Consistently high incidence of *Wolbachia* in global fig wasp communities

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Abstract

1. *Wolbachia* are very common and widespread endosymbionts of arthropods. They can have a considerable effect on their host's fitness. It has been estimated that as many as 40% of arthropod species are infected with *Wolbachia*, but particular taxonomic groups and ecological niches display significantly higher or lower incidences.
2. In this study, we combine an African sample with those of three previous studies from three other continents. We reanalyzed *Wolbachia* incidence in 172 fig wasp species (including pollinators, non pollinators, gallers and non gallers) associated with 81 fig trees of six subgenera, and estimated that > 80% of fig wasp species are infected with *Wolbachia*, which is far higher than putative global infection rates in arthropods.
3. We found that, within fig wasps, incidence does not vary consistently with respect to host taxonomy, ecology or geography. In particular, incidence seems remarkably constant across samples from different geographical locations.

Keywords: *Wolbachia* incidence, fig trees, pollinators, non pollinators, sex ratio

Introduction

Wolbachia pipientis belongs to the phylum Proteobacteria, class Alphaproteobacteria and order Rickettsiales, and were first described by Hertig (1936) in the host *Culex pipiens*. *Wolbachia* are perhaps best known for their remarkable manipulation of their hosts (Werren *et al*., 2008), including feminization of genetic males; parthenogenesis induction, which results in the development of unfertilized eggs; the killing of male progeny from infected females; and sperm–egg incompatibility, which is referred to as cytoplasmic incompatibility. Each of these reproductive alterations is adaptive for the bacterium by enhancing the production of infected females. These, and other profound effects on host biology, mean that *Wolbachia* can accelerate the evolution of their hosts (Werren, 1997; Jiggins & Hurst, 2011; Werren *et al*., 2008; Xu *et al.,* 2012), and also have implications for human disease and pest control (Werren *et al*., 2008; Bourtzis, 2008; Pfarr *et al*., 2006; Sarakatsanou *et al*., 2011; Iturbe-Ormaetxe *et al*., 2011).

The other remarkable fact about *Wolbachia* is their presence in so many host species. Until the early 1990s, *Wolbachia* were considered to be rare and unimportant bacteria. However, with the advent of molecular typing methods, *Wolbachia* were found to be the most widespread and common bacteria in insects, and subsequently also in other arthropods (mites, spiders, scorpions and isopods), as well as filarial nematodes (Werren *et al*., 2008). Indeed, a recent meta-analysis estimated that >65% of arthropod species harbour *Wolbachia* (Hilgenboecker *et al*., 2008), suggesting that they infect at least one million insect species alone (Werren *et al*., 2008; though see Zug and Hammerstein 2012).

The incidence level of a bacterium within a group of host species is the outcome of a dynamical process of infection loss and the transfection of new hosts. This process of transfection remains little understood in *Wolbachia*, partly because, like all Rickettsiales, they are obligatory intracellular bacteria, and so it had been assumed that they cannot survive outside host cells. However, experimentally, *Wolbachia* can remain viable out of their hosts (Fallon *et al*., 2008; Rasgon *et al*., 2006); an injection experiment showed that *Wolbachia* can move from somatic stem cells to germline, and eventually enter developing eggs (Frydman *et al.,* 2006). Therefore, it seems that some *Wolbachia* strains can briefly exist outside of host cells and traverse cell membranes, which could be important for horizontal transfer within and between species (Baldo *et al*., 2008). This transfection between taxa by microinjection into eggs indicates that they can become established within cells of diverse arthropods. But, not all hosts are equally permissive and *Wolbachia* strains can differ in their ability to transfect different host species (Werren *et al*., 2008; Werren & Windsor, 2000, Ahmed *et al*., 2010; Ahmed *et al*., unpublished).

Once infection is established, *Wolbachia* infection also varies widely in prevalence (i.e., the proportion of individuals in a population that are infected). Differences in prevalence might also vary with differences in the biology of host taxa (Wenseleers *et al*., 1998; Werren & Windsor, 2000; Hain & Cook, 2005; Ahmed *et al*., 2010), and many other environmental and ecological factors (Jia *et al*., 2009; Toju & Fukatsu, 2011).

Here, we present a systematic exploration of *Wolbachia* incidence and prevalence levels in a community of fig wasp species. Fig trees are involved in an obligate mutualism with fig-pollinating wasps in which the wasps disperse fig pollen, while fig flowers provide nutrition for wasp larvae (Cook & Rasplus, 2003). The mutualism is exploited by many non-pollinating wasp species, which feed on fig tissue or other wasps, and most fig species harbour several of these parasite species, their parasitoids or kleptoparasites and gallers (Cook & Rasplus, 2003). In most fig species, each syconium contains 50–500 wasps of a few different species developing together in close proximity. As such, the system is an ideal case study in the role of ecological interactions in *Wolbachia* transmission and persistence.

Below, we examine extensive data of fig wasp communities from four different continents, including previously reported data from Australia, China and Panama (Chen *et al*., 2011; Haine & Cook, 2005; Shoemaker *et al*., 2002), and new data from South Africa. Together, these data allow us to investigate the possible roles of various factors that could influence *Wolbachia* infection.

Materials and Methods

Sampling

We studied *Wolbachia* in 17 fig wasp species (12 pollinators belonging to the Agaonidae and 5 non-pollinators belonging to the Pteromalidae family of the order Hymenoptera) associated with two common fig trees subgenera (Sycomorus and Urostigma) of South Africa (Table S1). Species identity was confirmed using a binocular microscope. Sampling was mostly done on figs releasing wasps or on figs that were freshly pollinated, where the foundresses were sampled. The collected wasps were stored in 96% ethanol at –20ºC. Only female wasps were screened for *Wolbachia*. Next, all previously published studies related to *Wolbachia* infection incidences in fig associated wasps were searched, and their data were combined with those of the present study. Altogether, we analyzed 172 wasp species (a total of 844 individuals) from four defined wasp families (Agaonidae, Ormyridae, Pteromalidae and Torymidae) and some undefined families (of subfamily Sycophaginae and of genera *Aepocerus* and *Heterandrium*). The data included 70 known pollinator species and 102 non-pollinators from 30 distinct wasp genera, associated with 81 fig tree species from six distinct tree subgenera (Ficus, Pharmacosycea, Sycidium, Sycomorus, Synoecia, Urostigma) and included samples from four different continents (locations: South Africa, China, Panama and Australia) (Table S1).

These communities are largely independent, as there is no wasp species (pollinator or non-pollinator) in common between our South African sample and the previously published samples from Panama, Australia and China. However there are some possible common wasp species (*Ceratosolen sp.* and *Blastophaga sp.* in pollinators and *Platyneura sp.*, *Philotrypesis sp.* and *Sycoscapter sp.* in non pollinators) between China and Australia (Table 1).

Table 1. *Wolbachia* incidences in samples from fig wasp species (Hymenoptera: Chalcidoidea).

Categories	**Incidence of *Wolbachia* in all species**	**Incidence of *Wolbachia* in pollinator species**	**Incidence of *Wolbachia* in non-pollinator species**
All species	0.60(172)	0.66(70)	0.56(102)
According to fig wasp family			
Agaonidae	0.66(70)	0.66(70)	-
Pteromalidae	0.55(65)	-	0.55(65)
Torymidae	0.67(3)	-	0.67(3)
Ormyridae	1(1)	-	1(1)
Incertae sedis:			
Sycophaginae	0.58(26)	-	0.58(26)
Aepocerus	0.60(5)	-	0.60(5)
Heterandrium	0(1)	-	0(1)
According to geographical location			
Australia	0.67(64)	0.75(28)	0.61(36)
China (Asia)	0.53(47)	0.82(11)	0.44(36)
Panama (S. America)	0.59(44)	0.50(18)	0.65(26)
South Africa (Africa)	0.52(17)	0.54(13)	0.50(4)
According to host fig tree subgenus			
Ficus	0.78(9)	0.67(3)	0.84(6)
Pharmacosycea	0.56(9)	0.25(4)	0.80(5)
Sycidium	0.86(7)	1(4)	0.67(3)
Sycomorus	0.58(19)	0.66(6)	0.53(13)
Synoecia	0(1)	-	0(1)
Urostigma	0.58(125)	0.64(53)	0.53(72)

Shown are the proportion of sampled species that contained one or more sampled individuals infected with *Wolbachia*. Numbers in parentheses are the total number of wasp species sampled.

