



Pollinator sharing in dioecious figs (*Ficus*: Moraceae)

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As one of the most specialized pollination syndromes, the fig (*Ficus*)–fig wasp (Agaonidae) mutualism can shed light on how pollinator behaviour and specificity affect plant diversification through processes such as reproductive isolation and hybridization. Pollinator sharing among species has important implications for *Ficus* species delimitation and the evolutionary history of the mutualism. Although agaonid wasp pollinators are known to visit more than one host species in monoecious figs, pollinator sharing has yet to be documented in dioecious figs. The present study investigated the frequency of pollinator sharing among sympatric, closely-related dioecious figs in *Ficus* sections *Sycocarpus* and *Sycidium*. Molecular and morphological species identification established the associations between pollinating agaonid wasp species and host fig species. Cytochrome oxidase I was sequenced from 372 *Ceratosolen* pollinators of *Ficus* section *Sycocarpus* and 210 *Kradibia* pollinators of *Ficus* section *Sycidium*. The association between fig species and morphologically distinct clades of pollinator haplotypes was predominantly one-to-one. In *Ceratosolen*, six of 372 pollinators (1.5%) visited fig species other than the predominant host. No pollinator sharing was detected between the two *Sycidium* host species, although a rare hybrid shared *Kradibia* pollinators with both parental species. These findings point to low rates of pollinator sharing among closely-related dioecious fig species in sympatry, and perhaps lower rates than among monoecious figs. Such rare events could be evolutionarily important as mechanisms for gene flow among fig species. Differences in rates of pollinator sharing among fig lineages might explain the conflicting phylogenetic patterns inferred among monoecious figs, dioecious figs, and their respective pollinators. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 103, 546–558.

ADDITIONAL KEYWORDS: agaonid wasps – *Ceratosolen* – cytochrome oxidase I – *Kradibia* – specificity – reproductive isolation.

INTRODUCTION

Pollinators visit flowers to obtain pollen, nectar, oils, brood sites or perceived mating opportunities. The potential for gene flow among sympatric plant species and the maintenance of species integrity depends on the degree to which pollinators consistently visit flowers of the same species versus different species (Waser & Ollerton, 2006). Pollinator specificity can act as a pre-zygotic mechanism of reproductive isolation among plant species (Grant, 1949, 1994) and is a

product of behavioural, morphological, and chemical interactions. Such pollinator-mediated reproductive isolation has been observed in diverse angiosperm lineages with varying degrees of sympatry and of gene flow in hybrid zones. Classic examples include *Aquilegia* (Grant, 1952; Fulton & Hodges, 1999), *Mimulus* (Schemske & Bradshaw, 1999; Ramsey, Bradshaw & Schemske, 2003), *Ipomopsis* (Campbell, Alarcon & Wu, 2003; Campbell, 2004), *Penstemon* (Kimball, 2008), and *Asclepias* (Kephart & Theiss, 2004). Pollinator specificity is considered to be strongest in obligate pollinator–nursery mutualisms (Ollerton, 2006) in which pollination services are exchanged for brood sites. The best studied among

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these mutualisms are those between yucca plants–yucca moths (Pellmyr, 2003), fig plants–fig wasps (Janzen, 1979), and *Glochidion* trees–*Epicephala* moths (Kato, Takimura & Kawakita, 2003). Studies have shown that plant volatile cues play a significant role in pollinator-mediated plant reproductive isolation (Grison-Pige *et al.*, 2002b; Okamoto, Kawakita & Kato, 2007). The morphological match between host and pollinator are also known to contribute to reproductive isolation (van Noort & Compton, 1996; Godsoe *et al.*, 2008; Smith *et al.*, 2009).

Fig plants (Moraceae: *Ficus*) and their wasp pollinators (Hymenoptera: Agaonidae) represent an extreme along the plant–pollinator specialization continuum where specificity has chemical and morphological components. The life cycle of the agaonid wasp begins and ends within an enclosed inflorescence, or syconium, of its host *Ficus* plant (Weiblen, 2002). After maturation and mating within the fig, a female wasp emerges, carrying pollen, and searches for another fig in which to lay her eggs. Volatile chemical signals attract her to a receptive fig (Song *et al.*, 2001; Grison-Pige, Bessiere & Hossaert-McKey, 2002a; Chen & Song, 2008; Proffit, Schatz & Bessiere, 2008). She then gains access to her new host through a tight, bract-covered opening called the ostiole. Specialized appendages on her head help her to pry her way through the bracts. As she oviposits down the styles of the tiny flowers within the fig, she deposits pollen that she has carried from her birth fig onto the stigmas. Some flowers will produce seed and others will house the developing wasp larvae. In monoecious figs, both flower types are found within the same syconia. In dioecious figs, seed-producing and wasp/pollen-producing figs are located on separate plants. Pollinator visitation depends on a foundress wasp locating and recognizing a specific host via chemical cues (Bronstein, 1987; van Noort, Ware & Compton, 1989), as well as a morphological match between wasp and fig (VanNoort & Compton, 1996; Weiblen, 2004), allowing access to flowers within the enclosed inflorescence. Successful pollination additionally depends on a number of other factors, including wasp behaviour and pollen compatibility. The present study aimed to examine the effectiveness of pre-zygotic isolating mechanisms associated with pollinator visitation. We examined pollinator specificity by identifying wasp species that successfully located, accepted, and gained access to the syconia of sympatric and dioecious fig species. A previous study examining monoecious fig pollinator visitation found that a single wasp species comprised 99% of pollinators attracted to *Ficus pertusa*, and only this species gained access to *F. pertusa* syconia (Bronstein, 1987).

