

# Multi-Species Fig Wasp Communities Reveal Extreme Deviation From Reciprocal Partner Specificity

Kevin Quinteros <sup>\*1</sup>, Finn Piatscheck<sup>2</sup>, Jordan D. Satler<sup>1</sup>, John D. Nason<sup>1</sup>, and Tracy A. Heath<sup>1</sup>

<sup>1</sup>Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011

<sup>2</sup>Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama

January 15, 2023

## Abstract

Symbiotic interactions underpin key ecosystem functions and have played a significant role in shaping the diversity and organization of the Earth's biota. Such strong interactions between species subsequently affect population dynamics and coevolution of associated symbionts. Yet, the exact nature of associations between host/symbiont are often unknown due to the lack of targeted studies, insufficient sampling, and taxonomic impediments. Among classical symbioses, figs and their pollinating wasps constitute a highly diverse (+800 species) mutualism. However, there is a striking lack of studies with the specific aims of assessing host-pollinator association and how this varies across the geographic range of the host. Here, we present the first targeted investigation using high throughput sequence data to assess pollinator diversity and host-pollinator associations across the geographic range of multiple *Ficus* species. Our results confirm that associations between species of *Ficus* hosts and their wasp mutualists have changed over time and space. Thus, our work demonstrates that highly specific, one-to-one symbiotic interactions may often break down when faced with ecological or evolutionary changes. Host-switching and pollinator-sharing are also prevalent and ubiquitous throughout the fig and fig-wasp system. This, in turn, provides opportunities for introgression and gene flow between *Ficus* species, thus these results have important implications on the co-evolutionary trajectory of the fig and fig wasp mutualism.

## 1 Introduction

Symbiotic interactions like mutualisms and parasitisms underpin key ecosystem functions and have played a significant role in shaping the diversity and organization of the Earth's biota [Leigh Jr, 2010; Bronstein et al., 2006; Futuyma and Slatkin, 1983; Thompson, 1994]. High levels of intimacy and partner specificity found in many symbioses create conditions in which partners impose reciprocal selection for reproductive success [Thrall et al., 2007]. Such strong interactions between species subsequently affect population dynamics and coevolution [Thrall et al., 2007]. In some cases, highly intimate interactions over evolutionary time may drive cospeciation, such as with pocket Gophers (*Geomys*) and their chewing lice (*Geomydoecus*) [Demastes and Hafner, 1993; Clark et al., 2000]. Given these dynamics, it is important to ask: "How many species are involved in a host/symbiont interaction?" This question arises because species interaction strength will differ when comparing a system with one host and one symbiont species (specialist) to systems with multiple hosts or symbiont species (generalist) [Herre et al., 1999; Darwell et al., 2014]. Limited knowledge on taxonomy and species diversity can hinder the understanding of community composition and dynamics of host/symbiont associations [Kaarinen et al., 2010; Smith et al., 2011; Wheeler, 2010]. A classic example of a

---

\*kevinq@iastate.edu

coevolved mutualism with limited taxonomic knowledge is provided by the obligate relationship between fig trees (*Ficus*) and fig-pollinating wasps (Hymenoptera: Agaonidae). Here, we use a broad-scale multi-species approach to investigate the host/symbiont interaction dynamics in fig and fig wasp communities.

The obligate nursery-pollination mutualism of fig trees and their pollinating fig wasps has generally been regarded as a classic system for studying coevolution [Marussich and Machado, 2007]. Female fig wasps are dependent on receptive fig syconia—the urn-shaped inflorescence commonly referred to as fig “fruit”—for brood development, while fig trees are reciprocally dependent on fig wasps for pollination [Janzen, 1979]. Figs with receptive female flowers have evolved elaborate floral fragrances to attract species-specific fig-wasp pollinators [Chen and Song, 2008; Wang et al., 2013]. These fragrances enable the dispersal of female fig wasp pollinators. Despite their minuscule size ( $\sim 5$  mm), female fig wasps are excellent dispersers using wind currents to reach host-specific fig trees that are often several kilometers from their natal trees [Nason et al., 1998; Harrison and Rasplus, 2006; Ahmed et al., 2009; Ramírez B., 1969]. The long held dogma in the fig and fig wasp mutualism has been one of extreme reciprocal partner specificity with each fig species having only one fig wasp pollinator species throughout its range (1:1 rule). Recent studies, however, have undermined this long-held assumption with multiple reports of a single fig species hosting multiple pollinators [Michaloud et al., 1996; Haine et al., 2006; Su et al., 2008; Compton et al., 2009; Lin et al., 2011; Moe et al., 2011; Chen et al., 2012; Darwell et al., 2014; Yu et al., 2019], as well as examples of fig species sharing pollinators [Lopez-Vaamonde et al., 2002; Cook and Rasplus, 2003; Marussich and Machado, 2007; Su et al., 2008; Cornille et al., 2012; Wang et al., 2016; Satler et al., 2022]. Often, a fig species associated with multiple pollinators occurs largely in allopatry, though there are several examples of sympatric pollinator species sharing the same host fig species [Machado et al., 2005; Satler et al., 2022].

Most research on fig and fig-wasp associations have focused on single *Ficus* species with wide-spread species ranges. These studies have shown that over the span of a *Ficus* species’ range, it may be associated with different pollinator species [Lin et al., 2011; Chen et al., 2012; Darwell et al., 2014; Yu et al., 2019]. Host/pollinator associations have also been revealed by studies investigating the phylogeography and ecology of the mutualism, as well as wasp reproductive behavior [Wang et al., 2009; Chen et al., 2011; Yu and Nason, 2013; Wang et al., 2013; Wei et al., 2014]. When multi-species fig and fig-wasp associations are examined within a community context, sampling is often conducted within a very restricted and localized geographic scale [Machado et al., 2005; Souto-Vilarós et al., 2018]. These studies are limited, since they do not address whether the same fig and wasp associations are maintained throughout the entire ranges of multi-species *Ficus* communities. Studying fig and fig-wasp associations across wide geographic sampling can allow us to further understand the evolutionary dynamics of this mutualism, since we are not limited to studying the system within a fraction of a typical *Ficus* species range. The need for these types of studies are illustrated by recent reviews that have estimated that a third or half of the over 800 *Ficus* species worldwide are associated with multiple pollinator species [Cook and Segar, 2010]. The rapid revision of *Ficus* and fig wasp taxonomy due to the emergence of genomic data has further demonstrated the need for a broad-scale, multi-species approach. Genomic data have revealed multiple examples of cryptic species of fig-wasps pollinating the same host [Lopez-Vaamonde et al., 2002; Su et al., 2008]. Currently, only  $\sim 150$  pollinating fig-wasp species are described, but as more specimens are sequenced, the number of unique species may grow to more than 1,000 [Cook and West, 2005]. These studies emphasize the shortcomings of focusing on a single *Ficus* host and localized sampling when studying figs and their associated wasp pollinators.

A multi-species approach, with careful sampling over a wide geographic area, enables us to investigate the number of wasp pollinators associated with each *Ficus* species, and how these assemblages vary across the host range. Here, we focus on neo-tropical monoecious *Ficus* communities throughout Mexico and within the Barro Colorado Nature Monument (BCNM). We investigated the diversity of fig wasp pollinators associated with a multi-species fig community both at a broad and localized geographic scales. The phylogenetic relationships and host-pollinator associations for the BCI *Ficus* community has been extensively documented [Molbo et al., 2003; Marussich and Machado, 2007; Satler et al., 2019, 2022]. Using high-throughput sequencing data (RAD and ultra-conserved elements) and wide-sampling, we aim to answer the following questions:

1. Host *Ficus* perspective:

- (a) How many fig-wasp pollinators are associated with an individual *Ficus* species throughout the

- majority of a single host-species range?
  - (b) Are a fig’s pollinator wasps allopatric or sympatric in distribution?
  - (c) Are a fig’s pollinator wasps shared with other *Ficus* host species?
2. Pollinator perspective:
- (a) How many host associations are observed for a single fig-wasp species over a broadly sampled geographic range?
  - (b) Is a fig-wasp pollinator allopatric or sympatric with other fig-wasp species associated with the same host?
3. Community-level perspective (Multiple *Ficus* and fig wasp pollinator species)
- (a) What are the relative proportions of unique- versus shared-pollinator associations at local and broad geographic scales?

## 2 Methods

### 2.1 Host Fig Sampling, Sequencing and Phylogenetic Analysis

Due to the geopolitics of western mainland Mexico, our sampling of *Ficus* species in this area was restricted to regions we could access safely. Additionally, our sampling in Panama was restricted to the Barro Colorado Nature Monument (BCNM). We sampled 26 tentative *Ficus* species (188 individuals) representing both free-standing (subgenera *Pharmacosycea*) and strangler fig (subgenera *Urostigma*) trees. Species assignment during sampling was based on morphological traits and was subsequently confirmed with molecular data. Sampling was conducted throughout western mainland Mexico between 2017-2019 and from previously geo-referenced individuals in the BCNM (Figure 1). Leaf samples were collected from each tree and preserved using color indicating silica gel before being transported to Iowa State University and stored at -80°C until DNA extraction and library construction.

Genomic DNA was extracted using a modified CTAB protocol [Doyle and Doyle, 1987] or Qiagen DNeasy® Plant Pro Kit (Qiagen Inc., Valencia CA). Extractions were sent to Floragenex Inc. (Eugene, OR, USA) for restriction-site associated DNA (RAD) library preparation. Single-end RAD libraries were generated with the PstI restriction enzyme following standard protocol [Baird et al., 2008]. Libraries were sequenced on an Illumina HiSeq 3000 using 100 bp single-end sequencing. Raw sequence reads were processed and *de novo* assembled using Ipyrad (version 0.9.31) [Eaton and Overcast, 2020]. No mismatches were allowed in barcodes when demultiplexing samples, with strict filtering used for removing any adapter contamination. Up to five low-quality base calls were allowed in a read. We used a clustering threshold of 85% when assembling reads into loci within species. Within individuals, we allowed up to 5% Ns and 5% heterozygous sites in a locus. Alleles were clustered across individuals using an 85% threshold. For clustered loci, we allowed up to 20% SNPs, up to 20% heterozygous sites, and up to 8 total indels.