DNA extraction, PCR and Sequencing

DNA was extracted from whole wasps using the Chelex procedure (West *et al*., 1998). Extractions were performed in 500µl with 10% Chelex with proteinase K treatment. We screened for *Wolbachia* by polymerase chain reaction (PCR) for the *wsp* (*Wolbachia* surface protein) gene, with *wsp*81F and *wsp*691R (Zhou *et al*., 1998).

For quality control purposes, negative controls were randomly included to check for contamination and positive controls were used in *Wolbachia* diagnosis PCR assays: Cytochrome *b* (Erasmus *et al*., 2007) was amplified as a positive control for samples that did not show any infection. Samples that were unsuccessful in amplifying with cytochrome *b* were excluded from our data. We performed the PCR with an Eppendorf thermocycler, with 3min at 94ºC, 35 cycles of: 1min at 94ºC, 1min at 52ºC, 1min at 72ºC, and final elongation of 5min at 72ºC. The same conditions were used for cytochrome *b*, with only the annealing temperature reduced to 48ºC. 5µl of the PCR product were run on a 1.5% agarose gel to determine the presence and size of the amplicon. The PCR products were ~600bp for *wsp*, and ~450-500 bp for cyt *b.* The *wsp*81F and *wsp*691R primers were only used to test for *Wolbachia* infection. To determine the subgroup of *Wolbachia* present, different combinations of primers were used, namely, 136F and 691R for subgroup A and 81F and 522R for subgroup B (Zhou *et al*., 1998). One sample per population was sequenced to double check that the correct fragments were amplified. After infection was confirmed with *wsp* PCR, a 50µl reaction was set up. The PCR products were purified using the High Pure PCR Purification Kit (Roche). The purified products were used directly in standard fluorescent cycle-sequencing PCR reactions, cleaning of sequences and analysis were done with BioEdit (Hall, 1999). The sequences were subjected to a BLAST search.

Data analysis

Estimator of distribution of *Wolbachia* prevalences

Data were analyzed according to the methods described by Hilgenboecker *et al.* (2008). The method assumes that the distribution of infection frequencies (prevalences) between species can be described by a beta distribution, whose probability density function is given by:

$$pdf(q) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} q^{\alpha-1}(1-q)^{\beta-1}$$

Where $\Gamma(.)$ is Euler's Gamma function. The shape parameters for this distribution, α and β, can be estimated from our data as described by Hilgenboecker *et al.* (2008), and the complete distribution is plotted in Fig. 2. The proportion of species infected at a frequency of c or above is then estimated as:

$$\text{Incidence} = \int_c^1 pdf(q)dq.$$

Test for predictors of *Wolbachia* infection

To see if *Wolbachia* are non-randomly distributed with regards to continent of origin, subgenus of *Ficus* or whether the host is a pollinator or not, we carried out a contingency table analysis using a generalized linear model with Poisson errors and testing against the χ^2 distribution. A saturated model (Crawley, 2005) was fitted explaining the number of species in each cell as a function of continent of origin, subgenus of tree, wasp being a pollinator or not, and wasp being infected by *Wolbachia* or not. The model was reduced by deleting non-significant interactions (Crawley, 2005). Non-random distribution of *Wolbachia* is indicated when interactions containing *Wolbachia* are significant. All statistical analyses were carried out in R (version 2.11.0).

Wolbachia incidence and sex ratio of fig wasps

To see if *Wolbachia* cause a female bias in pollinating wasps' sex ratios we modeled the average observed sex ratios of ten of the pollinating species studied here as a function of their infection status. The average sex ratios of these ten fig wasp species: *Alfonsiella binghami*, *Alfonsiella pipithiensis, Allotriozoon heterandoromorphum, Courtella armata, Elisabethiella bergi breviceps, Elisabethiella comptoni, Elisabethiella glumosae, Elisabethiella stuckenbergi, Platyscapa awekei,* and *Platyscapa soraria*, were reported in Nelson & Greeff (2009) along with their average foundress number, average number of female offspring and expected sex ratio, correcting for the effect of male dispersal. Since pollinator clutch size (Kjellberg *et al*., 2005) and the number of foundresses (Hamilton, 1979) also affect the sex ratio these were included in the linear model explaining observed sex ratios, as was the expected sex ratio.

Results

High incidence of *Wolbachia* in fig wasps compared to all hymenoptera and insects

Of the 172 populations of fig wasp in our sample, we found that 60% (103/172) were infected (0.599: 95% CI: 0.521- 0.673) which is significantly higher than the 25% (39/155 species) of other Hymenoptera (Binomial test: $P < 0.001$) and insects in general 17% (26/154 species) in the study of Werren *et al*. (1995), 20% (121/591 species) in Werren & Windsor (2000) and 17% (4/24 species) in Jiggins *et al*. (2001) (Fig. 1). These estimates of *Wolbachia* incidence within samples are certain to miss low prevalence infections, that cannot be detected from small samples. Accordingly, we followed the method of Hilgenboecker *et al*. (2008) to estimate the total incidence of *Wolbachia* in fig wasps.

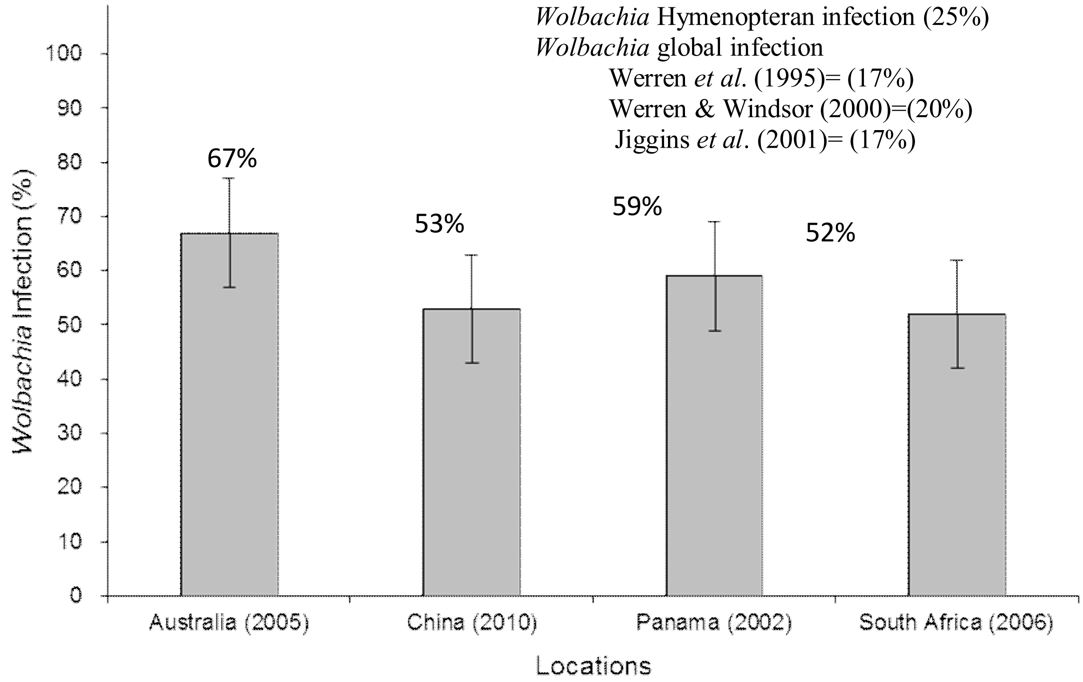

Fig. 1. *Wolbachia* incidences (%) in samples from 172 fig wasp species hosted by 81 fig trees from four different continents.