However, the last decade of study on the fig–fig wasp pollination mutualism and the proliferation of

molecular data has changed our view of fig–fig wasp specificity from a highly species-specific 1 : 1 association to a more complex model that includes two or more pollinator species associated with particular fig species (Molbo *et al.*, 2003; Haine, Martin & Cook, 2006; Marussich & Machado, 2007; Jackson *et al.*, 2008; Peng, Duan & Yang, 2008; Su *et al.*, 2008; Zhang *et al.*, 2008; Compton, Grehan & van Noort, 2009) and pollinator sharing among multiple fig species (Molbo *et al.*, 2003; Machado *et al.*, 2005; Marussich & Machado, 2007; Su *et al.*, 2008). Molecular evidence of hybridization among fig species has also been documented (Parrish *et al.*, 2003; Machado *et al.*, 2005; Renoult *et al.*, 2009). However, the ubiquity of such events in the fig–fig wasp system is unclear. Pollinator sharing has implications for plant species integrity, the delimitation of *Ficus* species, and the importance of host switching in shaping the evolutionary history of the mutualism. Because the floral constancy of pollinators in other systems are often context-dependent and variable among populations (Moeller, 2005; Aldridge & Campbell, 2007, 2009; Hersch & Roy, 2007), it is important to determine whether the patterns seen in these studies are universal across the fig–fig wasp mutualism. Does the frequency of pollinator sharing differ across fig lineages, associated wasp lineages, geographically, or among plant breeding systems?

Although pollinator sharing and host switching have been invoked to explain molecular and phylogenetic patterns among figs and their pollinating wasps (Herre, Jander & Machado, 2008; Renoult *et al.*, 2009), few studies have reported direct evidence of a single species of wasp associated with more than one species (Herre *et al.*, 2008). Pollinator sharing is reported in monoecious neotropical *Ficus* subgenera *Urostigma* (Molbo *et al.*, 2003; Machado *et al.*, 2005; Marussich & Machado, 2007; Su *et al.*, 2008) and *Pharmacosycea* (Su *et al.*, 2008). The studies of *Urostigma* found shared pollinators in ten out of 25 *Ficus* species using molecular methods of wasp identification. By contrast, Weiblen, Yu & West (2001) reared only unique pollinators from each of 14 dioecious, palaeotropical *Ficus* species. Their study involved the morphological identification of fig wasps reared from 8–19 crops per fig species. Could the different findings of these studies be explained by morphological versus molecular species concepts? Alternatively, are they a result of biological differences between study systems?

The present study examined pollinator sharing in dioecious, Palaeotropical *Ficus* through molecular and morphological identification of agaonid wasps that are capable of accessing flowers within syconia. We focused on identifying the pollinators visiting dioecious fig species in New Guinea, a centre of *Ficus*

diversity where up to 70 species of figs are known to co-occur in lowland rainforests.

MATERIAL AND METHODS

TAXONOMY AND HOST ASSOCIATIONS

We chose to examine agaonid wasp species associated with sympatric, locally abundant and closely-related *Ficus* species because these provide conditions most likely to facilitate pollinator sharing. We included five species from dioecious *Ficus* subgenus *Sycomor*, section *Sycocarpus* (*Ficus hispidioides* S.Moore, *Ficus congesta* Roxb., *Ficus morobensis* C.C.Berg, *Ficus pachyrrhachis* K.Schum. & Lauterb., and two morphotypes of *Ficus bernaysii* King) that met these conditions. The two morphotypes of *F. bernaysii* were first recognized by local landowners and are distinguished by the colour, length, and density of epidermal hairs on petioles and young shoots. Because there is not yet taxonomic recognition or information on ecological or genetic divergence between these entities, we refer to them as morphotypes 'A' and 'B'. We also included *Ficus copiosa* Steud. and *Ficus wassa* Roxb. (Weiblen, 2000), which are sister species from dioecious *Ficus* subgenus *Ficus*, section *Sycidium*.

Sycomor figs make up a clade of predominantly dioecious plants estimated to have originated at least 45 Mya (Ronsted *et al.*, 2005). The focal species in section *Sycocarpus* are a monophyletic but highly unresolved subset (as a result of a lack of sequence diversity) of this clade estimated to have originated at least 15 Mya (Silvieus, Clement & Weiblen, 2008). Members of *Sycocarpus* are morphologically similar, although differentiated mainly by syconium, leaf, and epidermal hair characteristics (Berg & Corner, 2005). *Sycocarpus* species are pollinated by *Ceratosolen* wasps, the largest genus of Australasian agaonids (Wiebes, 1994). The *Ceratosolen* pollinators of *Sycocarpus* are monophyletic and appear to have codiversified with their hosts (Lopez-Vaamonde *et al.*, 2009). The pollinators of section *Sycidium* are *Kradibia*, a close relative of the *Ceratosolen* (Lopez-Vaamonde *et al.*, 2009). Among the 234 herbarium specimens of *F. copiosa* and *F. wassa* collected by the authors, two were identified as hybrids: one from New Guinea and the other from New Britain (G. D. Weiblen, unpubl. data). Germination of seed from the New Guinea individual suggests an F1 hybrid because the backcross displays a wide range of morphotypes spanning the continuum of variation between *F. copiosa* and *F. wassa* (A. M. Moe, unpubl. data).

FOUNDRESS COLLECTION

Foundresses were collected from a 4-km² area of lowland rainforest surrounding Ohu village, in

Madang Province, Papua New Guinea (latitude 5°13'38"S, longitude 145°40'44"E). Up to 25 receptive figs were removed from each of ten trees of each focal species from section *Sycocarpus* and section *Sycidium* and from a single *F. copiosa* × *F. wassa* individual. Figs were immediately opened and live foundresses were placed in airtight collection tubes containing silica gel and a cotton plug. Dried foundresses were stored at -80 °C. A leaf voucher was taken for each tree from which foundress wasps were collected. In total, 372 *Ceratosolen* pollinators of *Ficus* section *Sycocarpus* and 210 *Kradibia* pollinators of *Ficus* section *Sycidium* were identified using DNA barcodes. Before DNA extraction, the head of each wasp was removed and mounted as a voucher for morphological identification.