We used two methods to infer phylogenies that enabled us to determine individual species assignment and confirm host-pollinator associations between *Ficus* and pollinator species. First, we estimated a maximum likelihood (ML) phylogeny using concatenation in using RAxML v8.2.11 [Stamatakis, 2014]. We specified a general time reversible substitution model and gamma-distributed rate variation among sites. To assess nodal support, we generated 100 bootstrap replicates with the rapid bootstrapping algorithm [Stamatakis et al., 2008]. We used a multi-species coalescent based approach to infer a phylogeny using SVDquartets as implemented in PAUP\* (version 4.0a163) [Chifman and Kubatko, 2014; Swofford, 2003]. SVDquartets [Chifman and Kubatko, 2014] uses site patterns in the nucleotide data to estimate a phylogeny under the multi-species coalescent model. We used the full unlinked SNP data set for input, quartets were evaluated exhaustively, and 100 bootstrap replicates were run to provide nodal support values. and

## 2.2 Pollinator Sampling and Sequencing

To obtain pollinator wasps, we collected near-mature (late interphase) and mature *Ficus syconia*. The syconia were placed in plastic vials and pollinators were allowed to emerge, at which time they were preserved in 95% ethanol or RNALater. If wasps had not emerged within 24 hours, or a syconium was immature, then in some cases galled flowers were preserved for later removal of wasp larva or pupae. In total, we genotyped 304 individuals (genera *Pegoscapus* and *Tetrapus*), as well 22 non-pollinating fig wasps (genus *Idarnes*) were collected from several *Ficus* species to serve as an outgroup clade.

To generate genome-wide sequence data for the wasps, we used targeted enrichment of ultra-conserved elements (UCEs) following the workflow outlined in Faircloth et al. [2012]. To ensure independence among samples, a single wasp was selected per syconium and genomic DNA was extracted with a Qiagen DNeasy® Blood and Tissue Kit (Qiagen Inc., Valencia CA). Samples were fragmented to an average size range of 450 bp using a Covaris ME220 focused-ultrasonicator (Covaris Inc., Woburn MA) and Illumina libraries were prepared using a KAPA HyperPrep Kit (Roche Sequencing and Life Science). After library construction, samples were grouped in eight sets and hybridized with biotinylated RNA probes to capture targeted loci. We used the hymenopteran probe set *v2* to target 2590 ultra-conserved element (UCE) loci [Branstetter et al., 2017]. Following probe hybridization and library amplification, we confirmed size distributions using a Bioanalyzer, after which libraries were pooled into equimolar concentrations for sequencing. Illumina libraries were sent to GeneWiz (South Plainfield, NJ) and sequenced on two full lanes of an Illumina HiSeq 3000 using 150 base pair paired-end sequencing.

Phyluce *v1.6.7* [Faircloth, 2016] with SAMtools *v1.10.2* [Li et al., 2009; Cock et al., 2015] was used to process raw sequence reads and generate UCE loci for each individual. Raw sequence reads were cleaned with illumiprocessor [Faircloth, 2013], which is a wrapper around Trimmomatic *v0.32* [Bolger et al., 2014]. SPAdes *v3.12.0* [Bankevich et al., 2012] was used to assemble clean reads into contigs, which were aligned to our hymenopteran probe set to filter out nonspecific sequences. Resulting UCE loci were then aligned with MAFFT *v7.407* [Katoh and Standley, 2013] and edge trimmed with trimAl Capella-Gutierrez et al. [2009], with ambiguously aligned internal sites removed using Gblocks version 0.91b [Castresana, 2000]. Finally, we required a minimum of 70% sample coverage to retain a locus.

For the pollinator data set, we also inferred phylogenies using RAxML and SVDQuartets. We concatenated all recovered UCE loci into a single data matrix as input for both analyses. Nodal support for each analysis was assessed as previous described. Based on the results of the *Ficus* and pollinator phylogenies, we plotted association table within R *v4.0.3* R Core Team [2020] and created a tanglegram using the *cophylo* function with the *ape* R package Paradis and Schliep [2019].

## 3 Results

### 3.1 Host Sequencing and Phylogeny

We generated 1,419,845,018 raw sequence reads for the 188 *Ficus* samples, with individuals averaging 7,552,367 ( $\pm 7,791,081$ ) reads. Following data processing, individuals had on average 78,576 ( $\pm 71,535$ ) RAD clusters. Requiring 50% coverage for loci, we recovered 1,305,493 SNPs across 188 samples. All individuals tentatively identified as *F. velutina* sequenced poorly, so they were excluded from downstream phylogenetic analyses.

RAxML and SVDQuartets resulted in similar topologies, so we only present the SVDQuartets phylogeny (Figure 2). Both analyses recovered the subgenera *Pharmacosycea* and *Urostigma* as monophyletic groups. Our molecular data, for the most part, supported tentative species assignment based on morphological traits with some notable exceptions. *Ficus insipida* individuals collected throughout Mexico were placed in a group sister to *F. glabrata*, while *F. insipida* individuals collected from Panama were sister to both Mexican *F. insipida* and *F. glabrata* (Figure 2). Some individuals that were tentatively identified as *F. pertusa* formed a monophyletic clade that is sister to *F. pringlei*. The evolutionary relationships of all other individuals within our *Ficus* phylogeny agreed with previous published phylogenies of neo-tropical figs [Machado et al., 2005; Cruaud et al., 2012; Satler and Carstens, 2017].

## 3.2 Pollinator Sequencing and Phylogeny

We generated 2,008,598,323 raw sequence reads for the 304 fig wasp samples, with individuals averaging 5,503,009 ( $\pm 2,340,771$ ) reads. Following data processing, individuals had on average 1923 ( $\pm 348$ ) UCE loci. Requiring 50% coverage for loci, we recovered 1,305,493 SNPs across 304 samples.

RAxML and SVDQuartets phylogenies had congruent topologies, so we only present the SVDQuartets phylogeny (Figure 2). The SVDQuartets phylogeny separated fig-wasp pollinators into two main clades (genera *Pegoscapus* and *Tetrapus*). *Pegoscapus* fig wasps are active pollinators (actively collect pollen) for the *Urostigma* figs. *Tetrapus* wasps are passive pollinators for *Pharmacosycea* figs. Thirty-three different fig wasp species were identified with twenty-seven being *Pegoscapus* pollinators and six being *Tetrapus* pollinators. Our phylogeny supports previously published topologies of *Pegoscapus* and *Tetrapus* wasps [Machado et al., 2005; Marussich and Machado, 2007; Satler et al., 2019, 2022]. In addition, we have identified putative novel host-pollinator associations.

## 3.3 Host-Pollinator Associations

### 3.3.1 Host *Ficus* perspective

Our genetically inferred host-pollinator associations (Figures 3 and 4) confirm that *Ficus* species can be associated with multiple fig-wasp pollinators throughout their species range. Among our *Pharmacosycea* figs, *F. glabrata* and *F. maxima* are both pollinated by two *Tetrapus* species (Figure 3). With one pollinator, *T. sp. 2*, being shared among both *F. glabrata* and *F. maxima* figs. Additionally, all three *Tetrapus* species occur in sympatry within BCNM (Panama). In contrast, *F. insipida* (Mexico), which was sampled along a transect from northern to southern Mexico ( $>800$  km) was associated with only had one pollinator species across the sampled range. Among the *Urostigma* figs, nine *Ficus* species had multiple fig-wasp pollinators throughout their ranges (Figure 4). As with the *Pharmacosycea* figs, we observed *Urostigma* species occurring in sympatry share pollinators. *Ficus colubrinae* and *F. perforata* are both pollinated by *P. silvestrii*, while each having their own exclusive fig wasp pollinator. Similarly, *F. bullenei* and *F. popenoei* are both pollinated by *P. gemellus* A, while *F. bullenei* is exclusively pollinated by *P. gemellus* C and *F. popenoei* is pollinated only by *P. gemellus* B.

After RAD loci assembly and cleaning, we curated three *Urostigma* species with broad geographic sampling (*F. petiolaris*, *F. crocata*, and *F. obtusifolia*). *Ficus crocata* had the largest number (five) of associated pollinators throughout its species range with *P. sp. 5* being the most prevalent (Figure 5). *P. grandii* was the only *F. crocata* pollinator that did not pollinate other fig species. *Ficus obtusifolia* had two sympatric pollinators within BCNM and one allopatric pollinator through out its sampled range in Mexico (Figure 6). Finally, *F. petiolaris* had four pollinators throughout its range with *P. sp. 1*, and *P. sp. 11* occurring in sympatric in the southern Mexico (Figure 7).

### 3.3.2 Pollinator perspective

One third of fig-wasp pollinator species had multiple fig hosts throughout their sampled species range (Figures 3 and 4). *Pegoscapus* sp. 1 and *P. sp. 12* both had three host-species associations throughout their range. We also explored *Pegoscapus* species with broad geographic ranges. *Pegoscapus* sp. 4 was sampled from northern to southern Mexico and showed two host associations with *F. cotinifolia* and *F. pringlei*. The host-association of *P. sp. 4* changed by region (Figure 8), with *P. sp. 4* pollinating *F. cotinifolia* in northern and southern Mexico, but *F. pringlei* in central Mexico. The host association of *P. sp. 5* also varied by region, since it pollinated both *F. crocata* and *F. pertusa* in central Mexico and only *F. crocata* in northern and southern Mexico (Figure 9).

Just as their fig-host counterparts, several fig-wasp pollinators exhibited overlapping host associations in sympatry. For example, *P. gemellus* A is associated with *F. bullenei* and *F. popenoei*. In total, this pattern of sympatric host sharing was observed in one *Tetrapus* (*T. sp. 2*) and five *Pegoscapus* pollinators (*P. gemellus* A, *P. insularis*, *P. sp. 1*, *P. sp. 10*, & *P. sp. 12*). All other pollinator species with multiple host had fig-wasp pollinator host-association that occurred in allopatry. Overall, these results suggest that the fig and fig wasp mutualism is more dynamic than previously known.

