

# Occurrence of internally ovipositing non-agaonid wasps and pollination mode of the associated agaonid wasps



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## ABSTRACT

Figs (*Ficus*, Moraceae) and their pollinating fig wasps (Hymenoptera, Agaonidae, Chalcidoidea) are a striking example of obligate mutualism and coevolution. Agaonid females enter the figs to lay their eggs, but also actively or passively transport pollen into the figs at the same time. We examined eight related fig tree species pollinated by host specific *Eupristina* agaonids to determine the relationships between pollination mode, host pollen, ovule ratios and the ability of the figs to recruit additional non-agaonid pollinators. Uniquely amongst the eight *Eupristina* species, the pollinator of *Ficus curtipes* has non-functional pollen pockets and no coxal combs, showing that it pollinates passively. Reflecting this, the anther-to-ovule ratio of *F. curtipes* is unusually high.

In addition to the agaonids, figs support many species of 'non-pollinating fig wasps' (NPFW) that are typically ovule gallers or parasitoids. These mainly oviposit from outside the figs but there are a few species of NPFW that are like agaonids and enter the figs to oviposit. Two of the eight *Eupristina* pollinated fig trees support host specific internally-ovipositing fig wasps belonging to the chalcidoid genera *Diaziella* (Sycoecinae) and *Lipothymus* (Otitellinae). Reflecting the trees' pollination modes, these fig wasps act as supplementary pollinators of *F. curtipes*, but not of *Ficus glaberrima*, where agaonid pollination is active.

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## 1. Introduction

The association between figs (*Ficus*, Moraceae) and their pollinating fig wasps (Hymenoptera, Agaonidae, Chalcidoidea) is generally thought to be an ideal system for investigating coevolution and the maintenance of mutualisms. Both figs and their pollinating fig wasps are completely dependent on each other for survival and reproduction, as figs can only be pollinated by pollinating fig wasps, and pollinating fig wasps can only reproduce within figs. Although previous studies reported that each fig species had its own species-specific pollinating fig wasp species (Ramirez, 1970; Wiebes, 1979; Bronstein, 1987; Herre et al., 1996; Anstett et al., 1997), recent work has found examples of multiple pollinating fig wasp species co-

occurring on the same fig species (Kerdelhué et al., 1999; Molbo et al., 2003, 2004). These findings suggest that the phenomenon of pollinator host-switching may be more complex than previously thought in fig-wasp mutualism (Machado et al., 2005).

Some Agaonids are passive pollinators, transporting pollen on their body surface, while others actively collect and store pollen and then distribute it on the styles of fig flowers while they are ovipositing. Active pollination is much more efficient, allowing their host figs to produce far fewer male flowers because less pollen is required. In about one-third of fig species (Kjellberg et al., 2001), pollination is passive. The mode of pollination occurring in a species of *Ficus* can be consistently predicted from the anther-to-ovule ratio (Kjellberg et al., 2001). On a representative number of fig species, these authors showed that an anther-to-ovule ratio of less than 0.16 indicates active pollination, while a ratio over 0.20 is characteristic of passively pollinated species.

Figs also host numerous non-pollinating fig wasp species that depend on figs for their development and reproduction without providing any benefit to their figs (Bronstein, 1991; Compton and

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**Table 1**  
The species of fig wasps and their biological characteristics.

Ficus	Species	Diagnosis	Coexisting role
<i>Ficus curtipes</i>	<i>Eupristina</i> sp. 1	Female: body black color with clear wings; male: wingless	Gall maker
<i>F. curtipes</i>	<i>Diaziella yangi</i>	Female: the large dark patch on the forewings; male: winged	Inquiline
<i>F. curtipes</i>	<i>Lipothymus</i> sp.	Female: body metallic color with clear wings; Male: wingless	Inquiline
<i>F. glaberrima</i>	<i>Eupristina</i> sp. 2	Female: body black color with clear wings; Male: wingless	Gall maker
<i>F. glaberrima</i>	<i>D. bizarrea</i>	Female: body metallic color with clear wings; Male: winged	Inquiline
<i>F. altissima</i>	<i>E. altissima</i>	Female: body black color with clear wings; Male: wingless	Gall maker
<i>F. benjamina</i>	<i>E. oningsbergeri</i>	Female: body black color with clear wings; Male: wingless	Gall maker
<i>F. stricta</i>	<i>E. cyclostigma</i>	Female: body black color with clear wings; Male: wingless	Gall maker
<i>F. microcarpa</i>	<i>E. verticillata</i>	Female: body black color with clear wings; Male: wingless	Gall maker
<i>F. macellandi</i>	<i>Eupristina</i> sp. 3	Female: body black color with clear wings; Male: wingless	Gall maker
<i>F. drupacea pubescens</i>	<i>Eupristina</i> sp. 4	Female: body black color with clear wings; Male: wingless	Gall maker