DNA EXTRACTION, POLYMERASE CHAIN REACTION (PCR), AND SEQUENCING

Sequences from the 3' end of cytochrome oxidase I (COI) were obtained directly from individual wasps. DNA was extracted using a Dneasy Tissue Kit (Qiagen). We amplified approximately 546 bp of mitochondrial COI using primers SW2618 and Pat (Simon *et al.*, 1994; Machado, 1998).

Amplification was performed on an Eppendorf mastercycler thermocycler with 1 min at 94 °C followed by 36 cycles of 30 s at 94 °C, 1 min at 45 °C, 30 s at 68 °C, and a final extension of 5 min at 72 °C. The amplified PCR products were purified using a Qiaquick PCR purification kit (Qiagen). Sequencing reactions were performed using a BigDye Terminator, version 3.1, Cycle Sequencing Ready reaction kit on an Eppendorf mastercycler thermocycler with 1 min at 96 °C followed by 26 cycles of 10 s at 96 °C, 5 s at 50 °C, 4 min at 60 °C, and then visualized on a ABI Prism 377 DNA Sequencer. Sequences were edited in SEQUENCHER and manually aligned.

MOLECULAR IDENTIFICATION

Molecular analyses were performed separately for *Ceratosolen* and *Kradibia* samples. *Ceratosolen* sequences served as an outgroup for *Kradibia* analyses and vice versa. To detect potential pollinator sharing among focal and nonfocal *Ficus* species, COI sequences from all *Sycocarpus*-pollinating *Ceratosolen* and *Sycidium*-pollinating *Kradibia* available in GenBank were included in the analyses. Haplotype frequencies were noted and redundant haplotypes removed from further analysis. Uncorrected *p*-distances were calculated in PAUP, version 4.0 (Swofford, 2001) to facilitate comparison with barcoding literature (Hebert, Ratnasingham & deWaard, 2003; Hebert *et al.*, 2004) and neighbour-joining trees were

constructed to examine clustering of genetic haplotypes. Maximum parsimony and Bayesian methods were used to construct best estimates of phylogeny and assess monophyly of genetic clusters. Under maximum parsimony, a heuristic search was performed in PAUP with 10 000 random addition sequence replicates. Parsimony bootstrap analysis was performed with 10 000 replicates using the ‘fast stepwise addition’ option. Bayesian estimates of phylogeny with posterior probabilities were obtained with MrBayes, version 3.1.2 (Huelsenbeck & Ronquist, 2001) by sampling 2000 trees from two simultaneous runs of four chains over 1×10^6 generations of Markov chain Monte Carlo analysis and a GTR + G + I model of evolution. The final standard deviation of split frequencies was 0.026 in *Ceratosolen* and 0.008 in *Kradibia*, indicating that separate runs had converged onto a stationary distribution.

MORPHOLOGICAL IDENTIFICATION

Slide-mounted wasp heads were cleared in 10% KOH solution and examined under a compound microscope to determine whether genetic clusters corresponded to published descriptions of named species (Wiebes, 1963; Wiebes, 1980a). Figures 1 and 2 show the diagnostic characters used to identify wasp species, including the prominence of mandibular teeth and glands, the shape of the projection in the epistomal margin, and the number of lamellae on the mandibu-

lar appendage. All pollinators found in *Ficus* species other than their predominant host were examined to confirm their identity and rule out the possibility of DNA contamination or other laboratory errors.

POLLINATOR SHARING RATIO

For each pollinator species, the number of individuals encountered with each of several sympatric fig species was counted to calculate a pollinator sharing ratio. The pollinator sharing ratio (PS) is defined as $v/(v+v')$ where v is the number of sampled foundresses visiting the predominant host and v' is the number of sampled foundresses from all other host species. A pollinator sharing ratio of zero indicates complete fidelity to a single host. A ratio of 0.5 indicates that pollinators are just as likely to visit an alternative host as they are to visit their predominant host. As the PS ratio approaches 1.0, visitation of the most common host declines in proportion to the number of alternative host species and the frequency of visits to alternative hosts. We used a binomial test to compare our pollinator sharing ratios for these dioecious figs with a pollinator sharing ratio obtained from rearing data from monoecious figs *sensu* Molbo *et al.* (2003). *Pegoscapus gemellus* sp. A wasps were reared from both *Ficus popenoei* Standl. and *Ficus bullenei* I.M. Johnst. Twenty-eight of 32 observations of *P. gemellus* sp. A came from *F. popenoei*, whereas the remainder were from *F. bullenei*, resulting in a