## 4 Discussion

A textbook example of symbiosis is provided by the fig and fig-wasp mutualism, which is often a crucial species in their respective ecological communities [Janzen, 1979]. Yet, the exact nature of associations between figs and their wasp pollinators are often unknown due to the lack of targeted studies, insufficient sampling, and taxonomic impediments [Cook and Rasplus, 2003; Cook and Segar, 2010; Darwell et al., 2014]. Here, we present the first targeted investigation using high throughput sequence data to assess pollinator diversity and host-pollinator associations across the geographic range of multiple *Ficus* species. Our study supports the breakdown of the one-to-one rule within this highly specialized mutualism [Michaloud et al., 1996; Haine et al., 2006; Su et al., 2008; Compton et al., 2009; Moe et al., 2011; Darwell et al., 2014; Yu et al., 2019]. Additionally, we show that pollinator sharing among *Ficus* species is more common than previously known. Over the geographic ranges of fig-wasp pollinators their host-associations can often change. This investigation adds to the growing body of evidence that diversity of fig wasp pollinators is greatly underestimated [Cook and Rasplus, 2003; Cook and Segar, 2010].

We genetically identified twenty-five different *Ficus* species and thirty-three pollinator species (*Tetrapus* and *Pegoscapus*). This allowed us to answer our first question: *How many fig-wasp pollinators are associated with an individual Ficus species throughout the majority of a single host-species range?* Based on our observations, *Ficus* species can often have multiple pollinators throughout their geographic range. For example, five pollinator-species associations were recorded for *F. crocata*, the largest number of any *Ficus* within the study (Figure 4). This high count of pollinator associations is not uncommon. Lack of pollinator specificity has been reported in several other *Ficus* species with broad geographic range [Su et al., 2008; Lin et al., 2011; Darwell et al., 2014; Su et al., 2022]. In contrast, we also observed high pollinator specificity in a number of *Ficus* lineages. *Ficus insipida* (Mexico) which was sampled from northern to southern Mexico only had one associated species. This suggest pollinator host-specificity varies among fig wasp pollinators. Species specificity of associations between figs and their pollinators is more dynamic and these ecological interactions are not as rigid as previously acknowledged [Janzen, 1979].

### 4.1 Host-Pollinator Associations: Host Perspective

Our study has revealed dynamic interactions among figs and their pollinating fig wasps, allowing us to ask the question: *If a Ficus species has multiple pollinator associations, do those associations occur in allopatry or sympatry?* Here, we report cases of both allopatric and sympatric multi-species pollinator associations in *Ficus* species. Within our Panamanian study site, we observed six species (*F. bullenei*, *F. popenoei*, *F. maxima*, *F. glabrata*, *F. colubrinae*, and *F. perforata*) with multi-pollinator associations occurring in sympatry. Additionally, species such as *F. crocata* exhibited associations with several pollinator species that occurred both in allopatry and sympatry (Figure 5). This leads to another question: *Are a fig's pollinator wasps shared with other Ficus host species?* Eleven pollinator species are “shared” or pollinate multiple *Ficus* species. *Ficus glabrata* and *F. bullenei* each have their own unique pollinator association, but also have a pollinator that is shared between them. This can have important implications for the system, since shared pollinators create opportunities for gene flow between closely related fig lineages. A growing body of research has recently revealed hybridization and introgression between *Ficus* species has played a significant role in the evolutionary history of the genus [Jackson et al., 2008; Renoult et al., 2009; Bruun-Lund et al., 2017; Wilde et al., 2020; Wang et al., 2021]. In all these documented cases, present-day pollinator sharing was observed between putative parental *Ficus* species. Our observations are consistent with these studies and suggest that ongoing pollinator sharing is a mechanism for gene flow among host lineages.

### 4.2 Host-Pollinator Associations: Pollinator Perspective

When shifting to understand the dynamics of this mutualism from the perspective of pollinating fig wasps, we were able to address the question: *How many host associations are observed for a single fig wasp species over a broadly sampled geographic range?* The number of hosts varied across pollinator species, though the majority of our sampled pollinators only had one host association. Among the eleven pollinators that had multiple host associations, *Pegoscapus* sp. 1 and *P.* sp. 12, were both tied for the highest number of host-association at three. These wasp species were only sampled in southern Mexico, thus all of their host species

were sympatric. Other multi-host pollinators that were observed over broad geographic ranges, exhibited spatially distributed changes in their number of associated hosts. For example, *P. sp. 4* pollinates both *F. pringlei* and *F. cotinifolia* (Figure 8), but its association with *F. pringlei* is restricted to the host’s range within Colima and Jalisco Mexico. In contrast, By contrast, *P. sp. 4* pollinates *Ficus cotinifolia* throughout the host’s species range from northern to southern Mexico. No *F. cotinifolia* samples were collected within central Mexico, but we assume that *P. sp. 4* is the common pollinator throughout its species range. Thus, the host associations of *P. sp. 4* might change according to locality.

This was similarly observed with *Pegoscapus sp. 5* which is the common pollinator of *F. crocata* (Figure 9). Within central Mexico, this species also pollinated *F. pertusa*. Both *Pegoscapus sp. 4* and *P. sp. 5*, showcase examples of host-switching within the mutualism. Host-switching has been shown to be ubiquitous and prevalent within the fig and fig wasp mutualism [Marussich and Machado, 2007; McLeish and van Noort, 2012; Satler et al., 2019; Su et al., 2022]. Host-switching has been proposed as a potential mechanism for speciation for both pollinators and their fig host [Satler et al., 2019]. Our data reaffirm that host switching is a prevalent mechanism within fig and fig wasp mutualism.

### 4.3 Community-Level Perspective (Multiple *Ficus* and Fig-Wasp Pollinator Species)

The relative proportions of unique versus shared-pollinator associations change when observed from local to broader geographic scales. Within our Panamanian BCNM fig and fig wasp community, only *F. crocata* has a single unique pollinator species. When the host-pollinator associations of *F. crocata* are recorded throughout its geographic range, the ratio of unique- versus shared-pollinator associations decreases. This was observed in multiple species within and out of BCNM, such as *F. petiolaris*, where the number of associated pollinators changes with geographic locality. Depending on geographic location, some species may have only one unique pollinator association. Despite ubiquitous pollinator sharing and host switching, most fig-wasp species exhibit relatively high host-species-specificity. For instance, we observed one-to-one pollinator-host interactions for *Pegoscapus sp.5* and *F. crocata*, as well as *Tetrapus sp. 4* and *F. insipida*. Both *Ficus crocata* and *F. insipida* are distributed over wide geographic species ranges, yet they each maintain an association with a unique pollinator species.

### 4.4 Challenges of Sampling

It is important to acknowledge some potential limitations of this study. Due to the geopolitics of Mexico and the biology of the fig and fig-wasp system, sampling high numbers of pollinators and *Ficus* individuals is extremely difficult. Thus, it is difficult to rule out accidental pollination by atypical fig wasp pollinators in our data set. This is especially true when our sample sizes are small (i.e.  $n=1$ ). Despite these challenges and potential pitfalls, this study still provides essential information on the dynamics of fig and fig wasp biology. Even if most of the samples in which  $n=1$  were the results of atypical pollinators, it shows that pollinator specificity, although still relatively high, is not perfect. The mistakes created by the wasps might be more common than previously acknowledged. Moreover, we hope that this investigation into the spatial dynamics of host-pollinator associations will motivate future research that will continue to elucidate patterns of ecological interactions in the fig and fig-wasp mutualism.

### 4.5 Conclusion

We present the first study to assess pollinator diversity and host-pollinator associations across the geographical range of multiple *Ficus* species using high-throughput sequence data. Our results confirm that associations between species of *Ficus* hosts and their wasp mutualists have changed over time and space. Thus, our work demonstrates that highly specific, one-to-one symbiotic interactions may often break down when faced with ecological or evolutionary changes. Host-switching and pollinator-sharing are also prevalent and ubiquitous throughout the fig and fig-wasp system. This, in turn, provides opportunities for introgression and gene flow between *Ficus* species, thus these results have important implications on the co-evolutionary trajectory of the fig and fig wasp mutualism.