Hawkins, 1992; Boucek, 1993; West and Herre, 1994; West et al., 1996; Cook and Rasplus, 2003; Pereira and Prado, 2005). They do not belong to the pollinating lineage Agaonidae (Rasplus et al., 1998). Most non-agaonid fig wasps oviposit through the fig wall from the exterior of the fig. However, some species enter figs and oviposit in the female flowers, just as the pollinating fig wasps do.

Biological data on these internally-ovipositing non-agaonid fig wasps is limited. Their hosts are often largely unknown for Asian species. Some internally ovipositing non-agaonid fig wasps *Diaziella* (Pteromalidae, Sycoecinae) and *Lipothymus* species (Pteromalidae, Otitesellinae) are known to be able to act as pollinators. They develop in figs that produce abundant pollen and consequently their *Waterstoniella* and *Eupristina* agaonids are passive pollinators (Jousselin et al., 2001; Zhang et al., 2008). In contrast, there is no evidence of a mutualism between an internally ovipositing non-agaonid fig wasp and its actively-pollinated host fig, *Ficus glaberrima* (Zhang et al., 2009). Here, we examine the anatomy of the *Eupristina* pollinators of eight closely related fig tree species, and relate this to mode of pollination and the possibility of non-agaonids being recruited for pollination.

## 2. Materials and methods

### 2.1. Study site and study species

Figs were collected from eight species of (subgenus *Urostigma*, subsection *Conosycea*), in the vicinity of the Xishuangbanna Tropical Botanical Garden (XTBG), in South-West China (101° 15' E, 21° 55' N), at the northern margin of tropical South-east Asia. The genus *Eupristina* belongs to Agaonidae. Two internally ovipositing non-agaonid fig wasp species (*Diaziella yangi* and *Diaziella bizarrea*) belong to Pteromalidae, Sycoecinae. Another internally ovipositing non-agaonid fig wasp species (*Lipothymus* sp.) belongs to Pteromalidae, Otitesellinae. The species of fig wasps and their biological characteristics are given in Table 1.

### 2.2. Occurrence of internally ovipositing non-agaonid wasps

We first assessed whether internally ovipositing non-agaonid wasps were regularly found in *Ficus* species pollinated by the agaonid genus *Eupristina*. For eight *Ficus* species pollinated by fig wasps of this genus, figs were sampled. Figs were collected when fig wasps are emerging and pollen is mature. They were kept in fine-mesh bags and fig wasps were allowed to emerge. Wasps were preserved in 70% alcohol for identification. In addition, all female and male flowers of figs were counted.

### 2.3. Direct evidence of mode of pollination

The mode of pollination was confirmed by using our own observations and published data. Receptive and mature figs were

collected. Then we bisected the fig lengthways, from the stalk to the ostiole, to reveal the lumen. Observation of pollen-loading and pollen-deposition behavior was performed under microscope. In order to further detect the pollination mode of *Eupristina* species, SEM photos of the pollen pockets and coxal combs were taken. Pollen pockets and coxal combs are two traits associated with the mode of pollination in agaonid fig wasps. Coxal combs are considered as the most reliable trait for inferring mode of pollination, as many species of agaonids still possess pollen pockets but do not actively collect and deposit pollen (Kjellberg et al., 2001).

## 3. Results

### 3.1. Frequencies of internally ovipositing non-agaonid fig wasps in fig crops pollinated by *Eupristina*

Among the eight species of *Ficus* collected at fig maturity. Interestingly, two *Ficus* species host one or two species of non-agaonid wasps that enter the fig to oviposit in addition to agaonid wasp belonging to the genus *Eupristina*. *Ficus* species collected and their associated wasps are given in Table 2. *Diaziella*, *Lipothymus* and *Eupristina* emerged simultaneously from figs. Each species of *Diaziella* and *Lipothymus* was specific to its associated fig.