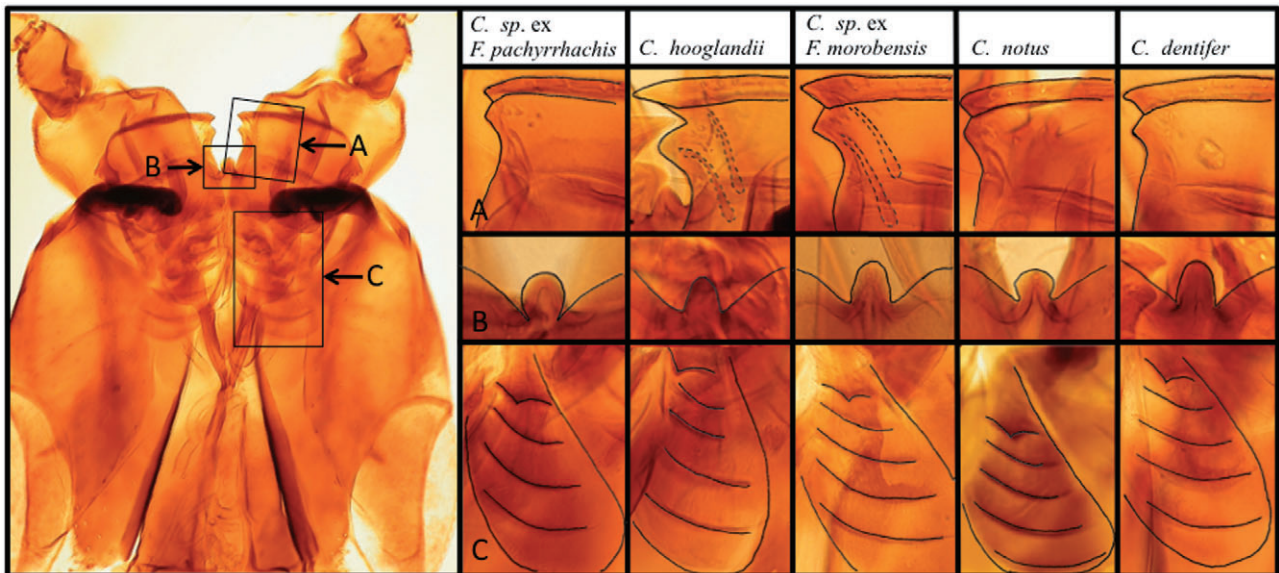


Figure 1. Diagnostic characteristics for *Ceratosolen* species. A, mandible. *Ceratosolen hooglandii* is distinguished by a large, over-extended upper tooth on the mandible that all other species lack. *Ceratosolen* sp. ex *Ficus morobensis* and *C. hooglandii* are distinguished by two large, prominent mandibular glands. B, epistomal projection. *Ceratosolen notus* is distinguished by a ‘knob-like’ projection in the epistomal margin, which is at least as wide as it is long. C, *Ceratosolen* sp. ex *Ficus pachyrrhachis* is distinguished by four lamellae on the mandibular appendage.

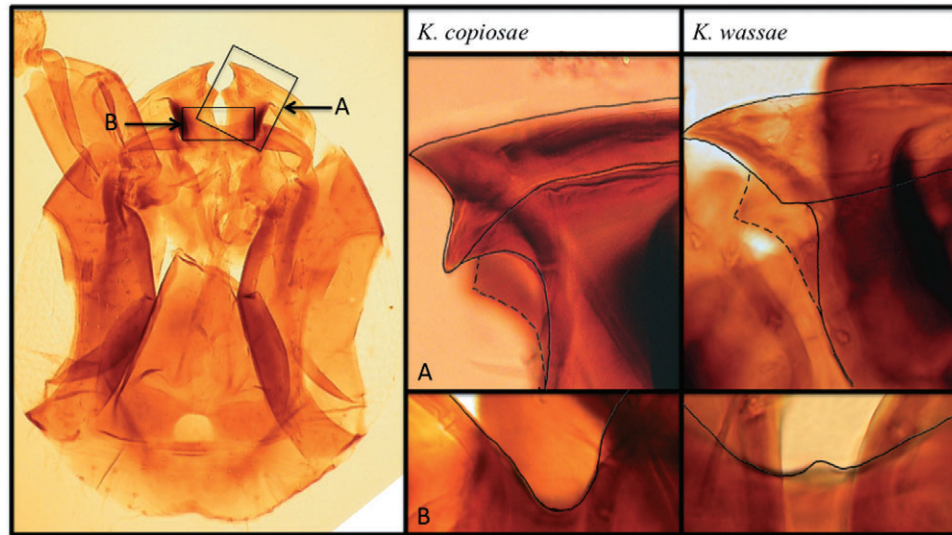


Figure 2. Diagnostic characteristics for *Kradibia* species. A, *Kradibia copiosae* has two strong teeth on the mandible. *Kradibia wassae* has a strong upper tooth and weak lower tooth on the mandible. B, *Kradibia copiosae* has a deeply bilobed epistomal margin with no projection and *K. wassae* has a shallowly bilobed epistomal margin with a slight projection.

Table 1. Frequency of associations between sympatric New Guinea *Ceratosolen* species and *Ficus* section *Sycocarpus* species

Pollinator species	n_f	n_h	v	v'	Common host	Alternate host	Pollinator sharing ratio
<i>Ceratosolen dentifer</i>	63	14	61	2	<i>Ficus hispidooides</i>	<i>Ficus bernaysii</i> B <i>Ficus congesta</i>	0.032*
<i>Ceratosolen hooglandii</i> clade 1	64	32	62	2	<i>Ficus bernaysii</i> A	<i>Ficus bernaysii</i> B	0.031*
<i>Ceratosolen hooglandii</i> clade 2	57	19	56	1	<i>Ficus bernaysii</i> B	<i>Ficus bernaysii</i> A	0.018*
<i>Ceratosolen notus</i>	75	27	75	0	<i>Ficus congesta</i>	–	NA
<i>Ceratosolen</i> sp. ex <i>Ficus morobensis</i>	55	24	54	1	<i>Ficus morobensis</i>	<i>Ficus bernaysii</i> B	0.018*
<i>Ceratosolen</i> sp. ex <i>Ficus pachyrrhachis</i>	58	8	58	0	<i>Ficus pachyrrhachis</i>	–	NA
Total	372	124	366	6			

Data include numbers of foundresses collected (n_f), numbers of foundress haplotypes (n_h), numbers of foundresses visiting the predominant host (v), numbers of foundresses visiting other fig species (v'). The pollinator sharing ratio ($PS = v'/(v + v')$) *Significant difference from monoecious fig expectations derived from Molbo *et al.* (2003).

pollinator sharing ratio of 0.125. This is likely to be an underestimate of the true pollinator sharing ratio because it assumes: (1) every pollinator sharing event resulted in a brood; (2) each brood is the progeny of a single foundress; and (3) shared host species were sampled equally.

RESULTS

Alignment of a 532-bp segment of COI yielded 155 *Ceratosolen* haplotypes and 31 *Kradibia* haplotypes (Tables 1, 2). Pairwise uncorrected p -distances (Fig. 3)

among named species (mean \pm SD, $11.7 \pm 2.2\%$) were markedly greater than pairwise p -distances within species ($0.8 \pm 0.6\%$). Substantial intraspecific divergence was observed in *Ceratosolen hooglandii* (mean \pm SD distance between divergent clades, $3.8 \pm 0.5\%$), *Ceratosolen wassae* ($4.0 \pm 0.2\%$) and *Ceratosolen copiosae* ($7.2 \pm 0.4\%$).