## References

- Ahmed, S., S. G. Compton, R. K. Butlin, and P. M. Gilmartin, 2009. Wind-borne insects mediate directional pollen transfer between desert fig trees 160 kilometers apart. *Proceedings of the National Academy of Sciences* 106.
- Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. A. Lewis, E. U. Selker, W. A. Cresko, and E. A. Johnson, 2008. Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. *PLoS ONE* 3:e3376. URL <https://dx.plos.org/10.1371/journal.pone.0003376>.
- Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M. Lesin, a. S. P. Sergey I. Nikolenko, A. D. Prjibelski, A. V. Pyshkin, A. V. Sirotkin, N. Vyahhi, G. Tesler, M. A. Alekseyev, and P. A. Pevzner, 2012. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* 19:455–477.
- Bolger, A. M., M. Lohse, and B. Usadel, 2014. Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics* .
- Branstetter, M. G., J. T. Longino, P. S. Ward, and B. C. Faircloth, 2017. Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods in Ecology and Evolution* 8:768–776. URL <https://onlinelibrary.wiley.com/doi/abs/10.1111/2041-210X.12742>.
- Bronstein, J. L., R. Alacrón, and M. Geber, 2006. The evolution of plant–insect mutualisms. *New Phytologist* 172:412–428.
- Bruun-Lund, S., W. L. Clement, F. Kjellberg, and N. Rønsted, 2017. First plastid phylogenomic study reveals potential cyto-nuclear discordance in the evolutionary history of *Ficus* L. (Moraceae). *Molecular Phylogenetics and Evolution* 109:93–104. URL <https://www.sciencedirect.com/science/article/pii/S1055790316304602>.
- Capella-Gutierrez, S., J. M. Silla-Martinez, and T. Gabaldon, 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972–1973. URL <https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btp348>.
- Castresana, J., 2000. Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. *Molecular Biology and Evolution* 17:540–552. URL <https://doi.org/10.1093/oxfordjournals.molbev.a026334>.
- Chen, C. and Q. Song, 2008. Responses of the pollinating wasp *ceratosolen solmsi marchali* to odor variation between two floral stages of *figus hispida*. *Journal of Chemical Ecology* .
- Chen, Y., S. G. Compton, M. Liu, and X.-Y. Chen, 2012. Fig trees at the northern limit of their range: the distributions of cryptic pollinators indicate multiple glacial refugia: Glacial Refugia of Pollinating Fig Wasps. *Molecular Ecology* 21:1687–1701. URL <https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2012.05491.x>.
- Chen, Y., Z.-X. Jiang, S. G. Compton, M. Liu, and X.-Y. Chen, 2011. Genetic diversity and differentiation of the extremely dwarf *Ficus tikoua* in Southwestern China. *Biochemical Systematics and Ecology* 39:441–448. URL <https://linkinghub.elsevier.com/retrieve/pii/S030519781100127X>.
- Chifman, J. and L. Kubatko, 2014. Quartet Inference from SNP Data Under the Coalescent Model. *Bioinformatics* 30:3317–3324. URL <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4296144/>.
- Clark, M. A., N. A. Moran, P. Baumann, and J. J. Wernegreen, 2000. Cospeciation Between Bacterial Endosymbionts (*Buchnera*) And a Recent Radiation of Aphids (*Uroleucon*) And Pitfalls of Testing for Phylogenetic Congruence. *Evolution* 54:517–525. URL <https://onlinelibrary.wiley.com/doi/10.1111/j.0014-3820.2000.tb00054.x>.



- Cock, P. J. A., J. K. Bonfield, B. Chevreux, and H. Li, 2015. SAM/BAM format v1.5 extensions for de novo assemblies. preprint, Bioinformatics. URL <http://biorxiv.org/lookup/doi/10.1101/020024>.
- Compton, S. G., K. Grehan, and S. v. Noort, 2009. A Fig Crop Pollinated by Three or More Species of Agaonid Fig Wasps. *African Entomology* 17:215–222. URL <https://bioone.org/journals/african-entomology/volume-17/issue-2/003.017.0212/A-Fig-Crop-Pollinated-by-Three-or-More-Species-of/10.4001/003.017.0212.full>. Publisher: Entomological Society of Southern Africa.
- Cook, J. M. and J.-Y. Rasplus, 2003. Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology & Evolution* 18:241–248. URL <https://linkinghub.elsevier.com/retrieve/pii/S0169534703000624>.
- Cook, J. M. and S. T. Segar, 2010. Speciation in fig wasps. *Ecological Entomology* 35:54–66. URL <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2311.2009.01148.x>.
- Cook, J. M. and S. A. West, 2005. Figs and fig wasps. *Current Biology* 15:R978–R980. URL <https://www.sciencedirect.com/science/article/pii/S0960982205014661>.
- Cornille, A., J. G. Underhill, A. Cruaud, M. Hossaert-McKey, S. D. Johnson, K. A. Tolley, F. Kjellberg, S. v. Noort, and a. M. Proffit, 2012. Floral volatiles, pollinator sharing and diversification in the fig–wasp mutualism: insights from *Ficus natalensis*, and its two wasp pollinators (South Africa). *Proceedings of the Royal Society B Biological Sciences* 279:1731–1739.
- Cruaud, A., N. Rønsted, B. Chantarasuwan, L. S. Chou, W. L. Clement, A. Couloux, B. Cousins, G. Genson, R. D. Harrison, P. E. Hanson, M. Hossaert-McKey, R. Jabbour-Zahab, E. Jousset, C. Kerdelhué, F. Kjellberg, C. Lopez-Vaamonde, J. Peebles, Y.-Q. Peng, R. A. S. Pereira, T. Schramm, R. Ubaidillah, S. van Noort, G. D. Weiblen, D.-R. Yang, A. Yodpinyanee, R. Libeskind-Hadas, J. M. Cook, J.-Y. Rasplus, and V. Savolainen, 2012. An Extreme Case of Plant–Insect Codiversification: Figs and Fig-Pollinating Wasps. *Systematic Biology* 61:1029–1047. URL <https://academic.oup.com/sysbio/article-lookup/doi/10.1093/sysbio/sys068>.
- Darwell, C. T., S. al Beidh, and J. M. Cook, 2014. Molecular species delimitation of a symbiotic fig-pollinating wasp species complex reveals extreme deviation from reciprocal partner specificity. *BMC Evolutionary Biology* 14:189. URL <http://bmcevolbiol.biomedcentral.com/articles/10.1186/s12862-014-0189-9>.
- Demastes, J. W. and M. S. Hafner, 1993. Cospeciation of Pocket Gophers (*Geomys*) and Their Chewing Lice (*Geomydoecus*). *Journal of Mammalogy* 74:521–530. URL <https://www.jstor.org/stable/1382271>. Publisher: [American Society of Mammalogists, Oxford University Press].
- Doyle, J. and J. L. Doyle, 1987. Genomic plant DNA preparation from fresh tissue-CTAB method. *Phytochem Bull* 19:11–15.
- Eaton, D. A. R. and I. Overcast, 2020. ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics* 36:2592–2594. URL <https://doi.org/10.1093/bioinformatics/btz966>.
- Faircloth, B. C., 2013. Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming. <http://dx.doi.org/10.6079/J9ILL>.
- , 2016. PHYLUC is a software package for the analysis of conserved genomic loci. *Phylogenetics* 32:3.
- Faircloth, B. C., J. E. McCormack, N. C. Crawford, M. G. Harvey, R. T. Brumfield, and T. C. Glenn, 2012. Ultraconserved Elements Anchor Thousands of Genetic Markers Spanning Multiple Evolutionary Timescales. *Systematic Biology* 61:717–726.
- Futuyma, D. and M. Slatkin, 1983. *Coevolution*. Sinauer Associates, Sunderland, MA.

- Haine, E. R., J. Martin, and J. M. Cook, 2006. Deep mtDNA divergences indicate cryptic species in a fig-pollinating wasp. *BMC Evolutionary Biology* 6:83. URL <https://doi.org/10.1186/1471-2148-6-83>.
- Harrison, R. D. and J.-Y. Rasplus, 2006. Dispersal of fig pollinators in Asian tropical rain forests. *Journal of Tropical Ecology* 22:631–639. URL [https://www.cambridge.org/core/product/identifier/S0266467406003488/type/journal\\_article](https://www.cambridge.org/core/product/identifier/S0266467406003488/type/journal_article).
- Herre, E., N. Knowlton, U. Mueller, and S. Rehner, 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution* 14:49–53. URL <https://linkinghub.elsevier.com/retrieve/pii/S0169534798015298>.
- Jackson, A. P., C. A. Machado, N. Robbins, and E. A. Herre, 2008. Multi-locus phylogenetic analysis of neotropical figs does not support co-speciation with the pollinators: The importance of systematic scale in fig/wasp cophylogenetic studies. *Symbiosis* 45:16.
- Janzen, D. H., 1979. How to be a Fig. *Annual Review of Ecology and Systematics* 10:13–51.
- Kaartinen, R., G. N. Stone, J. Hearn, K. Lohse, and T. Roslin, 2010. Revealing secret liaisons: DNA barcoding changes our understanding of food webs. *Ecological Entomology* 35:623–638. URL <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2311.2010.01224.x>.
- Katoh, K. and D. M. Standley, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30:772–780.
- Leigh Jr, E., 2010. The evolution of mutualism. *Journal of Evolutionary Biology* 23:2507–2528.
- Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, and 1000 Genome Project Data Processing Subgroup, 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079. URL <https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btp352>.
- Lin, R.-C., C. K.-L. Yeung, J. J. Fong, H.-Y. Tzeng, and S.-H. Li, 2011. The Lack of Pollinator Specificity in a Dioecious Fig Tree: Sympatric Fig-pollinating Wasps of *Ficus septica* in Southern Taiwan: Sympatric Pollinators of *Ficus septica*. *Biotropica* 43:200–207. URL <https://onlinelibrary.wiley.com/doi/10.1111/j.1744-7429.2010.00686.x>.
- Lopez-Vaamonde, C., D. J. Dixon, J. M. Cook, and J.-Y. Rasplus, 2002. Revision of the Australian species of Pleistodontes (Hymenoptera: Agaonidae) fig-pollinating wasps and their host-plant associations. *Zoological Journal of the Linnean Society* 136:637–683. URL <https://doi.org/10.1046/j.1096-3642.2002.00040.x>.
- Machado, C. A., N. Robbins, M. T. P. Gilbert, and E. A. Herre, 2005. Critical review of host specificity and its coevolutionary implications in the fig/fig-wasp mutualism. *Proceedings of the National Academy of Sciences* 102:6558–6565. URL <https://pnas.org/doi/full/10.1073/pnas.0501840102>.
- Marussich, W. A. and C. A. Machado, 2007. Host-specificity and coevolution among pollinating and nonpollinating New World fig wasps: COPHYLOGENETIC ANALYSES AMONG FIG WASPS. *Molecular Ecology* 16:1925–1946. URL <https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2007.03278.x>.
- McLeish, M. J. and S. van Noort, 2012. Codivergence and multiple host species use by fig wasp populations of the *Ficus* pollination mutualism. *BMC Evolutionary Biology* 12:1. URL <https://doi.org/10.1186/1471-2148-12-1>.
- Michaloud, G., S. Carriere, and M. Kobbi, 1996. Exceptions to the One:One Relationship Between African Fig Trees and Their Fig Wasp Pollinators: Possible Evolutionary Scenarios. *Journal of Biogeography* 23:513–520. URL <https://www.jstor.org/stable/2845795>. Publisher: Wiley.

