.L., Couloux, A. *et al.* (2012) An extreme case of plant-insect codiversification: figs and fig-pollinating wasps. *Systematic Biology*, **61**, 1029–1047.

Dunn, D.W., Segar, S.T., Ridley, J., Chan, R., Crozier, R.H., Yu, D.W. *et al.* (2008) A role for parasites in stabilising the fig-pollinator mutualism. *PLoS Biology*, **6**, e59.

Farache, F.H.A., DO Ó, V.T. & Pereira, R.A.S. (2009) New occurrence of non-pollinating fig wasps (Hymenoptera: Chalcidoidea) in *Ficus microcarpa* in Brazil. *Neotropical Entomology*, **38**, 683–685.

Feng, G. & Huang, D.-W. (2010) Description of a new species of *Odontofroggatia* (Chalcidoidea, Epichrysomallinae) associated with *Ficus microcarpa* (Moraceae) with a key to species of the genus. *Zootaxa*, **2335**, 40–48.

Galil, J. & Eisikowitch, D. (1968) Flowering cycles and fruit types of *Ficus sycomorus* in Israel. *New Phytologist*, **67**, 745–758.

Garren, L.M. & Strauss, S.Y. (2009) Population-level compensation by an invasive thistle thwarts biological control from seed predators. *Ecological Applications*, **19**, 709–721.

Greeff, J.M. & Compton, S.G. (2002) Can seed protection lead to dioecy in *Ficus*? *Oikos*, **96**, 386–388.

Harrison, R.D. (2005) Fig and the diversity of tropical rainforest. *Bioscience*, **55**, 1053–1064.

Herre, E.A. (1993) Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science*, **259**, 1442–1445.

Herre, E.A., Jandér, K.C. & Machado, C.A. (2008) Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 439–458.

Jiang, Z.-F., Huang, D.-W., Chen, L.-L., Zhen, W.-Q., Fu, Y.-G. & Peng, Z.-Q. (2006) Rampant host switching and multiple female body color transitions in *Philotrypesis* (Hymenoptera: Chalcidoidea: Agaonidae). *Journal of Evolutionary Biology*, **19**, 1157–1166.

Joseph, K.J. (1957) Le parasitisme de *Philotrypesis caricae* L. (Hym., Chalcidiens Callimomidae) et l'influence de la vie parasitairesur le parasite. *Comptes Rendus de l'Académie des Sciences*, **244**, 1269–1272.

Jousselin, E., van Noort, S. & Greeff, J.M. (2004) Labile male morphology and intraspecific male polymorphism in the *Philotrypesis* fig wasps. *Molecular Phylogenetics and Evolution*, **33**, 706–718.

Kaufmann, S., Mckey, D.B., Hossaert-Mckey, M. & Horvitz, C.C. (1991) Adaptations for a two phase seed dispersal system involving vertebrates and ants in a hemiepiphytic fig (*Ficus microcarpa*: Moraceae). *American Journal of Botany*, **78**, 971–977.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, **17**, 164–170.

Knochel, D.G., Flagg, C. & Seastedt, T.R. (2010) Effects of plant competition, seed predation, and nutrient limitation on seedling survivorship of spotted knapweed (*Centaurea stoebe*). *Biological Invasions*, **12**, 3771–3784.

McKey, D. (1989) Population biology of figs: applications for conservation. *Experientia*, **45**, 661–673.

Miao, B.-G., Yang, D.-R., Liu, C., Peng, Y.-Q. & Compton, S.G. (2012) The impact of a gall midge on the reproductive success of *Ficus benjamina*, a potentially invasive fig tree. *Biological Control*, **59**, 228–233.

Müller-Schärer, H. & Schaffner, U. (2008) Classical biological control: exploiting enemy escape to manage plant invasions. *Biological Invasions*, **10**, 859–874.

Murray, M.G. (1987) The closed environment of the fig receptacle and its influence on male conflict in the old World fig wasp, *Philotrypesis pilosa*. *Animal Behaviour*, **35**, 488–506.