Neighbour-joining trees (Figs 4, 5) illustrate discrete genetic clusters of pollinator haplotypes characterized by large distances among clusters and small distances within clusters. Parsimony and Bayesian analysis confirmed the monophyly of these genetic

Table 2. Focal *Kradibia* species and their visitation of fig hosts

Pollinator species	n_f	n_h	v	v'	Common host	Alternate host
<i>Kradibia copiosae</i> clade 1	35	6	32	3	<i>Ficus copiosa</i>	<i>Ficus copiosa</i> × <i>Ficus wassa</i>
<i>Kradibia copiosae</i> clade 2	30	6	28	2	<i>Ficus copiosa</i>	<i>Ficus copiosa</i> × <i>Ficus wassa</i>
<i>Kradibia wassae</i> clade 1	142	18	47	95	<i>Ficus wassa</i>	<i>Ficus copiosa</i> × <i>Ficus wassa</i>
<i>Kradibia wassae</i> clade 2	3	1	3	0	<i>Ficus wassa</i>	–
Total	210	31	5	3		

n_f , number of foundresses collected; n_h , number of foundress haplotypes, v , number of foundresses visiting their common host, v' , number of foundresses visiting the *F. copiosa* × *F. wassa* hybrid. The pollinator sharing (PS) ratio is not applicable because it was not observed in samples from host species but only in a rare hybrid. Disproportionate sampling of the hybrid violates a key assumption of the PS ratio.

clusters corresponding to named pollinator species with high bootstrap support and posterior probabilities. Each pollinator species was predominantly associated with a single *Ficus* host species (Figs 4, 5). Additionally, clades of divergent *C. hooglandii* haplotypes showed strong associations with different *F. bernaysii* morphotypes. Among *Ceratosolen* pollinators, two of the five focal species (*Ceratosolen dentifer* and *Ceratosolen* sp. ex *F. morobensis*) were found visiting more than one species of fig, although encounters with alternate host species were rare (Table 1). *Ceratosolen dentifer* and *C. sp. ex F. morobensis* had pollinator sharing ratios of 0.032 and 0.018, respectively. Pollinator sharing ratios for *C. hooglandii* clades visiting the *F. bernaysii* morphotypes were in the range 0.018–0.031 (Table 1). In section *Sycidium*, no pollinator sharing was detected between *F. copiosa* and *F. wassa* (Table 2). However, both *Kradibia copiosae* and *Kradibia wassae* visited a single *F. copiosa* × *F. wassa* hybrid with a biased ratio of 1 : 19 for *K. copiosae* : *K. wassae*.

Binomial tests showed each *Ceratosolen* pollinator sharing ratio to be significantly less than that estimated for *Pegoscopus* ($P = 0.005$ – 0.011). Given the likely underestimation of the pollinator sharing ratio for *P. gemellus*, the observed difference seems likely to hold with comparable sampling of *P. gemellus* and its hosts.

Examination of pollinator head morphology (Figs 1, 2) confirmed the identity of described species (Wiebes, 1963, 1980a) corresponding to well supported clades of pollinator haplotypes. All pollinator sharing events were confirmed by head morphology. However, no consistent morphological differences in female heads were found to distinguish between intraspecific clades of *C. hooglandii*, *K. copiosae* or *K. wassae* haplotypes.

DISCUSSION

Low pollinator sharing ratios in *Ceratosolen* and *Kradibia* suggest that pollinators of dioecious fig

species are highly discriminatory among hosts, as indicated by the predominance of a single host species over rare alternate hosts. These results confirm previous findings in dioecious *Ficus* based on morphological species concepts (Weiblen *et al.*, 2001; Silvieus, 2007). Nonetheless, pollinators did occasionally visit fig species other than their predominant host. In section *Sycocarpus*, between four and six of 372 pollinators visited an alternate host, depending on whether *C. hooglandii* is considered a single species or two, resulting in a pollinator sharing ratio of 0.008–0.016. In section *Sycidium*, pollinator sharing was not observed directly between *F. copiosa* and *F. wassa*. We did not estimate a pollinator sharing ratio between parent and hybrid trees in section *Sycidium* because of highly unequal sampling among hosts (Table 2) given the rarity of hybrid trees at less than 1% of the *Sycidium* population. Estimates of parent and hybrid relative abundance would allow normalization of the pollinator sharing ratio according to the relative abundance of the respective tree species. Nonetheless, rare hybrids comprise evidence that pollinator sharing has occurred between the parental species in the past. Furthermore, the fact that pollinators of both parental species visited the hybrid is evidence of some variation in host fig choice. Asymmetric hybrid visitation, characterized by a predominance of *K. wassae*, may be a result of differences in pollinator body size and the ability to negotiate a hybrid ostiole. The size of the ostiole is likely correlated with the fig diameter and ripe figs of *F. copiosa* are 4.4–5.6 cm in diameter, whereas those of *F. wassa* are only 1.0–1.5 cm (Berg & Corner, 2005; Weiblen *et al.*, 2010). The intermediate diameter of *Ficus wassa* × *copiosa* hybrid figs, 2.7–3.4 cm (G. D. Weiblen, unpubl. data) could explain the preponderance of *K. wassae* over *K. copiosae* visitors. Body size of *K. copiosae* and *K. wassae* had a mean of 1.8 mm and 1.5 mm (Wiebes, 1980b), respectively, and head width had a mean of 0.58 mm and 0.46 mm (A. M. Moe, unpubl. data). Smaller *K. wassae* pollinators