- Moe, A. M., D. R. Rossi, and G. D. Weiblen, 2011. Pollinator sharing in dioecious figs (Ficus: Moraceae): POLLINATOR SHARING IN FICUS. *Biological Journal of the Linnean Society* 103:546–558. URL <https://academic.oup.com/biolinnean/article-lookup/doi/10.1111/j.1095-8312.2011.01669.x>.
- Molbo, D., C. A. Machado, J. G. Sevenster, L. Keller, and E. A. Herre, 2003. Cryptic species of fig-pollinating wasps: Implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. *Proceedings of the National Academy of Sciences* 100:5867–5872. URL <http://www.pnas.org/cgi/doi/10.1073/pnas.0930903100>.
- Nason, J. D., E. A. Herre, and J. L. Hamrick, 1998. The Breeding Structure of a Tropical Keystone Plant Resource. *Nature* 391:685–687. URL <https://www.nature.com/articles/35607>. Bandiera\_abtest: a Cg\_type: Nature Research Journals Number: 6668 Primary\_atype: Research Publisher: Nature Publishing Group.
- Paradis, E. and K. Schliep, 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–528.
- R Core Team, 2020. R: A Language and Environment for Statistical Computing. URL <https://www.R-project.org/>.
- Ramírez B., W., 1969. Fig Wasps: Mechanism of Pollen Transfer. *Science* 163:580–581. URL <https://www.jstor.org/stable/1726295>. Publisher: American Association for the Advancement of Science.
- Renoult, J. P., F. Kjellberg, C. Grout, S. Santoni, and B. Khadari, 2009. Cyto-nuclear discordance in the phylogeny of Ficus section Galoglychia and host shifts in plant-pollinator associations. *BMC Evolutionary Biology* 9:248. URL <https://doi.org/10.1186/1471-2148-9-248>.
- Satler, J. D. and B. C. Carstens, 2017. Do ecological communities disperse across biogeographic barriers as a unit? *Molecular Ecology* 26:3533–3545. URL <https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14137>. \_eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/mec.14137>.
- Satler, J. D., E. A. Herre, T. A. Heath, C. A. Machado, A. G. Zúñiga, and J. D. Nason, 2022. Genome-wide sequence data show no evidence of hybridization and introgression among pollinator wasps associated with a community of Panamanian strangler figs. *Molecular Ecology*.
- Satler, J. D., E. A. Herre, K. C. Jandér, D. A. R. Eaton, C. A. Machado, T. A. Heath, and J. D. Nason, 2019. Inferring processes of coevolutionary diversification in a community of Panamanian strangler figs and associated pollinating wasps\*. *Evolution* 73:2295–2311. URL <https://onlinelibrary.wiley.com/doi/abs/10.1111/evo.13809>.
- Smith, M. A., E. S. Eveleigh, K. S. McCann, M. T. Merilo, P. C. McCarthy, and K. I. V. Rooyen, 2011. Barcoding a Quantified Food Web: Crypsis, Concepts, Ecology and Hypotheses. *PLOS ONE* 6:e14424. URL <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0014424>.
- Souto-Vilarós, D., M. Proffit, B. Buatois, M. Rindos, M. Sisol, T. Kuyaiva, B. Isua, J. Michalek, C. T. Darwell, M. Hossaert-McKey, G. D. Weiblen, V. Novotny, and S. T. Segar, 2018. Pollination along an elevational gradient mediated both by floral scent and pollinator compatibility in the fig and fig-wasp mutualism. *Journal of Ecology* 106:2256–2273. URL <https://onlinelibrary.wiley.com/doi/10.1111/1365-2745.12995>.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. URL <https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btu033>.
- Stamatakis, A., P. Hoover, and J. Rougemont, 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Systematic Biology* 57:758–771. URL <https://doi.org/10.1080/10635150802429642>.

- Su, Z.-H., H. Iino, K. Nakamura, A. Serrato, and K. Oyama, 2008. Breakdown of the one-to-one rule in Mexican fig-wasp associations inferred by molecular phylogenetic analysis. *Symbiosis* 45:2008:9.
- Su, Z.-H., A. Sasaki, J. Kusumi, P.-A. Chou, H.-Y. Tzeng, H.-Q. Li, and H. Yu, 2022. Pollinator sharing, copollination, and speciation by host shifting among six closely related dioecious fig species. *Communications Biology* 5:1–15. URL <https://www.nature.com/articles/s42003-022-03223-0>. Number: 1 Publisher: Nature Publishing Group.
- Swofford, D., 2003. PAUP\*: Phylogenetic analysis using parsimony (and other method)(Version 4.0 bl0) EM/OL] Sinauer Associates, Sunderland-Massachusetts.
- Thompson, J., 1994. The coevolutionary process. University of Chicago Press, Chicago, IL.
- Thrall, P. H., M. E. Hochberg, J. J. Burdon, and J. D. Bever, 2007. Coevolution of symbiotic mutualists and parasites in a community context. *Trends in Ecology & Evolution* 22:120–126. URL <https://www.sciencedirect.com/science/article/pii/S0169534706003739>.
- Wang, G., C. H. Cannon, and J. Chen, 2016. Pollinator sharing and gene flow among closely related sympatric dioecious fig taxa. *Proceedings of the Royal Society B* 283.
- Wang, G., S. G. Compton, and J. Chen, 2013. The mechanism of pollinator specificity between two sympatric fig varieties: a combination of olfactory signals and contact cues. *Annals of Botany* 111.
- Wang, G., X. Zhang, E. A. Herre, D. McKey, C. A. Machado, W.-B. Yu, C. H. Cannon, M. L. Arnold, R. A. S. Pereira, R. Ming, Y.-F. Liu, Y. Wang, D. Ma, and J. Chen, 2021. Genomic evidence of prevalent hybridization throughout the evolutionary history of the fig-wasp pollination mutualism. *Nature Communications* 12:718. URL <https://www.nature.com/articles/s41467-021-20957-3>. Number: 1 Publisher: Nature Publishing Group.
- Wang, R., B. Ai, B.-Q. Gao, S. Yu, Y.-Y. Li, and X.-Y. Chen, 2009. Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. var. *pumila* (Moraceae). *Population Ecology* 51:307–315. URL <http://doi.wiley.com/10.1007/s10144-008-0126-0>.
- Wei, Z. D., N. Kobmoo, A. Cruaud, and F. Kjellberg, 2014. Genetic structure and hybridization in the species group of *Ficus auriculata*: can closely related sympatric *Ficus* species retain their genetic identity while sharing pollinators? *Molecular Ecology* 23:3538–3550.
- Wheeler, W., 2010. Distinctions between optimal and expected support. *Cladistics* 26:657–663. Publisher: Wiley Online Library.
- Wilde, B. C., S. Rutherford, M. v. d. Merwe, M. L. Murray, and M. Rossetto, 2020. First example of hybridisation between two Australian figs (Moraceae). *Australian Systematic Botany* 33:436–445. URL <https://bioone.org/journals/australian-systematic-botany/volume-33/issue-5/SB19048/First-example-of-hybridisation-between-two-Australian-figs-Moraceae/10.1071/SB19048.full>. Publisher: CSIRO Publishing.
- Yu, H. and J. D. Nason, 2013. Nuclear and chloroplast DNA phylogeography of *Ficus hirta* : obligate pollination mutualism and constraints on range expansion in response to climate change. *New Phytologist* 197:276–289. URL <https://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2012.04383.x>.
- Yu, H., E. Tian, L. Zheng, X. Deng, Y. Cheng, L. Chen, W. Wu, W. Tanming, D. Zhang, S. G. Compton, and F. Kjellberg, 2019. Multiple parapatric pollinators have radiated across a continental fig tree displaying clinal genetic variation. *Molecular Ecology* 28:2391–2405. URL <https://onlinelibrary.wiley.com/doi/10.1111/mec.15046>.

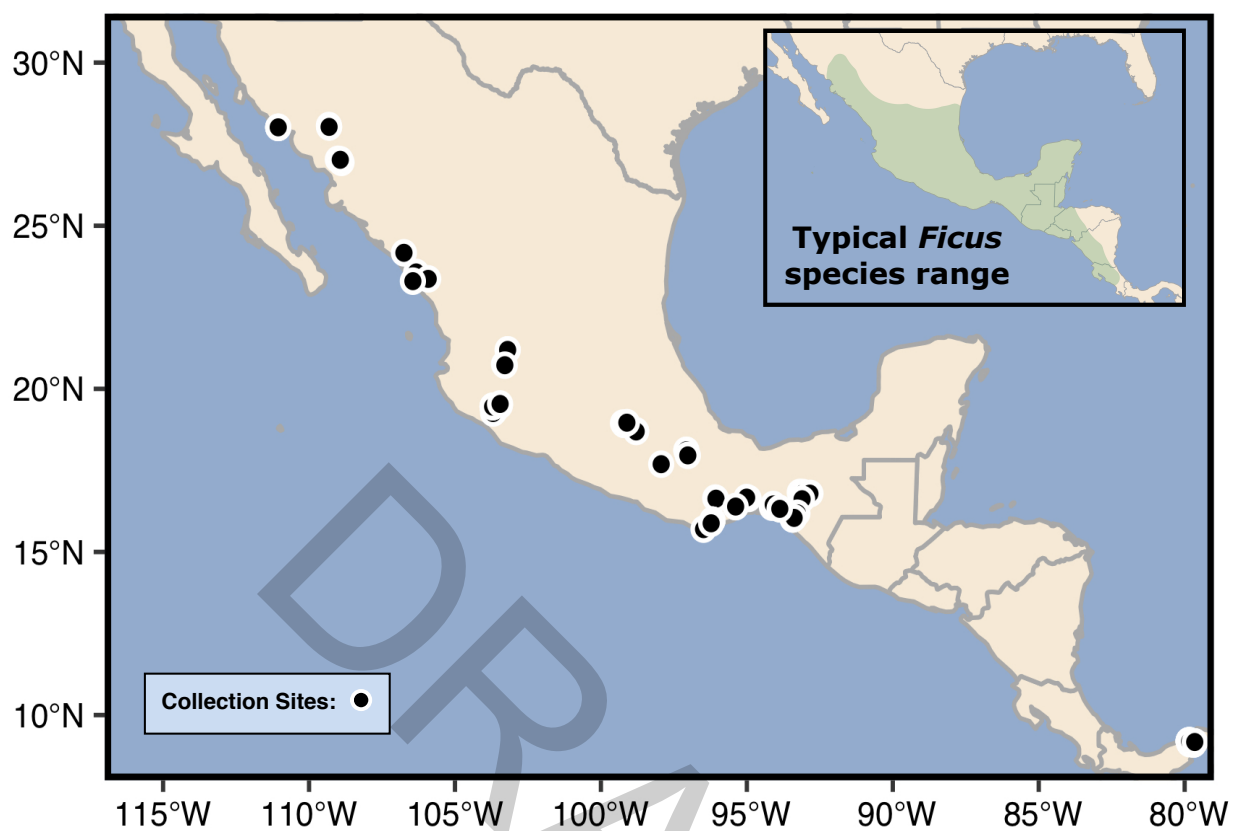


Figure 1: Collection map of *Ficus* individuals. The geographical distribution of an average fig are indicated by the green shading in the overlay map in the top right corner.