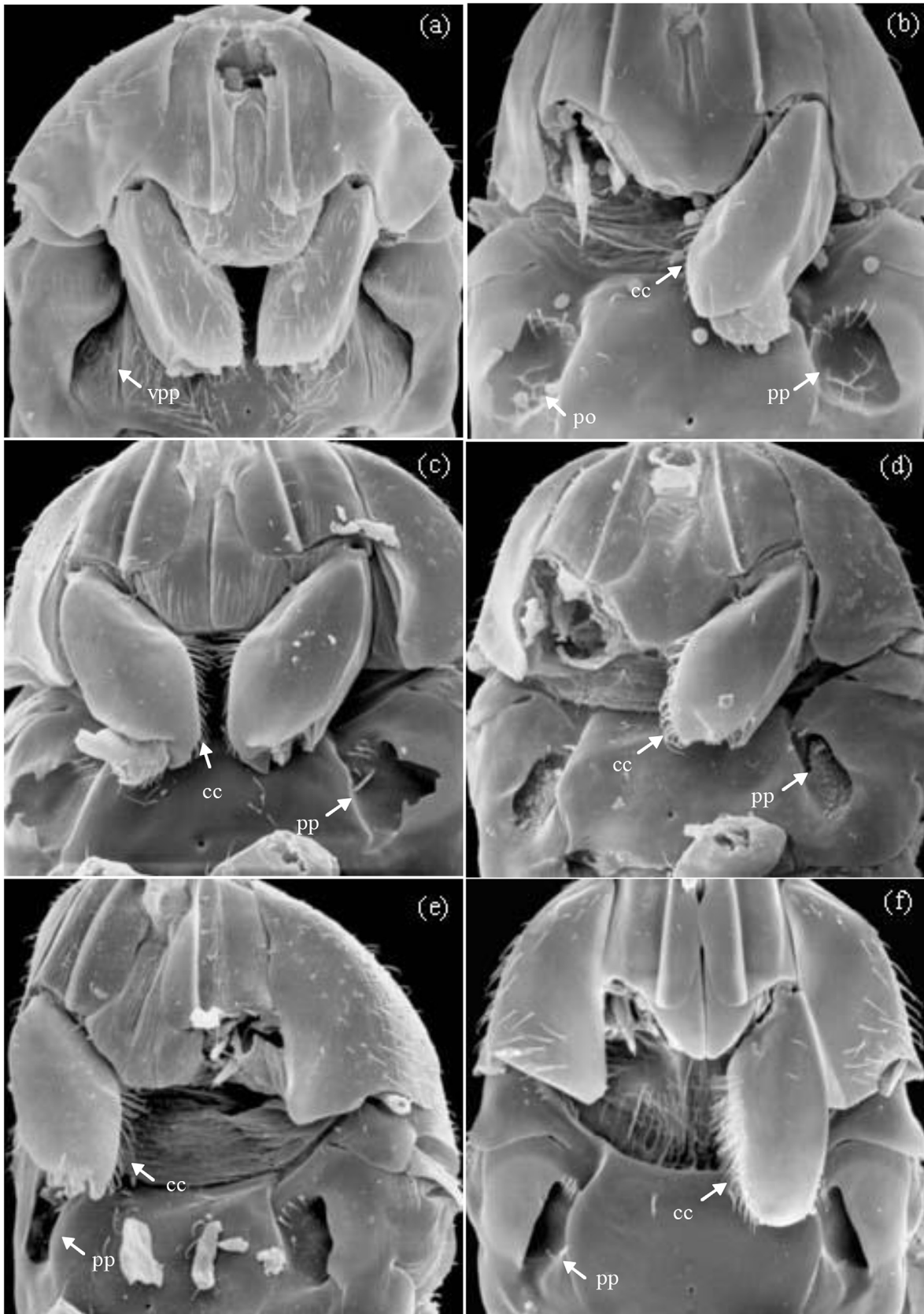
### 3.2. Pollination mode of the associated fig wasps

The anther-to-ovule ratio of *Ficus curtipes* was high ( $0.84 \pm 0.10$  (mean  $\pm$  SD,  $n = 67$ )), suggesting that this *Ficus* species is passively pollinated. This was consistent with the anatomy of adult female *Eupristina* sp. (Fig. 1a), the pollinator *Eupristina* sp. females of *F. curtipes* possessed obviously non-functional pollen pockets and had no the coxal combs. The females become completely dusted with pollen when they emergence from figs. When the fig wasps

**Table 2**

Occurrence of internally ovipositing wasps in *Ficus* species pollinated by *Eupristina* species. One crop was sampled from each tree.