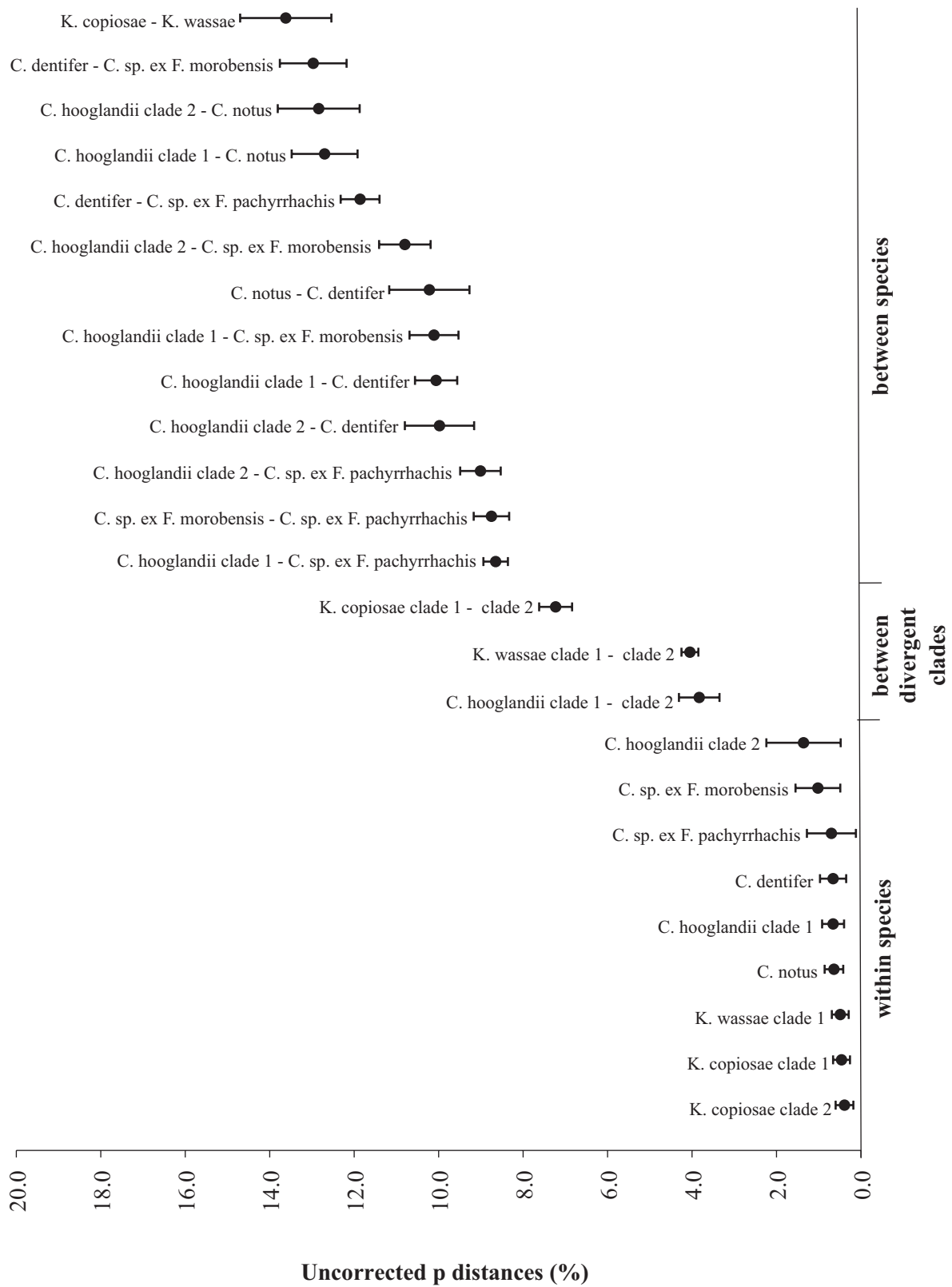


Figure 3. Pairwise uncorrected *p*-distances among foundress wasp haplotypes.