The mean infection frequency (prevalence) across our 172 sampled populations was 55.7%. If we assume a particular form for the distribution of infection frequencies across species, then we can go further and estimate the proportion of species that are infected above a given level (Hilgenboecker *et al.*, 2008). Accordingly, following Hilgenboecker *et al.* (2008), we assumed that the distribution of prevalence across species can be sufficiently well described by a beta distribution. Estimating the parameters of this distribution from our data, we estimate that 83.9% of species are infected at prevalence of 0.1% or above (and 88.6% of species at prevalence of 0.01% or above). These estimates of levels of *Wolbachia* infection in global fig wasp community are far higher than Hilgenboecker *et al.*'s (2008) estimate of global infection, which is 66% overall in arthropods (Table 2).

Table 2. Estimates of the mean infection frequency within species, the parameters α and β, and the overall incidence of *Wolbachia*

Groups	***n***	**μ**	***s***	**α**	**β**	**Average frequency within species (%)**	**Incidence *c* = 0. 001 (%)**	**Incidence *c* = 0. 0001 (%)**
Overall in global fig wasp community	172	0.56	0.21	0.15	0.12	55.7	83.9	88.6
Asia (Chinese fig wasps)	47	0.39	0.24	0.02	0.02	52.1	45.4	47.3
Africa (South African fig wasps)	17	0.36	0.16	0.19	0.33	36.3	81.2	87.8
Australia (Australian fig wasps)	64	0.70	0.19	0.08	0.04	66.0	82.5	85.5
South America (Panamanian fig wasps)	44	0.60	0.20	0.15	0.10	52.1	85.5	89.7

n: number of species sampled; **μ** = estimated mean infection frequency across all species; *s* = estimated standard deviation in infection frequencies; **α**, **β** = shape parameters for the beta distribution of between-species infection frequencies; *c* = the minimum infection frequencies, below which species are considered to be uninfected.

Most-or-few infection pattern within fig wasp species

According to Hilgenboecker *et al.* (2008), the distribution of within-species infection frequencies, across the arthropods, shows a 'most-or-few' infection pattern, with species tending to have either a very high or a very low *Wolbachia* prevalence, and very few species having intermediate prevalences. Results from our global fig wasps data show a similar pattern (Figs. 2 and 3). However, we estimate that more species are infected with infection frequencies of 90-100% than is true for the global arthropod dataset of Hilgenboecker *et al.* (2008) (Figs. 2 and 3). Our estimates are that 77% of fig wasp species are infected at <10% or >90%, and that 70% of

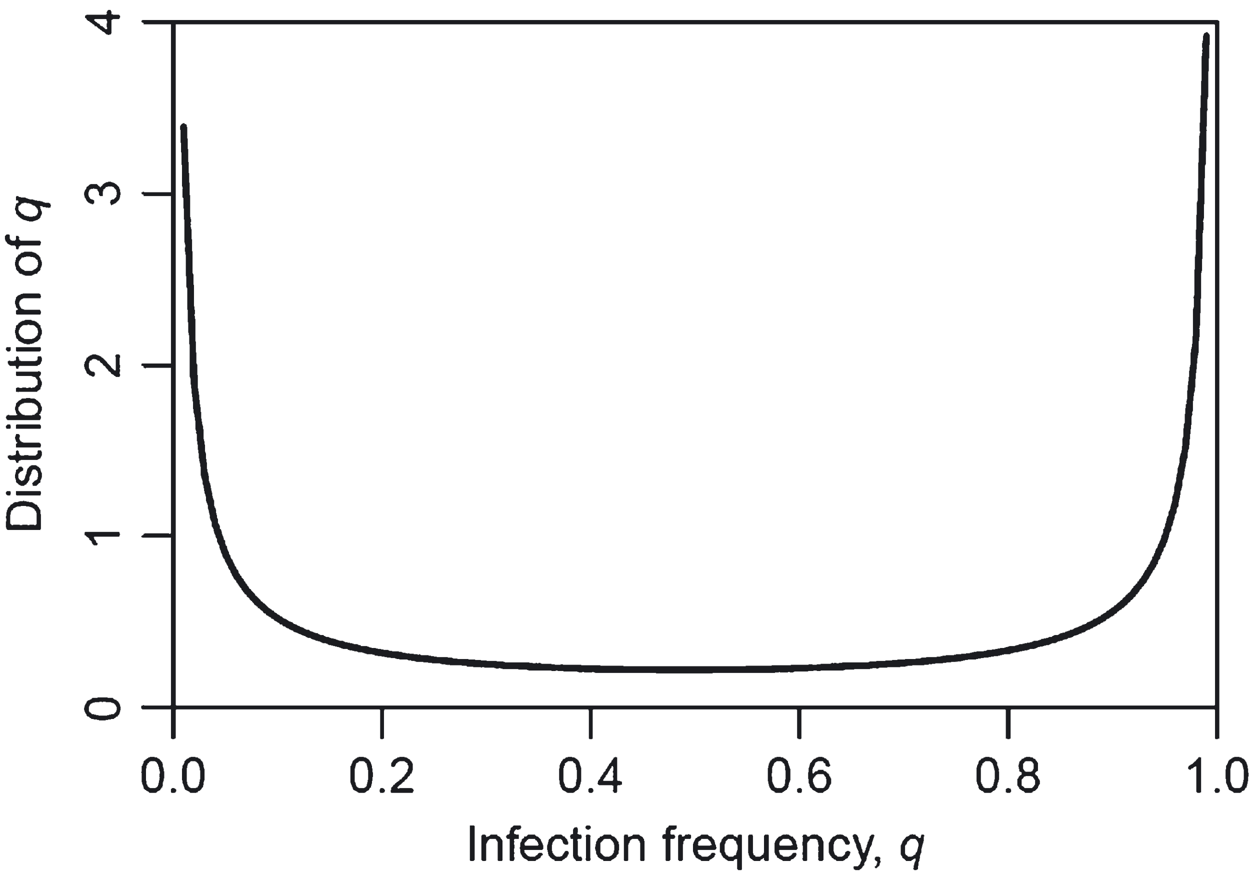

Fig. 2. Estimated distribution of the frequency of *Wolbachia* within species in overall global fig wasps community

fig wasp species are infected at <5% or >95%. Hilgenboecker *et al.* (2008) estimated that 72% of arthropod species are infected at <10% or >90%, and that 64% of arthropod species are infected at <5% or >95% (Fig. 3).

Evidence of globally constant *Wolbachia* infection

We screened 17 species of fig wasps associated with 12 South African fig tree species, and detected infection in 52% (9/17) of the species (Table S1). Combining our data with published samples of fig wasps from three other continents, we analyzed 172 fig wasp species of which 60% (103/172) were found to be infected (Fig. 1). The proportion of species whose samples