<i>Ficus</i> species	Number of trees	Internally-ovipositing fig wasps	
		Agaonids	Others
<i>Ficus curtipes</i>	4	<i>Eupristina</i> sp. 1	<i>Diaziella yangi</i> <i>Lipothymus</i> sp.
<i>F. glaberrima</i>	3	<i>Eupristina</i> sp. 2	<i>Diaziella bizarrea</i>
<i>F. altissima</i>	3	<i>Eupristina altissima</i>	
<i>F. benjamina</i>	2	<i>Eupristina koningsbergeri</i>	
<i>F. stricta</i>	2	<i>Eupristina cyclostigma</i>	
<i>F. microcarpa</i>	2	<i>Eupristina verticillata</i>	
<i>F. macellandi</i>	2	<i>Eupristina</i> sp. 3	
<i>F. drupacea pubescens</i>	1	<i>Eupristina</i> sp. 4	



**Fig. 1.** Ventral views of female agaonid mesosoma: (a) *Eupristina* sp.1 (host *Ficus curtipes*), (b) *Eupristina* sp.2 (host *Ficus glaberrima*), (c) *Eupristina koningsbergi* (host *Ficus benjamina*), (d) *Eupristina cyclostigma* (host *Ficus stricta*), (e) *Eupristina* sp.3 (host *Ficus macellandi*), (f) *Eupristina* sp. 4 (host *Ficus drupacea pubescens*). The head is pointing upward. Species pollinating actively are shown in b–f. Species pollinating passively is shown in a. Abbreviations: vpp, vestigial pollen pocket; pp, pollen pocket; po, pollen seen through pollen-pocket opening; cc, coxal comb.

**Table 3**  
Pollination behavior in *Eupristina* agaonids in relation to their anatomy and the pollen-ovule ratios of their host *Ficus*.

<i>Ficus</i> species	Sample size (figs)	Anthers/ovules (mean $\pm$ SD)	Pollinating fig wasps	Pollen pocket	Coxal comb	Pollination behavior (Sources)
<i>Ficus curtipes</i>	67	0.84 $\pm$ 0.10	<i>Eupristina</i> sp.1	vestigial	No	Passive (observed)
<i>F. glaberrima</i>	42	0.11 $\pm$ 0.02	<i>Eupristina</i> sp. 2	Yes	Yes	Active (observed)
<i>F. altissima</i>	30	0.09 $\pm$ 0.02	<i>Eupristina altissima</i>	Yes	Yes	Active (observed)
<i>F. benjamina</i>	26	0.10 $\pm$ 0.02	<i>Eupristina koningsbergeri</i>	Yes	Yes	Active (observed)
<i>F. stricta</i>	29	0.15 $\pm$ 0.02	<i>Eupristina cyclostigma</i>	Yes	Yes	Active (observed)
<i>F. microcarpa</i>	26	0.08 $\pm$ 0.03	<i>Eupristina verticillata</i>	Yes	Yes	Active (observed)
<i>F. macellandi</i>	34	0.14 $\pm$ 0.04	<i>Eupristina</i> sp. 3	Yes	Yes	Active (observed)
<i>F. drupacea pubescens</i>	Not recorded	0.09 (K)	<i>Eupristina</i> sp. 4	Yes	Yes	Active (observed)

Sources: data from (K): Kjellberg et al., 2001.

enter a receptive fig, pollen is progressively dispersed within the cavity. The fig females did not show pollination behavior.

The anther-to-ovule of other seven *Ficus* species was low, and the data on mode of pollination and on the fig traits were presented in Table 3, implying that these *Ficus* species are actively pollinated. These were consistent with the anatomy of their associated female agaonids (Fig. 1), which have well defined thoracic pollen pockets (to carry pollen) and coxal combs on their fore coxae (to manipulate pollen). Active pollination of these agaonids was confirmed by observation of pollen-loading and pollen-deposition behavior in mature and receptive figs respectively.