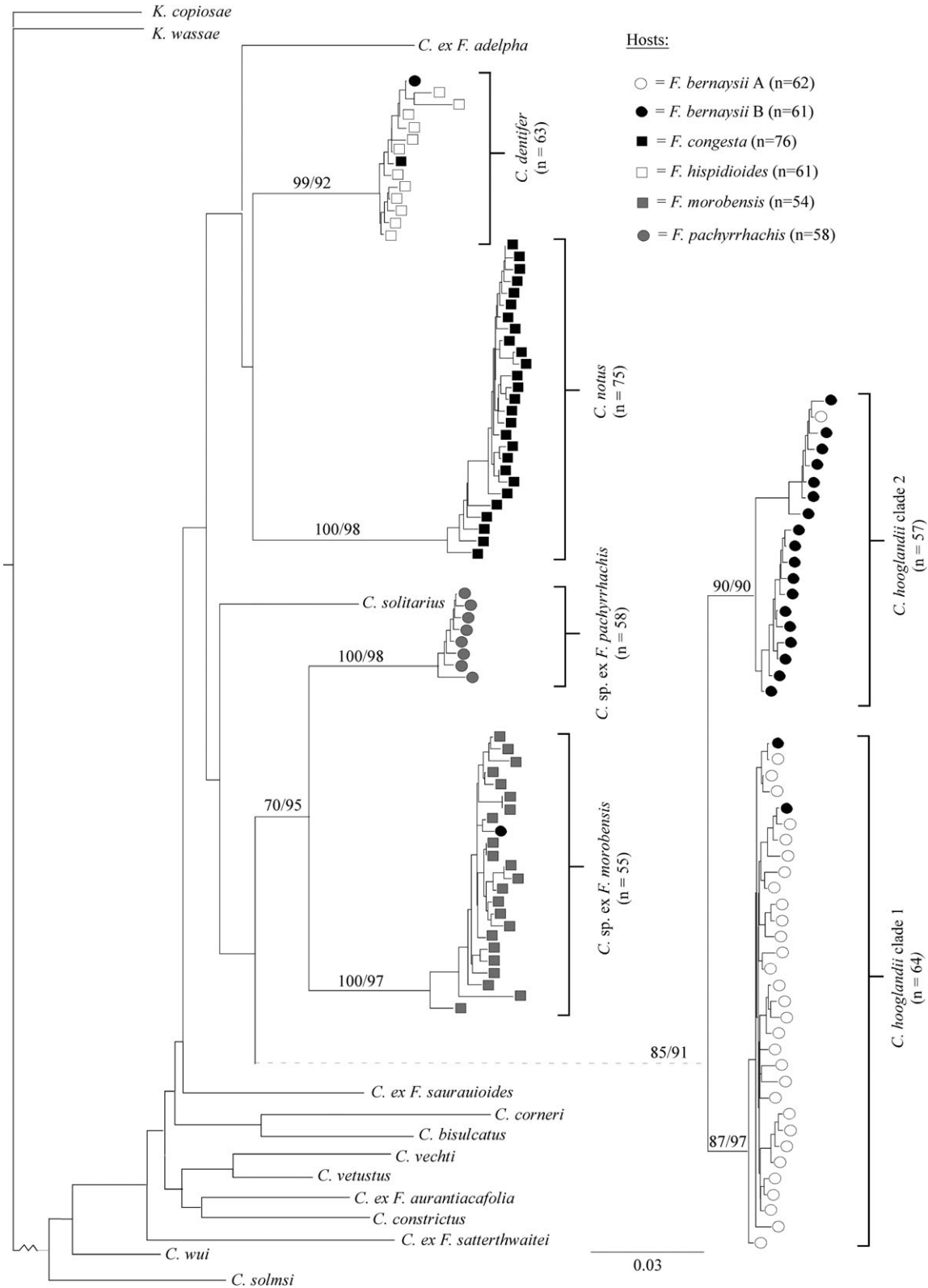


Figure 4. Cytochrome oxidase I neighbour-joining tree of *Ceratosolen* pollinator haplotypes from *Ficus* section *Sycocarpus*. Branches are labelled with bootstrap support/Bayesian posterior probabilities. Taxon symbols correspond to host *Ficus* species from which the pollinator haplotypes were collected.

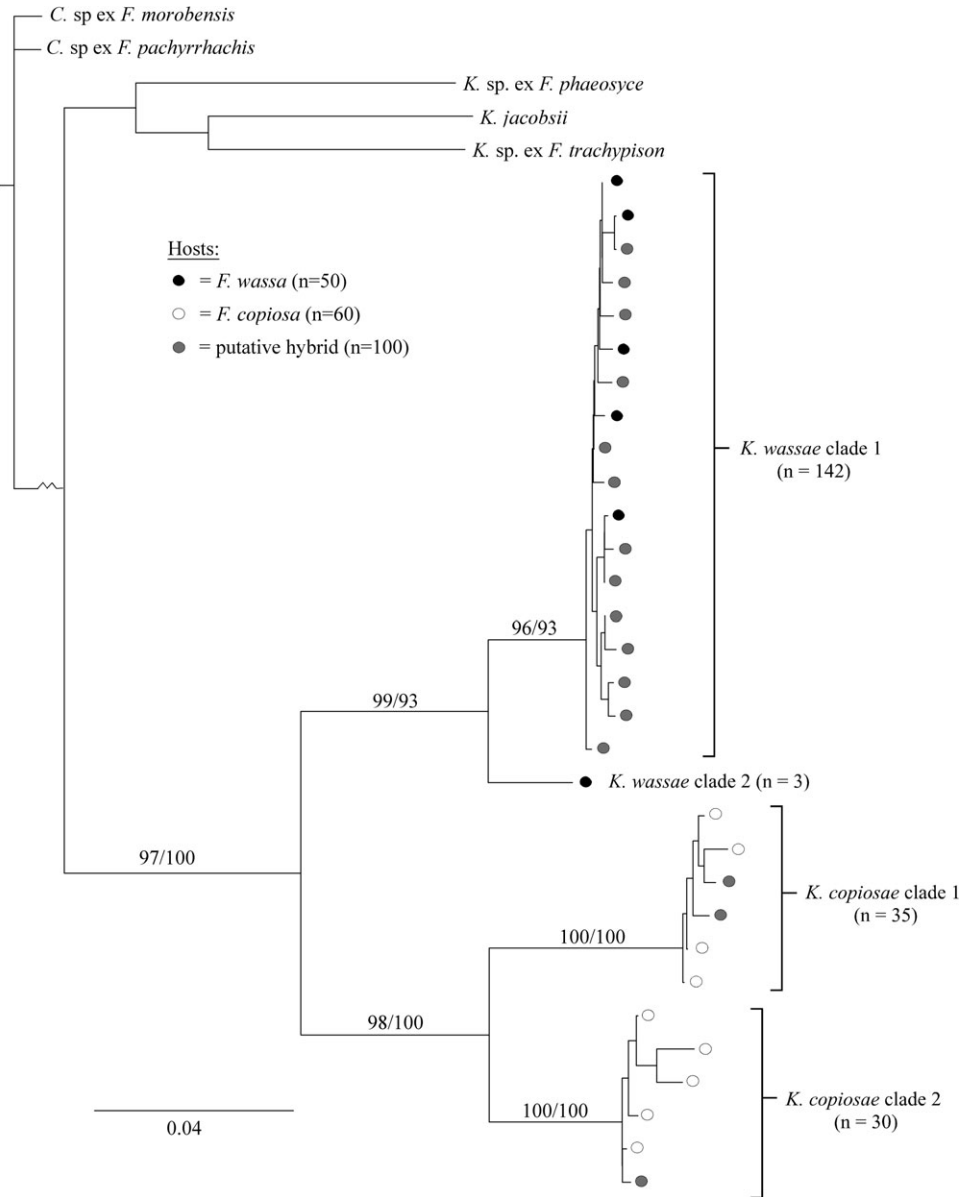


Figure 5. Cytochrome oxidase I neighbour-joining tree of *Kradibia* pollinator haplotypes from *Ficus* section *Sycidium*. Branches are labelled with bootstrap support/Bayesian posterior probabilities. Taxon symbols correspond to host *Ficus* species from which the pollinator haplotypes were collected.

would be more likely to successfully navigate hybrid ostioles than larger *K. copiosae* and this would tend to promote asymmetrical introgression from species with large figs to species with small figs.