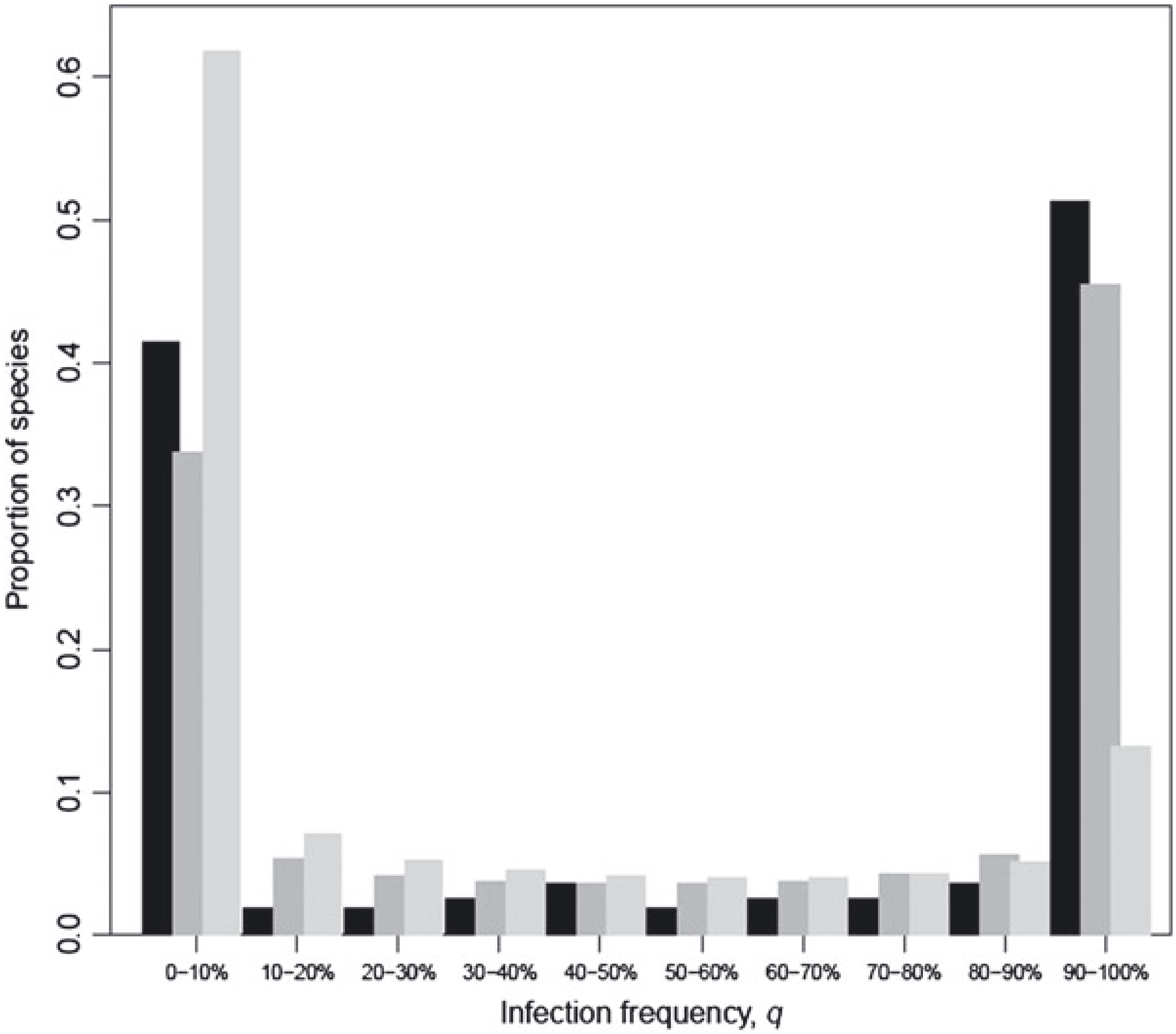

Fig. 3 Proportion of species with infection frequencies in particular intervals. Black bars describe the observed infection frequencies within samples from each of 172 species. The darker grey bars describe the *expected* proportion of species infected under best-fit beta distribution (i.e., with α=0.15, β= 0.12). The lighter grey bars describe the *expected* proportion of species under the parameter estimates of Hilgenboecker *et al.* (2008) for the best-fit beta distribution to their "B(iii)" arthropod data set.

contained infected individuals is similar in each of the continents, despite their different community composition. Applying the Hilgenboecker et al. (2008) estimator to the data sets from each continent suggests that 3 of the 4 estimates remain very similar to each other, with incidence estimates of 80-90% (Table 2). The exception is the Chinese sample where estimates are much lower at 40-50% (Table 2). However, the sample sizes in the Chinese data set are universally small (<10 individuals were sampled from each species, see Supplementary Table 1). Accordingly, estimates of the complete distribution of prevalences are certain to be very imprecise for this continent (Hilgenboecker et al. 2008). Together, then, these results suggest that the incidence of *Wolbachia* in fig wasps might be remarkably constant across the globe.

***Wolbachia* infection within fig wasp species is not predictable from host biology**

The apparent constancy of incidence levels is surprising given the different community compositions of our samples. Accordingly, we asked whether incidence levels varied in a consistent fashion with ecological variables, which themselves vary among the communities. We found that *Wolbachia* incidence levels were very similar between pollinators (66% of sampled species found to be infected; 46/70) and non pollinators (56%; 57/102) and between gallers (50%; 8/16) and non-gallers (57%; 49/86). A more formal analysis, testing for predictors of Wolbachia infection, showed that all interaction terms containing *Wolbachia* (four-way, three-way and two-way) were not significant ($p > 0.161$), suggesting that *Wolbachia* are randomly distributed not only with respect to continent, but also with *Ficus* subgenus and whether the wasp is a pollinator or not. The two-way interactions between tree subgenus, continent and pollinator or not were significant (in all cases $p < 0.026$ or less) and thus these were non-randomly associated with one another.

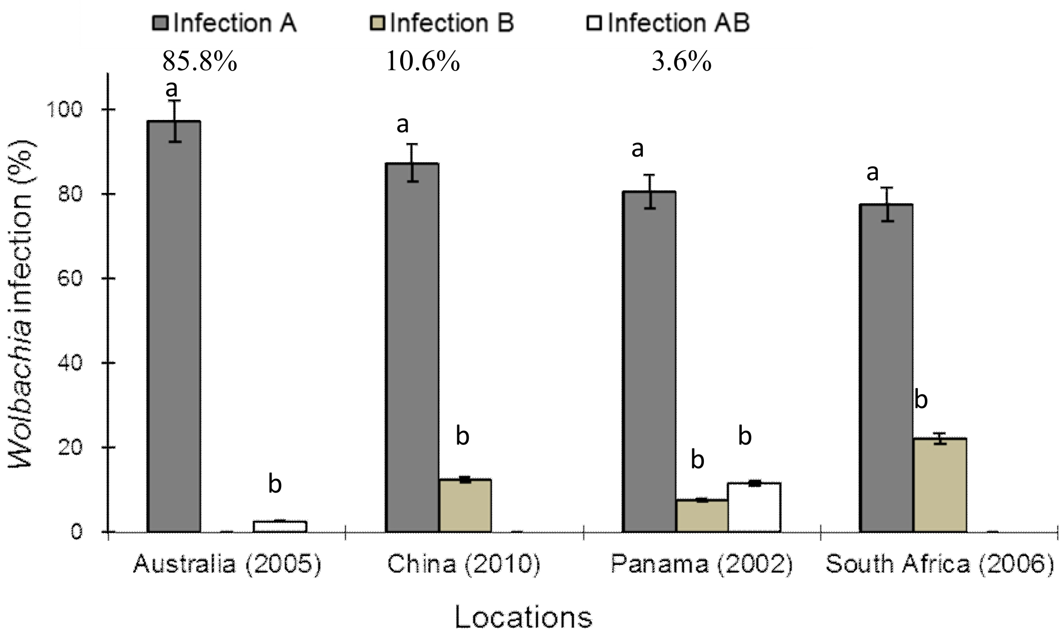

Fig. 4. *Wolbachia* supergroups (%) incidences detected from fig wasp species of four different continents.

Diversity of *Wolbachia* supergroups

Similarity in incidence levels would be surprising if *Wolbachia* strains differed among the communities. Accordingly, we determined which groups of *Wolbachia* were represented in our samples. All infected species of South African fig wasp harboured supergroup A *Wolbachia*, with the exception of two non-pollinator species (*Eukoebelea sycomori* and Sycoecine species 1), which harboured supergroup B *Wolbachia* (Table S1). These results are consistent with other studies of fig wasps and other Hymenopterans in which *Wolbachia* supergroup A predominated (West *et al*., 1998; Shoemaker *et al*., 2002; Haine & Cook, 2005; Chen *et al*., 2011). Unlike previous studies, we found no double infection (AB) in South African fig wasps, and all of them were singly infected. Notably, the two species infected with supergroup B *Wolbachia* were the sole two non-pollinators among our infected species, and even members of the Agonidae from the same figs showed no evidence of supergroup B infection.