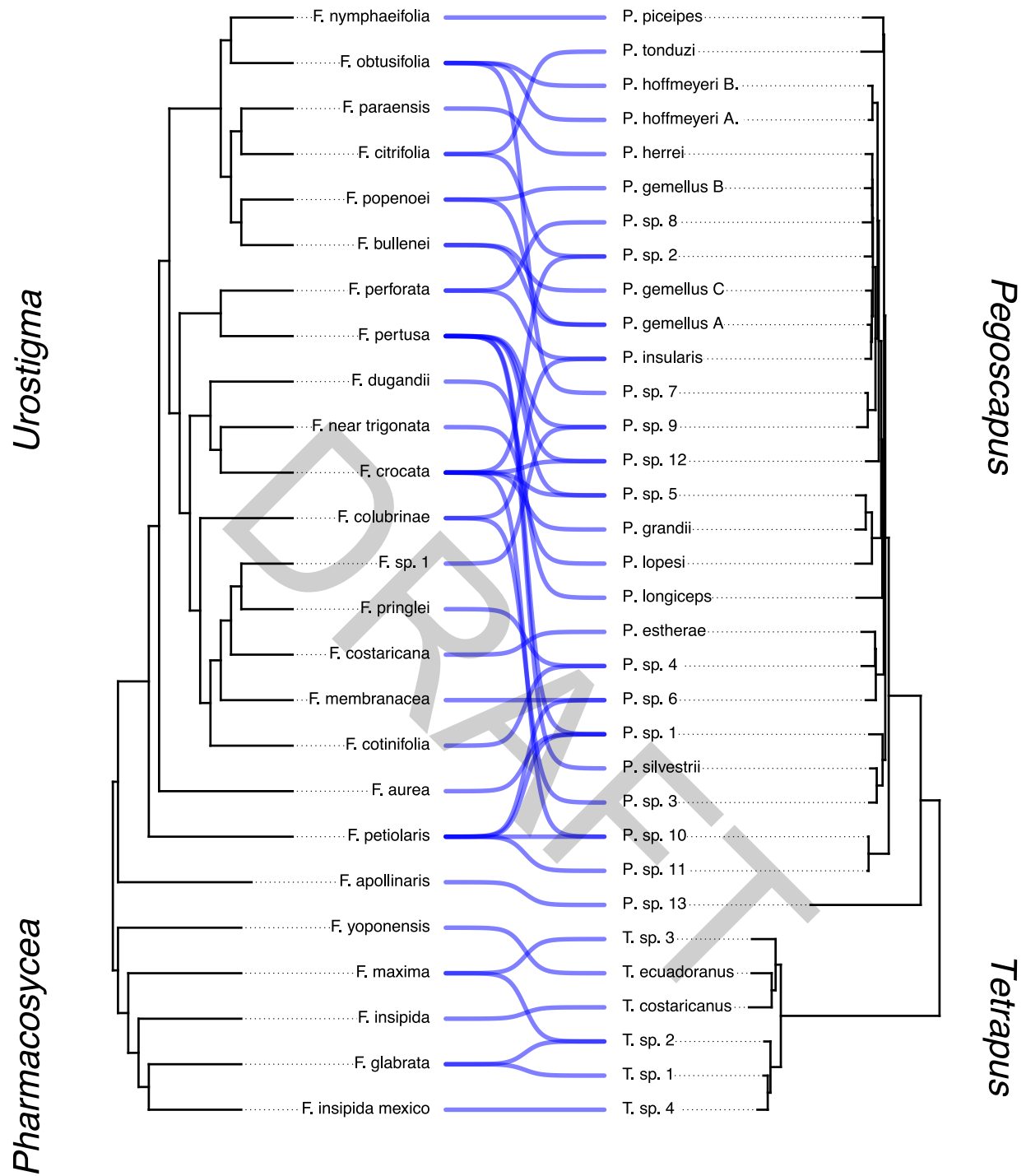


Figure 2: SVDquartets co-phylogeny of *Ficus* and fig-wasp pollinators. Twenty-five species of *Ficus* species were sampled along with thirty-three fig-wasp pollinators. The blue-line between *Ficus* species and fig-wasp species indicated a host-pollinator association.

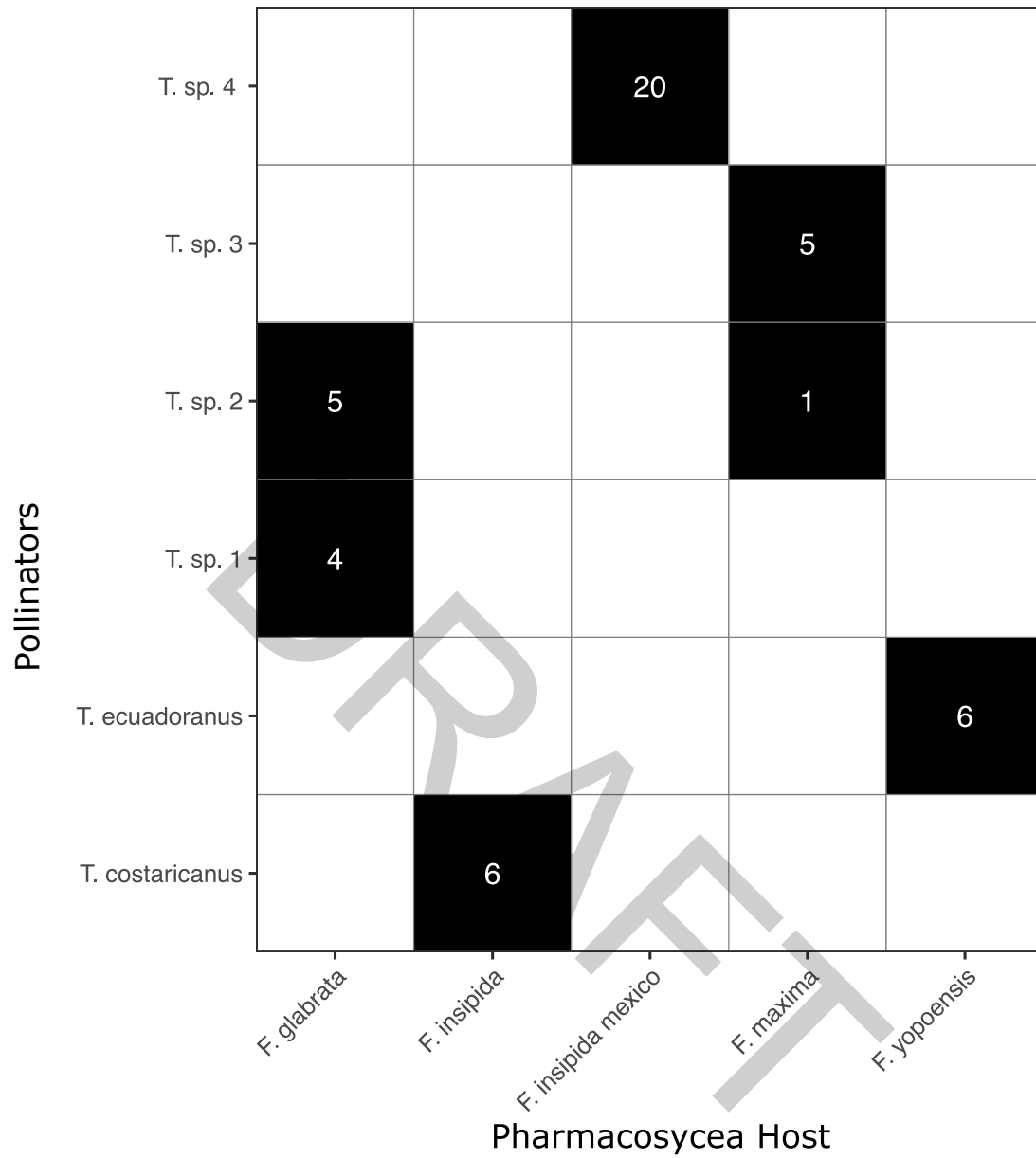


Figure 3: Host-pollinator associations for *Pharmacosycea* host and *tetrapus* pollinators. *Ficus* species are labeled on the columns and pollinators are labeled on the rows. Black squares indicate association between the *Ficus* species and fig-wasp pollinator. The count of pollinator samples are written in each square.