Although the pollinator sharing ratios reported in the present study are significantly lower than neotropical monoecious figs, pollinator sharing in dioecious figs may be biologically significant. Hundreds or thousands of wasps may emerge from any given fig and thousands of figs are pollinated by wasps at any given time in these forests. On this scale, even events as

rare as 0.8–1.6% could translate into a substantial number of interspecific pollination events. The reproductive outcome of pollinator sharing events is unknown. These events could result in fig hybridization, as has been suggested by molecular studies in African *Ficus* section *Galoglychia* (Renoult *et al.*, 2009). Pollinator sharing may result in poor pollination services or even parasitism, as was found in African *Sycomorus* figs that share *Ceratosolen* pollinators (Kerdelhué, Le Clainche & Rasplus, 1999). Two species were shared between *Ficus* syco-

morus and *F. mucoso*, although only one effectively pollinated *F. sycomorus*, whereas the other pollinated *F. mucoso*. Pollinator sharing could lead to the colonization of new host species and host switching, as suggested by wasp rearing and incongruent host-pollinator co-phylogenies from neotropical monoecious figs (Molbo *et al.*, 2003; Machado *et al.*, 2005; Marussich & Machado, 2007), or could be a reproductive dead end for pollinators, a possibility yet to be investigated. Cross-pollination experiments are needed to compare the fitness consequences of interspecies pollinations to those of intraspecies pollinations for both mutualistic partners.

Differences in the extent of pollinator sharing between monoecious figs and dioecious figs might be attributed to fundamental differences in breeding system influencing the evolution of host specificity and inferred cophylogenetic patterns. Highly congruent phylogenies of dioecious figs and pollinators have been interpreted as evidence of cospeciation (Weiblen & Bush, 2002; Silvieus *et al.*, 2008), whereas the highly incongruent phylogenies of monoecious figs and pollinators suggest the opposite (Machado *et al.*, 2005; Marussich & Machado, 2007; Su *et al.*, 2008). We propose two possible explanations for these differences. First, pollinators of functionally dioecious figs may be selected for a strong tendency to colonize figs that are highly similar to their birth fig, namely a gall fig as opposed to a seed fig, because pollinators of seed figs fail to reproduce (Valdeyron & Lloyd, 1979; Kjellberg *et al.*, 1987). Pollinators of dioecious figs discriminate between functionally male gall figs and functionally female seed figs, to some extent in certain host species (Anstett, Gibernau & Hossaert-McKey, 1998) but not in others (Patel *et al.*, 1995). A strong discriminatory behavioural response to variation in fig volatile attractants among host species could be a by-product of adaptations associated with seed fig avoidance. On the other hand, figs might be selected for specialized nursery function as it relates to the male component of fitness. A functionally female tree can achieve reproductive success with visits from only a few pollinators carrying conspecific pollen, whereas the fitness of a functionally male tree depends on successful rearing of wasps with behaviour affecting conspecific pollination. Therefore, floral morphology or chemistry that restricts rearing to faithful pollinators may be under positive selection. Such selection pressures may be absent or weaker in monoecious figs, where individual trees perform both male and female functions. Cross-pollination experiments in the field are needed to test these hypotheses.

Mitochondrial DNA divergence within three named wasp species could indicate cryptic or incipient speciation (Fig. 3), although it is difficult to tell without

expanded sampling of geographically distributed populations (Haine *et al.*, 2006; Lin, Yeung & Li, 2008; Moe & Weiblen, 2010). In the case of *K. wassa*, the rarity of individuals belonging to *K. wassa* clade 2 at our locality could be explained by phylogeography. Divergent *K. copiosa* haplotypes were more or less equally abundant in sympatry, and perhaps the depth of mitochondrial DNA divergence is evidence of cryptic species or incipient speciation. Divergence in *C. hooglandii* could be a case of ecological speciation, with each clade specializing on a different *F. bernaysii* morphotype. Differences between morphotypes are very subtle and, had local naturalists not pointed out epidermal hairs, we might have concluded that two cryptic species pollinate the same fig species. Many recent studies have discovered and described multiple cryptic wasp species pollinating a single species of fig (Molbo *et al.*, 2003; Machado *et al.*, 2005; Haine *et al.*, 2006; Peng *et al.*, 2008; Su *et al.*, 2008; Moe & Weiblen, 2010). A closer examination of the morphology and population genetics of host taxa from which cryptic wasp species are reported could shed light on the tempo and mode of speciation.

Named *Ceratosolen* and *Kradibia* form reciprocally monophyletic groups based on COI haplotypes, which correlate with morphology. Species identification using COI appears to be straightforward in these fig wasps such that DNA barcoding can serve as a useful diagnostic tool for the detection of 'cryptic' species, as has been broadly proposed for Lepidoptera (Hebert, deWaard & Landry, 2009) and birds (Tavares & Baker, 2008). Characters of the female head, which are highly variable among species and implicated in accessing the fig (van Noort & Compton, 1996), were sufficient to identify named species but not to discriminate between deeply divergent clades within named species or putative cryptic species.

CONCLUSIONS

Dioecious fig pollinators *Ceratosolen* and *Kradibia* demonstrated high levels of host specificity and low pollinator sharing ratios. Pollinator sharing in dioecious fig lineages appears to be less common than that reported for neotropical monoecious fig lineages. However, even low rates of pollinator sharing could be evolutionarily significant. The impact of rare pollinator sharing on fig species delimitation and on coevolution in the fig and fig wasp mutualism will depend on the reproductive consequences of pollinator sharing, which can be examined through direct experimentation and manipulative pollination in the field. Although molecular and morphological species concepts could yield different numbers of pollinator species associated with particular fig species, rates of

pollinator sharing in New Guinea were not sensitive to such differences. Deep mitochondrial divergence of lineages within named species of pollinators suggests incipient speciation. Divergent clades are strongly associated with different host morphotypes in the case of *Ceratosolen hooglandii*, which points to the possibility of plant–pollinator codivergence. Broader geographic sampling and molecular characterization of *F. bernaysii* are needed to further investigate this possibility.

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