Turning to the global sample, among 103 fig wasp species that were found to be infected, we were able to sequence 80 successfully. Among those 80 fig wasp species, 71/80 (85.8%±4.3) were infected with A supergroup and 5/80 (10.6%±4.6) with B supergroup. 4/80 (3.6%±2.7) were doubly infected with AB. Infection with a single supergroup is more frequent among fig wasps than double infection (T= 16.75, df=3, P>0.0001) in all four continents. In cases of single supergroup infection, supergroup A is significantly more common (T= 8.82, df = 3, P>0.003) (Fig. 3). Which is consistent with Chinese, Panamanian and Australian results (Chen *et al.*, 2011; Haine & Cook, 2005; Shoemaker *et al.*, 2002) as well as results from hymenopterans in general (Werren & Windsor, 2000).

***Wolbachia* presence does not predict the sex ratio of fig wasps**

High incidence of *Wolbachia* can arise from successful manipulation of host reproduction which alters the hosts' sex ratio. To test for evidence of this in our data, we tested for a relationship between host sex ratio and the presence of *Wolbachia*. In the model containing all four terms, infection status did not significantly affect sex ratio (P = 0.92). To be certain this was not the result of including too many terms, all possible models with combinations of three, two and single terms containing infection status were checked and *Wolbachia* was never significant (P > 0.14 or higher).

Discussion

We found that 52% of South African fig wasps harbor *Wolbachia* in our samples, which is consistent with previous studies on fig wasps from three other continents. The overall *Wolbachia* infection in fig wasp species of these four continents is significantly higher than the most recent statistical survey of *Wolbachia* in global arthropod species (Hilgenboecker *et al*., 2008; Zug & Hammerstein 2012). Analysis of *Wolbachia* infection from these four independent places allows us to confirm that the high infection level is a feature of fig wasps in general, regardless of geographical or ecological origins.

Why do fig wasps have such a high level of *Wolbachia* infection? Hymenopterans are highly prone to *Wolbachia* infection (Werren & Windsor, 2000; Rokas *et al*., 2002; Werren *et al*., 1995; West *et al*., 1998) and two common explanations for their high infection frequencies focus on their social and/or parasitoid lifestyles (Foster & Ratnieks, 2000; Vavre *et al*., 1999).

Fig wasps are quite aggregated when confined to the fig, and their community includes a variety of plant gallers as well as parasitoids/inquilines; these all develop along with pollinators and non-pollinators within an enclosed inflorescence called the syconium (Cook & Rasplus, 2003; Herre *et al*., 2008; Cruad *et al*., 2010). This syconium is a crowded space within which hundreds of wasps of various species and ecological types (gallers, parasitoids etc.) develop together, but each in their gall (Krishnan *et al*., 2010; Ghara *et al*., 2011). Thus, high prevalence of *Wolbachia* may result from high rates of interspecific horizontal transfer, facilitated by complex ecological interactions and the high density of fig wasps in the compact fig syconium (Cordaux *et al*., 2001; Dyson *et al*., 2002; Sintupachee *et al*., 2006; Vavre *et al*., 1999; Ahmed *et al*., 2010; Ahmed *et al*., unpublished). An alternative explanation is that *Wolbachia* are transmitted vertically with infrequent loss. But this explanation seems less likely, given the lack of evidence to support any cospeciation between the wasps and their *Wolbachia* (Shoemaker *et al.*, 2002; Schilthuizen & Stouthamer, 1997; Werren & Windsor, 2000; Shoemaker *et al.*, 2002). Also, while no known parasitoids have been confirmed to transmit *Wolbachia* between fig wasp species (Shoemaker *et al.*, 2002), many aspects of fig wasp biology do support horizontal transmission, these include interactions during larval development (Haine & Cook 2005; Huigens *et al*., 2000) and further opportunities when the mature wasps emerge and mate, when some species engage in fights (Rigaud & Juchault, 1995).

Recently Ahmed *et al.* (2010) reported that 89% of *Bemisia tabaci* cryptic species and 60% of their parasitoids are infected with *Wolbachia*, and this is among the highest infection of *Wolbachia* in any particular community after fig wasps. *B. tabaci* have at least 28 cryptic species (De Barro *et al*., 2011) and parasitoids from genera *Encarsia* and *Eretmocerus* (Li *et al*., 2011) and also live closely together in a narrow niche, possibly facilitating frequent horizontal transmission (Ahmed *et al*., 2010; Ahmed *et al*., 2012; Ahmed *et al*., unpublished).

Another reason why *Wolbachia* infections persist well in fig wasps may be low levels of host-parasite conflicts (Haine & Cook, 2005). Shoemaker *et al.* (2002) proposed low conflict in fig-pollinating wasps, because selection on wasp nuclear genes favours production of female-biased sex ratios, owing to local mate competition. Consequently, there is less discrepancy in the optimal wasp sex ratios from host and parasite viewpoints. However, while all fig-pollinating wasps have female-biased sex ratios (Herre *et al*., 1997), many non-pollinating species also have slightly female-biased ratios (West & Herre, 1998; Fellowes *et al*., 1999) and there is no significant difference in *Wolbachia* incidence between the two groups. Moreover it is possible that *Wolbachia* have a direct positive fitness effect on fig wasp species (Haine & Cook, 2005; Shoemaker *et al*., 2002).

Another possible explanation for high *Wolbachia* incidence is haplodiploid sex determination, which might allow more opportunities for *Wolbachia* to invade their host, and facilitate host sex ratio manipulation by *Wolbachia* (Werren *et al*., 2008; Charlat *et al*., 2003). However, we found no correlation between *Wolbachia* infection and female biased sex ratios in our data. This might be because other confounding factors are also influencing the sex ratio. These factors might include the effects of male fighting and dispersal (Greeff, 2002; Wakano, 2005), of non-pollinator wasps (Santinelo-Pereira & Pires-do-Prado, 2005), absolute and relative clutch size (Kjellberg *et al*., 2005; Greeff & Newman, 2011) and the number of foundresses per fig (Herre *et al*., 1997; Molbo *et al*., 2003). All of these factors play a role in wasp sex ratios, and might explain cases in which sex ratios within pollinating fig wasps are more female-biased than theoretical models predict (Herre *et al*., 1997; Kinoshita *et al*., 1998).

Our study compared *Wolbachia* incidence in fig wasps from four continents, and the data suggested a surprisingly invariant incidence of infection in the four different continents (Fig. 1; Table 2). The sole exception was the model-based estimate of incidence from China, and we have argued that this estimate is highly unreliable due to the small sample sizes involved. Furthermore, over the data set as a whole, we found no effect on *Wolbachia* incidence of either fig wasp biology (pollinator versus non-pollinator) or fig tree taxonomy. These results are consistent with earlier findings that *Wolbachia* infection is not predicted by taxonomic group, climatic and ecological factors like host plants, temperature or snowfall affect (Werren & Windsor, 2000; Wenseleers *et al.,* 1998; Jia *et al*., 2009; Toju & Fukatsu, 2011; Van-Opijnen *et al*., 1999).

If incidence levels are found to be globally constant, then this is consistent with the hypothesis that *Wolbachia* infections are at a global equilibrium – existing in a steady state between the transfection of new hosts and the clearance of infection by existing hosts (Werren & Windsor, 2000). Nevertheless, this hypothesis can only be confirmed by monitoring of the same communities over long periods of time.

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Supplementary Table 1. *Wolbachia* detection in fig wasp species from Asia (China), Africa (South Africa), Australia and South America (Panama).