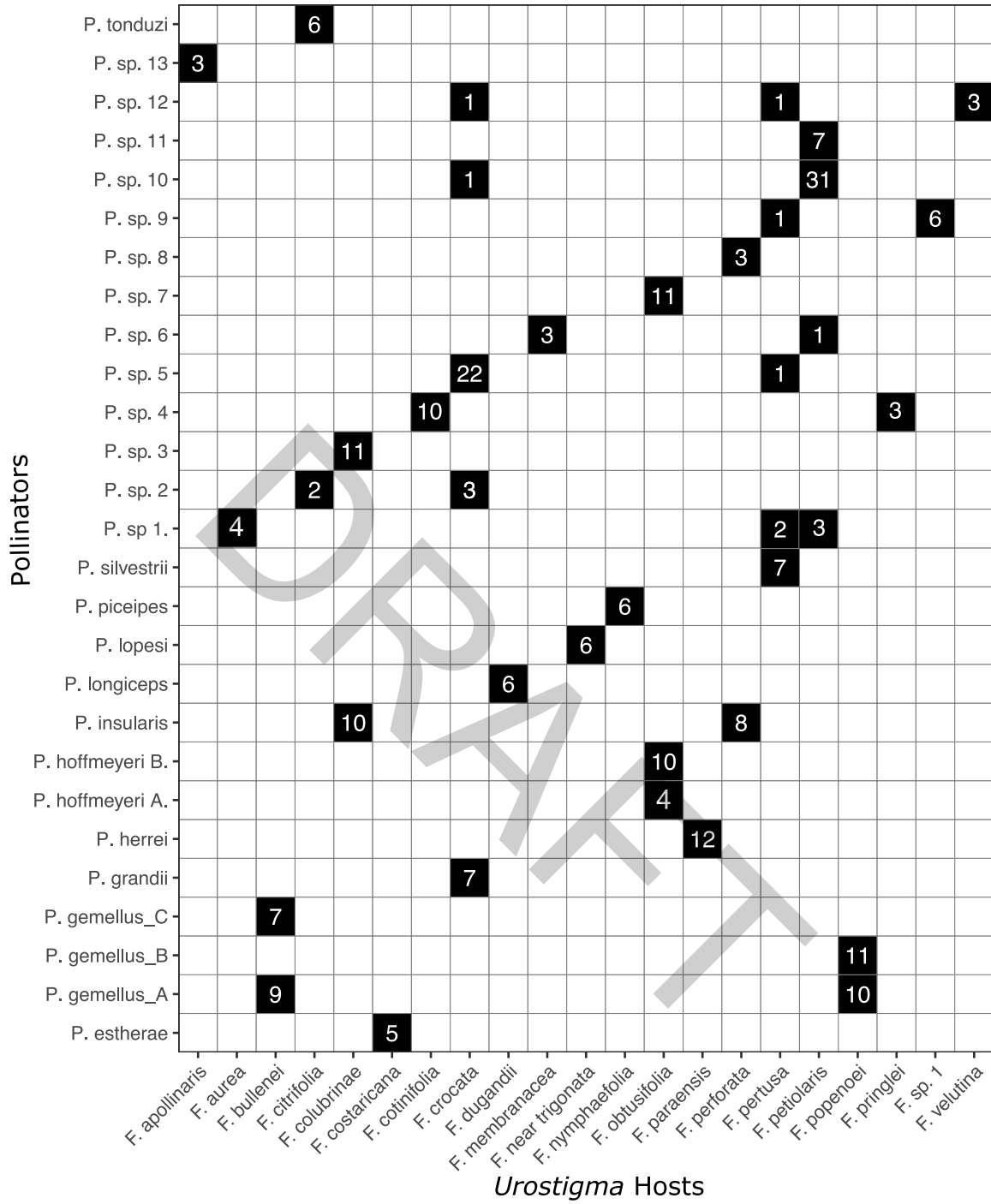


Figure 4: Host-pollinator associations for *Urostigma* host and *Pegoscapus* pollinators. *Ficus* species are labeled on the columns and pollinators are labeled on the rows. Black squares indicate association between the *Ficus* species and fig-wasp pollinator. The count of pollinator samples are written in each square. Note: Although, all *F. velutina* individuals samples sequenced poorly, and thus not included in our phylogenetic analysis, pollinators collected from these individuals are still included in our host-pollinator association table.



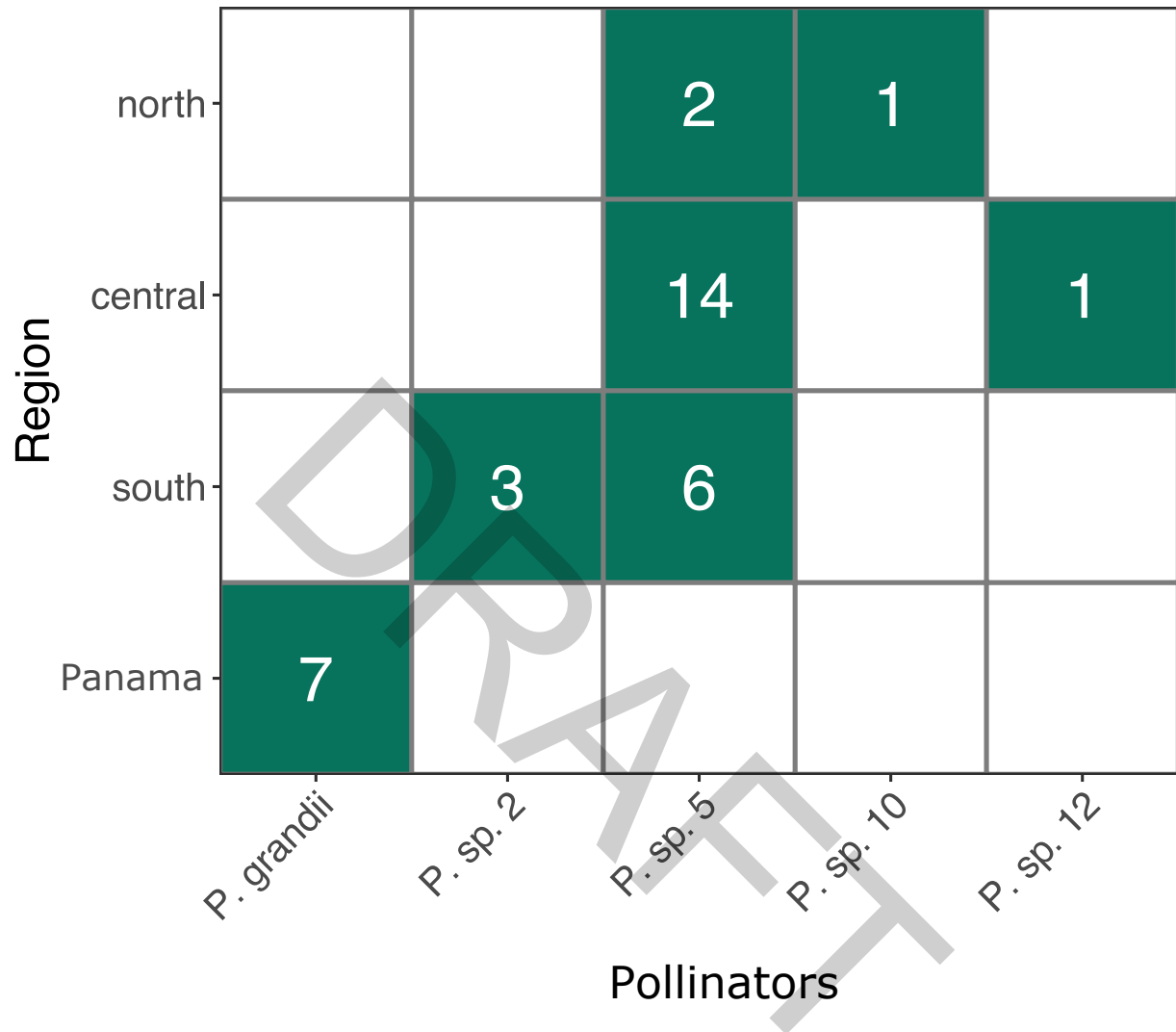


Figure 5: Pollinators of *F. crocata* arranged by their sampling region. A filled in square between region and pollinator species represent the region in which the pollinator species was sampled. The count of pollinator samples are written in each square. North represents the northern Mexican states of Sonora and Sinaloa. Central represents the central Mexican states of Colima and Jalisco. South represents the southern Mexican states of Morelos, Oaxaca and Chiapas.