Nadel, H., Frank, J.H. & Knight, R.J. Jr. (1992) Escapees and accomplices: the naturalisation of exotic *Ficus* and their associated faunas in Florida. *Florida Entomologist*, **75**, 29–38.

van Noort, S., Wang, R. & Compton, S.G. (2013) Fig wasps (Hymenoptera: Chalcidoidea: Agaonidae, Pteromalidae) associated with Asian fig trees (*Ficus*, Moraceae) in South Africa: Asian followers and African colonists. *African Invertebrates*, **54**, 381–400.

Pearson, D.E., Gallaway, R.M. & Maron, J.L. (2011) Biotic resistance via granivory: establishment by invasive, naturalized, and native asters reflects generalist preference. *Ecology*, **92**, 1748–1757.

Pereira, R.A.S., de Pádua Teixeira, S. & Kjellberg, F. (2007) An inquiline fig wasp using seeds as a resource for small male production: a potential first step for the evolution of new feeding habits? *Biological Journal of the Linnean Society*, **92**, 9–17.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & the R Development Core Team (2013) *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-110.

R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

Segar, S.T. & Cook, J.M. (2012) The dominant exploiters of the fig/pollinator mutualism vary across continents, but their costs falls consistently on the male reproductive function of figs. *Ecological Entomology*, **37**, 342–349.

Shanahan, M., So, S., Compton, S.G. & Corlett, R. (2001) Fig-eating by vertebrate frugivores: a global view. *Biological Reviews*, **76**, 529–572.

Starr, F., Starr, K. & Loope, L. (2003) *Ficus microcarpa Chinese banyan Moraceae* [WWW document]. URL http://www.hear.org/starr/hiplants/reports/pdf/ficus_microcarpa.pdf [accessed on 15 January 2013].

Suleman, N., Raja, S. & Compton, S.G. (2013) Parasitism of a pollinator fig wasp: mortalities are higher in figs with more pollinators, but are not related to local densities of figs. *Ecological Entomology*, **38**, 478–484.

Sun, X.-J., Xiao, J.-H., Cook, J.M., Feng, G. & Huang, D.-W. (2011) Comparisons of host mitochondrial, nuclear and endosymbiont bacterial genes reveal cryptic fig wasp species and the effects of *Wolbachia* on host mtDNA evolution and diversity. *BMC Evolutionary Biology*, **11**, 86–93.

Tan, H.T.W. & Yeo, C.K. (2009) *The Potential of Native Woody Plants for Enhancing the Urban Waterways and Water Bodies Environment in Singapore*. Raffles Museum of Biodiversity Research and Singapore-Delft Water Alliance National, University of Singapore, Singapore.

Tzeng, H.-Y., Tseng, L.-J., Ou, C.-H., Lu, K.-C. & Lu, F.-Y. (2008) Confirmation of the parasitoid feeding habit in Sycoscapter, and their impact on pollinator abundance in *Ficus formosan*a. *Symbiosis*, **45**, 129–134.

West, S.A., Herre, E.A., Compton, S.G., Godfray, H.C.J. & Cook, J.M. (1997) A comparative study of virginity in fig wasps. *Animal Behaviour*, **54**, 437–450.

Wiebes, J.T. (1979) Co-evolution of figs and their insect pollinators. *Annual Review of Ecology, Evolution, and Systematics*, **10**, 1–12.

Yu, H. & Compton, S.G. (2012) Moving your sons to safety: galls containing male fig wasps expand into the centre of figs, away from enemies. *PLoS One*, **7**, e30833.

Zhou, M-J., Xiao, J-H., Li Y-W., Wu, W-S., Niu, L-M., Hu H-Y. *et al*. (2012) Molecular approaches identify known species, reveal cryptic species, and verify host specificity of Chinese *Philotrypesis*. *Molecular Ecology Resources*, **12**, 598–606.

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