No.	**Fig trees Host (Family Moraceae and genus Ficus)**				**Fig wasps (Order Hymenoptera, Superfamily: Chalcidoidea)**			**Number infected (number screened)**	***Wolbachia* Supergroups**
	Fig Species	**Geographical Locations**	**Subgenus**	**Section**	**Wasp species (their biology)**	**Family**	**Genus**		
1	*Ficus sycomorus*	South Africa	*Sycomorus*	*Sycomorus*	*Ceratosolen arabicus* (P*)	Agaonidae	*Ceratosolen*	8(12)	A
2	*Ficus sycomorus*	South Africa	*Sycomorus*	*Sycomorus*	*Eukoebelea sycomori* (N)	Sycophaginae	*Eukoebelea*	12(14)	B
3	*Ficus stuhlmannii*	South Africa	*Urostigma*	*Galoglychia*	*Alfonsiella binghami* (P*)	Agaonidae	*Alfonsiella*	0(18)	—
4	*Ficus stuhlmannii*	South Africa	*Urostigma*	*Galoglychia*	*Sycoecine species* 1 (N)	Pteromalidae	*Sycoecine*	0(10)	—
5	*Ficus craterostoma*	South Africa	*Urostigma*	*Galoglychia*	*Alfonsiella pipithiensis* (P*)	Agaonidae	*Alfonsiella*	3(9)	A
6	*Ficus petersii*	South Africa	*Urostigma*	*Galoglychia*	*Alfonsiella species* 1 (P*)	Agaonidae	*Alfonsiella*	0(9)	—
7	*Ficus petersii*	South Africa	*Urostigma*	*Galoglychia*	*Alfonsiella species* 1 (P*)	Agaonidae	*Alfonsiella*	0(10)	—
8	*Ficus lutea*	South Africa	*Urostigma*	*Galoglychia*	*Allotriozoon heterandromorphum* (P*)	Agaonidae	*Allotriozoon*	0(5)	—
9	*Ficus lutea*	South Africa	*Urostigma*	*Galoglychia*	*Philotrypesiz species* (N)	Pteromalidae	*Philotrypesis*	0(2)	—
10	*Ficus sansibarica sansibarica*	South Africa	*Urostigma*	*Galoglychia*	*Courtella armata* (P*)	Agaonidae	*Courtella*	8(9)	A
11	*Ficus trichopoda*	South Africa	*Urostigma*	*Galoglychia*	*Elisabethiella bergi breviceps* (P*)	Agaonidae	*Elisabethiella*	2(3)	A
12	*Ficus abutilifolia*	South Africa	*Urostigma*	*Galoglychia*	*Elisabethiella comptoni* (P*)	Agaonidae	*Elisabethiella*	4(8)	A
13	*Ficus glumosa*	South Africa	*Urostigma*	*Galoglychia*	*Elisabethiella glumosae* (P*)	Agaonidae	*Elisabethiella*	9(20)	A
14	*Ficus glumosa*	South Africa	*Urostigma*	*Galoglychia*	*Sycoecine species* 2 (N)	Pteromalidae	*Sycoecine*	8(10)	B
15	*Ficus burkei*	South Africa	*Urostigma*	*Galoglychia*	*Elisabethiella stuckenbergi* (P*)	Agaonidae	*Elisabethiella*	19(19)	A
16	*Ficus salicifolia*	South Africa	*Urostigma*	*Urostigma*	*Platyscapa awekei* (P*)	Agaonidae	*Platyscapa*	0(26)	—
17	*Ficus ingens*	South Africa	*Urostigma*	*Urostigma*	*Platyscapa soraria* (P*)	Agaonidae	*Platyscapa*	0(18)	—
18	*Ficus montana*	Indo-Australia	*Sycidium*	*Sycidium*	*Liporrhophalum tentacularis* (P*)	Agaonidae	*Liporrhopalum*	6 (6)	A,B
19	*Ficus carica*	Australia	*Ficus*	*Ficus*	*Blastophaga psenes* (P*)	Agaonidae	*Blastophaga*	4 (4)	A
20	*Ficus septica*	Australia	*Sycomorus*	*Sycocarpus*	*Ceratosolen bisulcatus* (P*)	Agaonidae	*Ceratosolen*	0 (2)	—
21	*Ficus septica*	Australia	*Sycomorus*	*Sycocarpus*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	2 (2)	A
22	*Ficus septica*	Australia	*Sycomorus*	*Sycocarpus*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	a*
23	*Ficus racemosa*	Australia	*Sycomorus*	*Sycomorus*	*Ceratosolen sp.* (P*)	Agaonidae	*Ceratosolen*	4 (4)	A
24	*Ficus racemosa*	Australia	*Sycomorus*	*Sycomorus*	*Platyneura sp.* (N& G)	Sycophaginae	*Platyneura*	0 (1)	—
25	*Ficus pungens*	Australia	*Sycomorus*	*Bosscheria*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
26	*Ficus congesta*	Australia	*Sycomorus*	*Sycocarpus*	*Ceratosolen sp.* (P*)	Agaonidae	*Ceratosolen*	0 (2)	—
27	*Ficus microcarpa*	Australia	*Urostigma*	*Urostigma*	*Eupristina sp.* (P*)	Agaonidae	*Eupristina*	2 (2)	A

28	*Ficus microcarpa*	Australia	*Urostigma*	*Urostigma*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
29	*Ficus benjamina*	Australia	*Urostigma*	*Urostigma*	*Eupristina sp.* (P*)	Agaonidae	*Eupristina*	0 (2)	—
30	*Ficus xylosycia*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes riecki* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
31	*Ficus xylosycia*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	2 (2)	A
32	*Ficus watkinsiana*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes nigriventris* (P*)	Agaonidae	*Pleistodontes*	9 (17)	A
33	*Ficus watkinsiana*	Australia	*Urostigma*	*Malvanthera*	*Eukoebelea sp.* (N& G)	Sycophaginae	*Eukoebelea*	5 (7)	A
34	*Ficus watkinsiana*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (3)	—
35	*Ficus triradiata*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes schizodontes* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
36	*Ficus triradiata*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.*(N)	Pteromalidae	*Sycoscapter*	0 (1)	—
37	*Ficus subpuberula*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes astrobocheilus* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
38	*Ficus subpuberula*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
39	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes imperialis* (P*)	Agaonidae	*Pleistodontes*	71 (71)	A
40	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Herodotia sp.* (N& G)	Pteromalidae	*Herodotia*	1 (1)	a*
41	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Eukoebelea sp.* (N& G)	Sycophaginae	*Eukoebelea*	2 (2)	A
42	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Philotrypesis sp.* (Yellow) (N)	Pteromalidae	*Philotrypesis*	1 (1)	A
43	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Philotrypesis sp.* (Black) (N)	Pteromalidae	*Philotrypesis*	3 (3)	A
44	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Pseudidarnes sp.* (N& G)	Sycophaginae	*Pseudidarnes*	0 (5)	—
45	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	2 (2)	a*
46	*Ficus rubiginosa*	Australia	*Urostigma*	*Malvanthera*	*Watshamiella sp.* (N)	Pteromalidae	*Watshamiella*	0 (3)	—
47	*Ficus pleurocarpa*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes deuterus* (P*)	Agaonidae	*Pleistodontes*	10 (10)	A
48	*Ficus pleurocarpa*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes regalis* (P*)	Agaonidae	*Pleistodontes*	0 (7)	—
49	*Ficus pleurocarpa*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	2 (2)	A
50	*Ficus platypoda*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes cuneatus* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
51	*Ficus platypoda*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
52	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes greenwoodi* (P*)	Agaonidae	*Pleistodontes*	0 (14)	—
53	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes xanthocephalus* (P*)	Agaonidae	*Pleistodontes*	0 (9)	—
54	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Eukoebelea sp.* (N& G)	Sycophaginae	*Eukoebelea*	0 (4)	—
55	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Megastigmus sp.* (N)	Torymidae	*Megastigmus*	0 (1)	—
56	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	2 (2)	a*
57	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Pseudidarnes sp.* (N& G)	Sycophaginae	*Pseudidarnes*	0 (2)	—
58	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	5 (5)	A
59	*Ficus obliqua*	Australia	*Urostigma*	*Malvanthera*	*Watshamiella sp.* (N)	Pteromalidae	*Watshamiella*	0 (2)	—