#### 4. Discussion

Jousselin et al. (2001) reported seven *Ficus* species pollinated by wasps of the genus *Waterstoniella* in Brunei Darussalam, on the island of Borneo, their result showed that six species of *Ficus* that were passively pollinated by the agaonid genus *Waterstoniella* hosted internally ovipositing non-agaonid wasps (the genera *Diaziella* (Otitesellinae) and *Lipothymus* (Sycoecinae)). In contrast, the colonize rate of internally ovipositing non-agaonid wasps in *Ficus* species pollinated by the genus *Eupristina* was low. Our study shows that two species of *Ficus* pollinated by the genus *Eupristina* host one or two specific species of internally ovipositing non-agaonid wasps of the genera *Lipothymus* or *Diaziella* in Xishuangbanna, China.

Based on our observations, only about one species (*F. curtipes*) is passively pollinated, the other (*F. glaberrima*, *Ficus altissima*, *Ficus benjamina*, *Ficus stricta*, *Ficus macrocarpa*, *Ficus macellandi*, *Ficus drupacea pubescens*) is actively pollinated. Action pollinating is probably generally beneficial for both wasp and fig. Indeed, previous report showed that active pollination benefited the fig by allowing a strong reduction in pollen production (Kjellberg et al., 2001). It was also generally thought that ovule fertilization benefited the fig wasp, either because it limited fig abortion (Janzen, 1979) or because it ensured better survivorship of their larvae (Jousselin et al., 2003; Tarachai et al., 2008). In the genus *Eupristina*, most species pollinate actively and only one species pollinate passively. This pattern suggests that rare passively pollinating species in mainly actively pollinating species represent relatively recent cases of loss of active pollination. This conclusion is consistent with previous report (Kjellberg et al., 2001).

Some non-agaonid fig wasps (*Diaziella* and *Lipothymus* species) that enter figs to oviposit are capable of pollinating them, and thereby establishing a mutualism with their host plants (Jousselin et al., 2001; Zhang et al., 2008). In all reported cases the host plants are nonetheless also associated with a 'typical' agaonid pollinator, and so are not dependent upon these substitute pollinators. Furthermore, the agaonids are passive pollinators and their host figs produce large amounts of pollen that becomes scattered within the figs when adult females are preparing to leave their natal figs. Pollinators of such figs do not require any specific

adaptations to achieve effective pollination, so long as foundresses enter figs during their receptive period, which they are forced to do because this is the only time that the ostiolar bracts loosen to form a passage. In contrast, active pollinators, such as the *Eupristina* species associated with *F. glaberrima*, have complex morphological and behavioral adaptations that ensure that the relatively small amounts of pollen available to them can be collected and transferred. There is no evidence of mutualism between *D. bizarrea* and its actively-pollinated host fig, *F. glaberrima* (Personal communication). The contrast between the result and those of Jousselin et al. (2001) and Zhang et al. (2008) confirms that the ability of non-agaonids to act as substitute pollinators is dependent of the mode of pollination of their associated agaonid fig wasps.

When the associated agaonid fig wasp pollinates actively, only a very small quantity of pollen is produced by the fig (Galil and Meiri, 1981; Kjellberg et al., 2001). The internally ovipositing non-agaonids do not present any pollen collecting behavior, the low level of seed production is supposed to be due to incidentally passive pollen transfer on the body of these fig wasps (Newton and Lomo, 1979; Compton et al., 1991). In contrast, in passively pollinated figs, abundant pollen is produced in cavity of fig, which ensures the passive coating of fig wasps with pollen as they emerge, the internally ovipositing non-agaonids become efficient pollinators (Jousselin et al., 2001; Zhang et al., 2008).

Our study suggests that the mode of interaction between internally ovipositing non-agaonids and figs relies on the mode of pollination of the associated agaonid wasp. Studies of the molecular phylogenies of *Ficus*, pollinating and externally ovipositing non-pollinating fig wasps have already given valuable insights into the putative factors that are responsible for the origin and evolution of the fig-wasp mutualism (Machado et al., 1996; Rasplus et al., 1998; Marussich and Machado, 2007). Similarly, internally ovipositing non-agaonid wasps and pollinating and externally ovipositing non-pollinating fig wasps will be very interesting models for comparative analysis. The combination of mode of pollination will open a whole fig-wasp world of exciting possibilities. Further studies of internally ovipositing non-agaonid wasps present not only a tremendous advance in general fig knowledge, but an exceptionally good set of opportunities for future work.

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