60	*Ficus macrophylla*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes froggatti* (P*)	Agaonidae	*Pleistodontes*	0 (10)	—
61	*Ficus macrophylla*	Australia	*Urostigma*	*Malvanthera*	*Eukoebelea sp.* (N& G)	Sycophaginae	*Eukoebelea*	0 (1)	—
62	*Ficus macrophylla*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (3)	—
63	*Ficus macrophylla*	Australia	*Urostigma*	*Malvanthera*	*Watshamiella sp.* (N)	Pteromalidae	*Watshamiella*	2 (2)	A
64	*Ficus lilliputiana*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes proximus* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
65	*Ficus lilliputiana*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
66	*Ficus hesperidiiformis*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes plebejus* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
67	*Ficus hesperidiiformis*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
68	*Ficus glandifera*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes blandus* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
69	*Ficus glandifera*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
70	*Ficus destruens*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes rigisamos* (P*)	Agaonidae	*Pleistodontes*	14 (14)	A
71	*Ficus crassipes*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes addicotti* (P*)	Agaonidae	*Pleistodontes*	14 (14)	A
72	*Ficus crassipes*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes nitens* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
73	*Ficus crassipes*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	2 (2)	A
74	*Ficus crassipes*	Australia	*Urostigma*	*Malvanthera*	*Watshamiella sp.* (N)	Pteromalidae	*Watshamiella*	1 (1)	A
75	*Ficus cerasicarpa*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes macrocainus* (P*)	Agaonidae	*Pleistodontes*	1 (1)	A
76	*Ficus cerasicarpa*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
77	*Ficus brachypoda*	Australia	*Urostigma*	*Malvanthera*	*Pleistodontes athysanus* (P*)	Agaonidae	*Pleistodontes*	8 (8)	A
78	*Ficus brachypoda*	Australia	*Urostigma*	*Malvanthera*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (2)	—
79	*Ficus opposita*	Australia	*Sycidium*	*Sycidium*	*Kradibia sp.* (P*)	Agaonidae	*Kradibia*	2 (2)	A
80	*Ficus coronulata*	Australia	*Sycidium*	*Sycidium*	*Ceratosolen sp.* (P*)	Agaonidae	*Ceratosolen*	4 (4)	a*
81	*Ficus carica*	Australia	*Ficus*	*Ficus*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	1 (1)	A
82	*Ficus fistulosa*	China	*Sycomorus*	*Sycocarpus*	*Platyneura sp.* (N)	Sycophaginae	*Platyneura*	1 (1)	A
83	*Ficus hispida*	China	*Sycomorus*	*Sycocarpus*	*Ceratosolen solmsi* (P*)	Agaonidae	*Ceratosolen*	1 (1)	A
84	*Ficus hispida*	China	*Sycomorus*	*Sycocarpus*	*Philotrypesis pilosa* (N)	Pteromalidae	*Philotrypesis*	1 (1)	A
85	*Ficus hispida*	China	*Sycomorus*	*Sycocarpus*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	0 (5)	—
86	*Ficus hispida*	China	*Sycomorus*	*Sycocarpus*	*Apocrypta bakeri* (N)	Pteromalidae	*Apocrypta*	0 (5)	—
87	*Ficus racemosa*	China	*Sycomorus*	*Sycomorus*	*Platyneura mayri* (N)	Sycophaginae	*Platyneura*	1 (1)	A
88	*Ficus racemosa*	China	*Sycomorus*	*Sycomorus*	*Platyneura agraensis* (N)	Sycophaginae	*Platyneura*	0 (1)	—
89	*Ficus semicordata*	China	*Sycomorus*	*Hemicardia*	*Platyneura dunia* (N)	Sycophaginae	*Platyneura*	1 (1)	A
90	*Ficus semicordata*	China	*Sycomorus*	*Hemicardia*	*Sycoscapter trifemmensis* (N)	Pteromalidae	*Sycoryctes*	0 (7)	—
91	*Ficus squamosa*	China	*Sycomorus*	*Sycocarpus*	*Ceratosolen sp.* (P*)	Agaonidae	*Ceratosolen*	1 (1)	A
92	*Ficus altissima*	China	*Urostigma*	*Urostigma*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
93	*Ficus benjamina*	China	*Urostigma*	*Urostigma*	*Eupristina koningsbergeri* (P*)	Agaonidae	*Eupristina*	1 (1)	A
94	*Ficus benjamina*	China	*Urostigma*	*Urostigma*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
95	*Ficus benjamina*	China	*Urostigma*	*Urostigma*	*Philotrypesis tridentate* (N)	Pteromalidae	*Philotrypesis*	1 (1)	A

96	*Ficus concinna*	China	*Urostigma*	*Urostigma*	*Plastycapca sp.* (P*)	Agaonidae	*Platyscapa*	1 (1)	A
97	*Ficus concinna*	China	*Urostigma*	*Urostigma*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	0 (1)	—
98	*Ficus curtipes*	China	*Urostigma*	*Urostigma*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	0 (1)	—
99	*Ficus curtipes*	China	*Urostigma*	*Urostigma*	*Lipothymus sp.* (N)	Pteromalidae	*Lipothymus*	0 (1)	—
100	*Ficus drupacea*	China	*Urostigma*	*Malvanthera*	*Ceratosolen. sp.* (P*)	Agaonidae	*Ceratosolen*	0 (1)	—
101	*Ficus drupacea*	China	*Urostigma*	*Malvanthera*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	0 (1)	—
102	*Ficus microcarpa*	China	*Urostigma*	*Urostigma*	*Eupristina verticillata* (P*)	Agaonidae	*Eupristina*	1(1)	B
103	*Ficus microcarpa*	China	*Urostigma*	*Urostigma*	*Philotrypesis sp.* 1 (N)	Pteromalidae	*Philotrypesis*	0 (1)	—
104	*Ficus microcarpa*	China	*Urostigma*	*Urostigma*	*Philotrypesis sp.* 2 (N)	Pteromalidae	*Philotrypesis*	0 (2)	—
105	*Ficus microcarpa*	China	*Urostigma*	*Urostigma*	*Sycoscapter sp.* 1 (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
106	*Ficus microcarpa*	China	*Urostigma*	*Urostigma*	*Sycoscapter sp.* 2 (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
107	*Ficus microcarpa*	China	*Urostigma*	*Urostigma*	*Sycobia sp.* 1 (N)	Pteromalidae	*Sycobia*	0 (1)	—
108	*Ficus superba*	China	*Urostigma*	*Urostigma*	*Platyscapa corneri* (P*)	Agaonidae	*Platyscapa*	1 (1)	A
109	*Ficus virens*	China	*Urostigma*	*Urostigma*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
110	*Ficus virens*	China	*Urostigma*	*Urostigma*	*Lipothymus sp.* (N)	Pteromalidae	*Lipothymus*	0 (1)	—
111	*Ficus virens*	China	*Urostigma*	*Urostigma*	*Sycoscapter sp.*(N)	Pteromalidae	*Sycoscapter*	1 (1)	A
112	*Ficus virens*	China	*Urostigma*	*Urostigma*	*Ormyrus sp.* (N)	Ormyridae	*Ormyrus*	1 (1)	A
113	*Ficus virens*	China	*Urostigma*	*Urostigma*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	0 (2)	—
114	*Ficus cyrtophylla*	China	*Sycidium*	*Sycidium*	*Blastophaga sp.* (P*)	Agaonidae	*Blastophaga*	1 (1)	A
115	*Ficus subulata*	China	*Sycidium*	*Palaeomorphe*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	0 (2)	—
116	*Ficus subulata*	China	*Sycidium*	*Palaeomorphe*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	1 (1)	A
117	*Ficus tinctoria tinctoria*	China	*Sycidium*	*Palaeomorphe*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	1 (2)	A
118	*Ficus auriculata*	China	*Sycomorus*	*Sycomorus*	*Ceratosolen emarginatus* (P*)	Agaonidae	*Ceratosolen*	1 (1)	A
119	*Ficus auriculata*	China	*Sycomorus*	*Sycomorus*	*Philotrypesis longicaudata* (N)	Pteromalidae	*Philotrypesis*	2 (2)	A
120	*Ficus hirta hirta*	China	*Ficus*	*Eriosycea*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
121	*Ficus hirta hirta*	China	*Ficus*	*Eriosycea*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	1 (1)	A
122	*Ficus hirta roxburghii*	China	*Ficus*	*Eriosycea*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	1 (1)	A
123	*Ficus ischnopoda*	China	*Ficus*	*Ficus*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
124	*Ficus langkokensis*	China	*Ficus*	*Eriosycea*	*Blastophaga sp.* (P*)	Agaonidae	*Blastophaga*	1 (1)	A
125	*Ficus langkokensis*	China	*Ficus*	*Eriosycea*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	1 (1)	A
126	*Ficus variolosa*	China	*Ficus*	*Ficus*	*Blastophaga silvestriana* (P*)	Agaonidae	*Blastophaga*	0 (1)	—
127	*Ficus nervosa nervosa*	China	*Pharmacosycea*	*Oreosycea*	*Philotrypesis sp.* (N)	Pteromalidae	*Philotrypesis*	1 (1)	A
128	*Ficus hederacea*	China	*Synoecia*	*Kissosycea*	*Sycoscapter sp.* (N)	Pteromalidae	*Sycoscapter*	0 (1)	—
129	*Ficus paraensis*	Panama	*Urostigma*	*Americana*	*Pegoscapus herrei* (P*)	Agaonidae	*Pegoscapus*	9(9)	A
130	*Ficus obtusifolia*	Panama	*Urostigma*	*Americana*	*Pegoscapus hoffmeyeri* (P*)	Agaonidae	*Pegoscapus*	5(15)	A
131	*Ficus obtusifolia*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 1 (N)	Sycophaginae	*Idarnes*	6(6)	A,B

132	*Ficus obtusifolia*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 2 (N)	Sycophaginae	*Idarnes*	1(1)	A
133	*Ficus obtusifolia*	Panama	*Urostigma*	*Americana*	*Aepocerus sp.* 1 (N& G)	N/P	*Aepocerus*	0(6)	—
134	*Ficus obtusifolia*	Panama	*Urostigma*	*Americana*	*Aepocerus sp.* 2 (N& G)	N/P	*Aepocerus*	0(7)	—
135	*Ficus obtusifolia*	Panama	*Urostigma*	*Americana*	*Physothorax sp.* (N& P)	Torymidae	*Physothorax*	1(4)	B
136	*Ficus obtusifolia*	Panama	*Urostigma*	*Americana*	*Heterandrium sp.* (N& G)	N/P	*Heterandrium*	0(2)	—
137	*Ficus citrifolia*	Panama	*Urostigma*	*Americana*	*Pegoscapus tonduzi* (P*)	Agaonidae	*Pegoscapus*	0(10)	—
138	*Ficus citrifolia*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 3 (N)	Sycophaginae	*Idarnes*	1(1)	A
139	*Ficus popenoei*	Panama	*Urostigma*	*Americana*	*Pegoscapus gemellus* (P*)	Agaonidae	*Pegoscapus*	41(41)	A
140	*Ficus popenoei*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 4 (N)	Sycophaginae	*Idarnes*	6(6)	A
141	*Ficus popenoei*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 5 (N)	Sycophaginae	*Idarnes*	2(2)	A
142	*Ficus nymphaeifolia*	Panama	*Urostigma*	*Americana*	*Pegoscapus piceipes* (P*)	Agaonidae	*Pegoscapus*	0(20)	—
143	*Ficus trigonata*	Panama	*Urostigma*	*Americana*	*Pegoscapus grandii* (P*)	Agaonidae	*Pegoscapus*	0(15)	—
144	*Ficus trigonata*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 6 (N)	Sycophaginae	*Idarnes*	5(6)	B
145	*Ficus trigonata*	Panama	*Urostigma*	*Americana*	*Aepocerus sp.* (N& G)	N/P	*Aepocerus*	3(3)	A
146	*Ficus triangularis*	Panama	*Urostigma*	*Americana*	*Pegoscapus lopesi* (P*)	Agaonidae	*Pegoscapus*	9(10)	A
147	*Ficus triangularis*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 7 (N)	Sycophaginae	*Idarnes*	1(1)	A, B
148	*Ficus dugandii*	Panama	*Urostigma*	*Americana*	*Pegoscapus longiceps* (P*)	Agaonidae	*Pegoscapus*	13(13)	A
149	*Ficus dugandii*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 8 (N)	Sycophaginae	*Idarnes*	6(6)	A
150	*Ficus dugandii*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 9 (N)	Sycophaginae	*Idarnes*	0(1)	—
151	*Ficus dugandii*	Panama	*Urostigma*	*Americana*	*Aepocerus sp.* (N & G)	N/P	*Aepocerus*	1(1)	A
152	*Ficus dugandii*	Panama	*Urostigma*	*Americana*	*Physothorax sp.* (N& P)	Torymidae	*Physothorax*	2(2)	A
153	*Ficus turbinata*	Panama	*Urostigma*	*Americana*	*Pegoscapus sp.* (P*)	Agaonidae	*Pegoscapus*	0(2)	—
154	*Ficus turbinata*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 10 (N)	Sycophaginae	*Idarnes*	1(1)	A
155	*Ficus pertusa*	Panama	*Urostigma*	*Americana*	*Pegoscapus silvestrii* (P*)	Agaonidae	*Pegoscapus*	0(2)	—
156	*Ficus bullenei*	Panama	*Urostigma*	*Americana*	*Pegoscapus gemellus* (P*)	Agaonidae	*Pegoscapus*	3(17)	A
157	*Ficus bullenei*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 11 (N)	Sycophaginae	*Idarnes*	0(1)	—
158	*Ficus colubrinae*	Panama	*Urostigma*	*Americana*	*Pegoscapus orozcoi* (P*)	Agaonidae	*Pegoscapus*	0(1)	—
159	*Ficus colubrinae*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 12 (N)	Sycophaginae	*Idarnes*	0(1)	—
160	*Ficus perforata*	Panama	*Urostigma*	*Americana*	*Pegoscapus insularis* (P*)	Agaonidae	*Pegoscapus*	9(20)	A
161	*Ficus perforata*	Panama	*Urostigma*	*Americana*	*Idarnes sp.* 13 (N)	Sycophaginae	*Idarnes*	0(1)	—
162	*Ficus costaricana*	Panama	*Urostigma*	*Americana*	*Pegoscapus estherae* (P*)	Agaonidae	*Pegoscapus*	17(17)	A
163	*Ficus costaricana*	Panama	*Urostigma*	*Americana*	*Idarnes sp.*14 (N)	Sycophaginae	*Idarnes*	0(4)	—
164	*Ficus costaricana*	Panama	*Urostigma*	*Americana*	*Aepocerus sp.* (N & G)	N/P	*Aepocerus*	1(1)	A
165	*Ficus yoponensis*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Tetrapus ecuadoranus* (P*)	Agaonidae	*Tetrapus*	0(1)	—
166	*Ficus yoponensis*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Critogaster sp.* (N)	Pteromalidae	*Critogaster*	0(1)	—

167	*Ficus maxima*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Tetrapus americanus* (P*)	Agaonidae	*Tetrapus*	0(1)	—
168	*Ficus maxima*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Critogaster sp.* (N)	Pteromalidae	*Critogaster*	1(1)	A
169	*Ficus insipida*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Tetrapus costaricanus* (P*)	Agaonidae	*Tetrapus*	28(28)	A
170	*Ficus insipida*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Critogaster sp.* (N)	Pteromalidae	*Critogaster*	3(3)	A, B
171	*Ficus glabrata*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Tetrapus sp.* (P*)	Agaonidae	*Tetrapus*	0(1)	—
172	*Ficus glabrata*	Panama	*Pharmacosycea*	*Pharmacosycea*	*Critogaster sp.* (N)	Pteromalidae	*Critogaster*	1(1)	A

South African samples are reported for the first time in this study, Panamanian fig wasp data were taken from Shoemaker *et al*. (2002), Australian from Haine & Cook (2005) and Chinese from Chen *et al*. (2010); (N) Non pollinator, (P*) Pollinator, (G) Non pollinator galler, (P) Non pollinator parasitoids; Dashed line means no *Wolbachia* infection; a* Sample confirmed positive by PCR and were not sequenced; N/P not placed in a defined family.