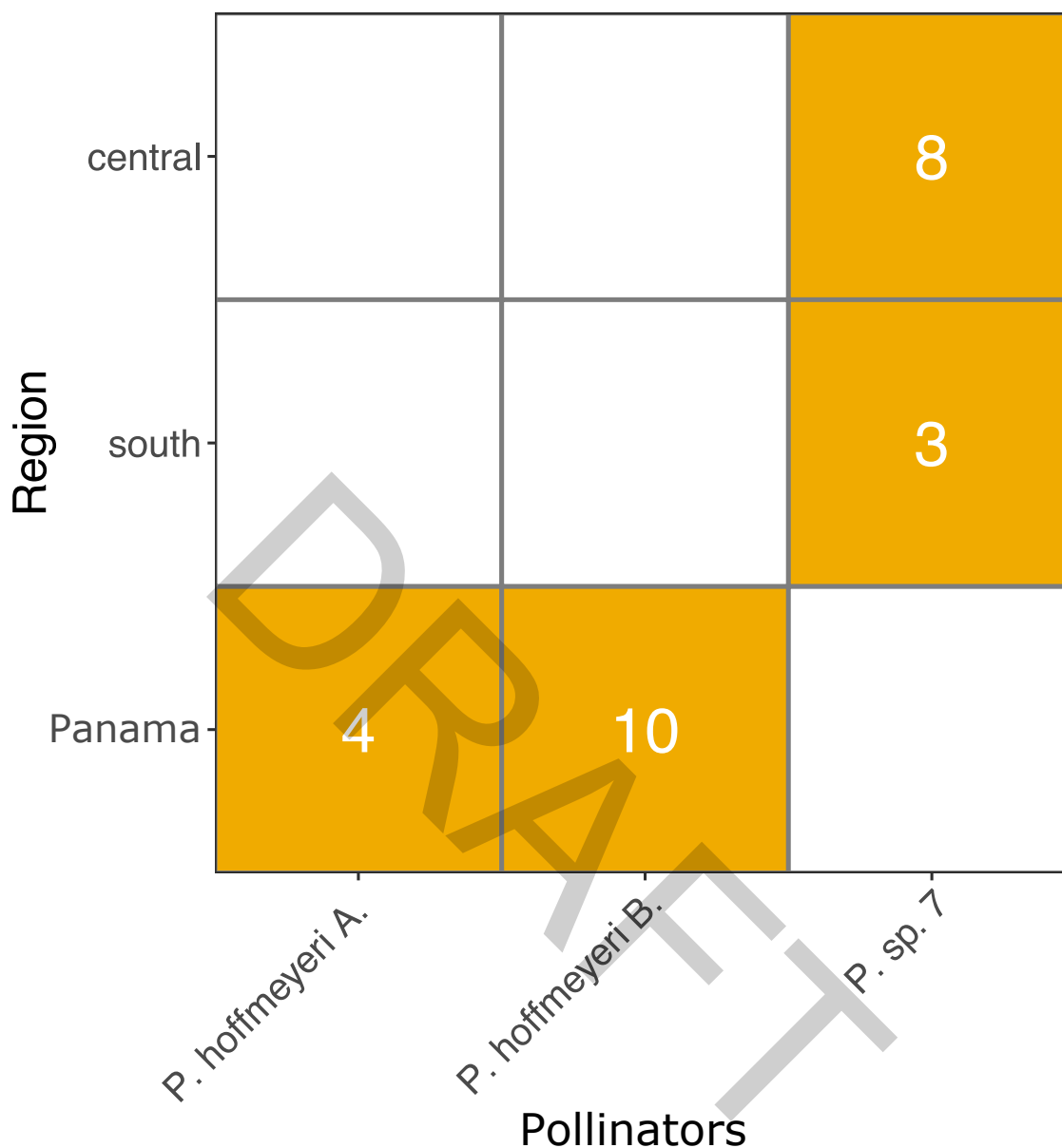


Figure 6: Pollinators of *F. obtusifolia* arranged by their sampling region. A filled in square between region and pollinator species represents the region in which the pollinator species was sampled. The count of pollinator samples are written in each square. North represents the northern Mexican states of Sonora and Sinaloa. Central represents the central Mexican states of Colima and Jalisco. South represents the southern Mexican states of Morelos, Oaxaca and Chiapas.

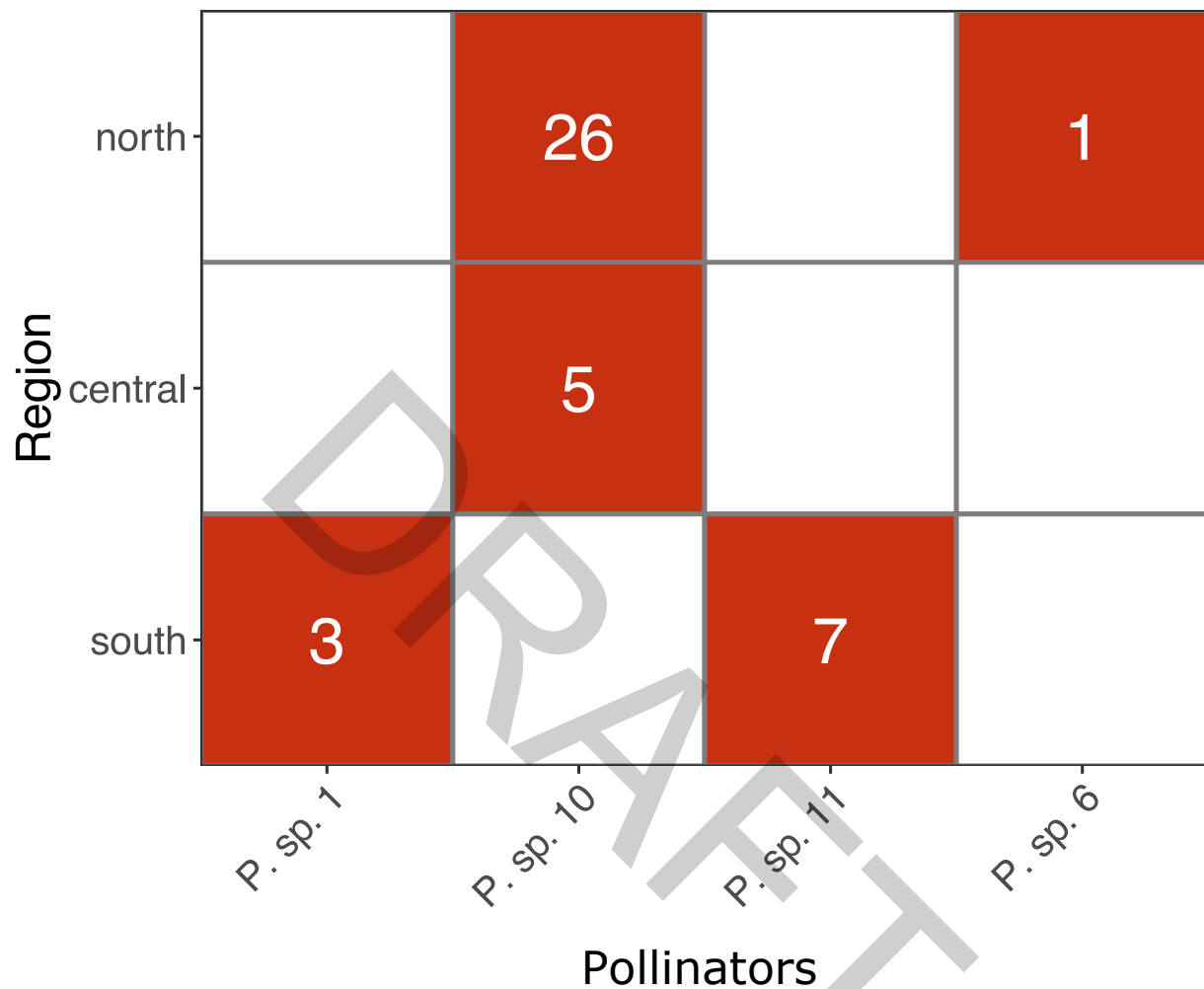


Figure 7: Pollinators of *F. petiolaris* arranged by their sampling region. A filled in square between region and pollinator species represents the region in which the pollinator species was sampled. The count of pollinator samples are written in each square. North represents the northern Mexican states of Baja, California, Sonora and Sinaloa. Central represents the central Mexican states of Zacatecas, Colima and Jalisco. South represents the southern Mexican states of Morelos, and Oaxaca.

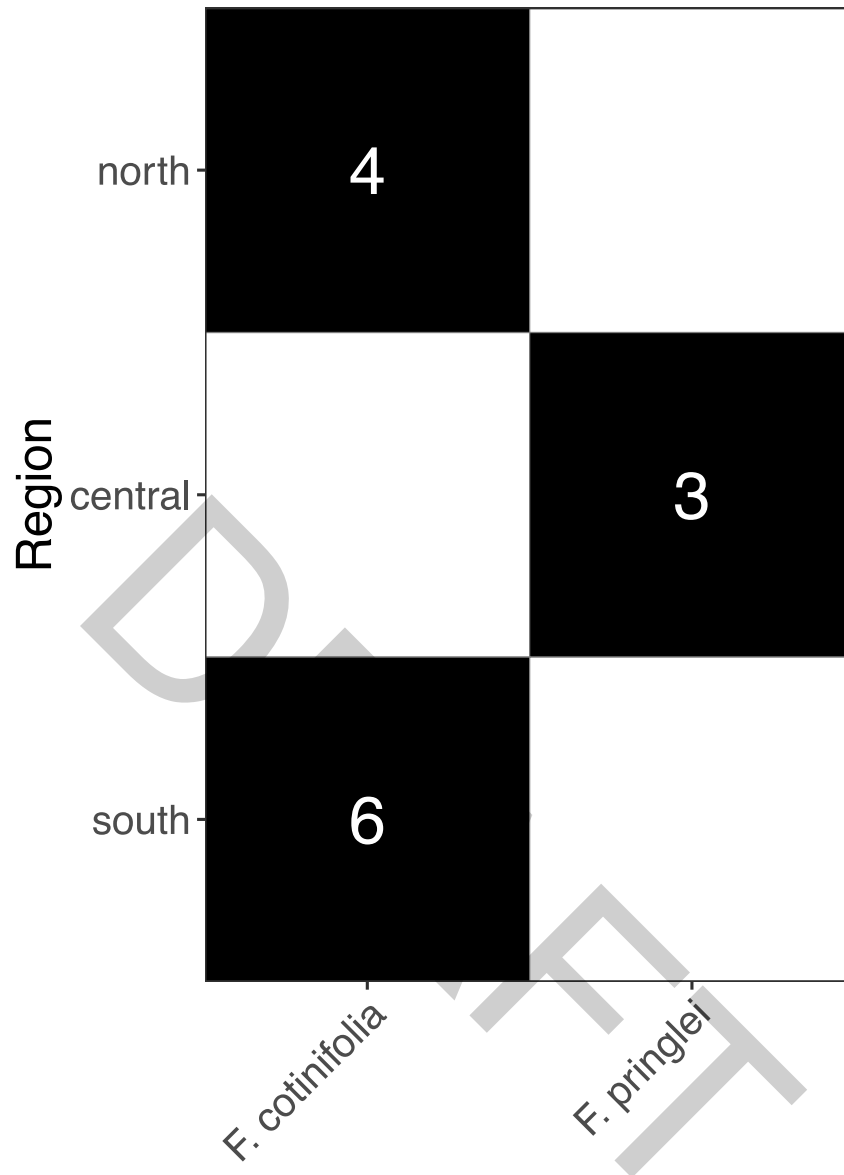


Figure 8: Fig hosts of *Pegoscapus sp.4* arranged by sampling region. A filled in square between region and fig species represents the region in which the fig species was sampled. The count of pollinator samples are written in each square. North represents the northern Mexican states of Sonora and Sinaloa. Central represents the central Mexican states of Colima and Jalisco. South represents the southern Mexican states of Morelos, Oaxaca and Chiapas.

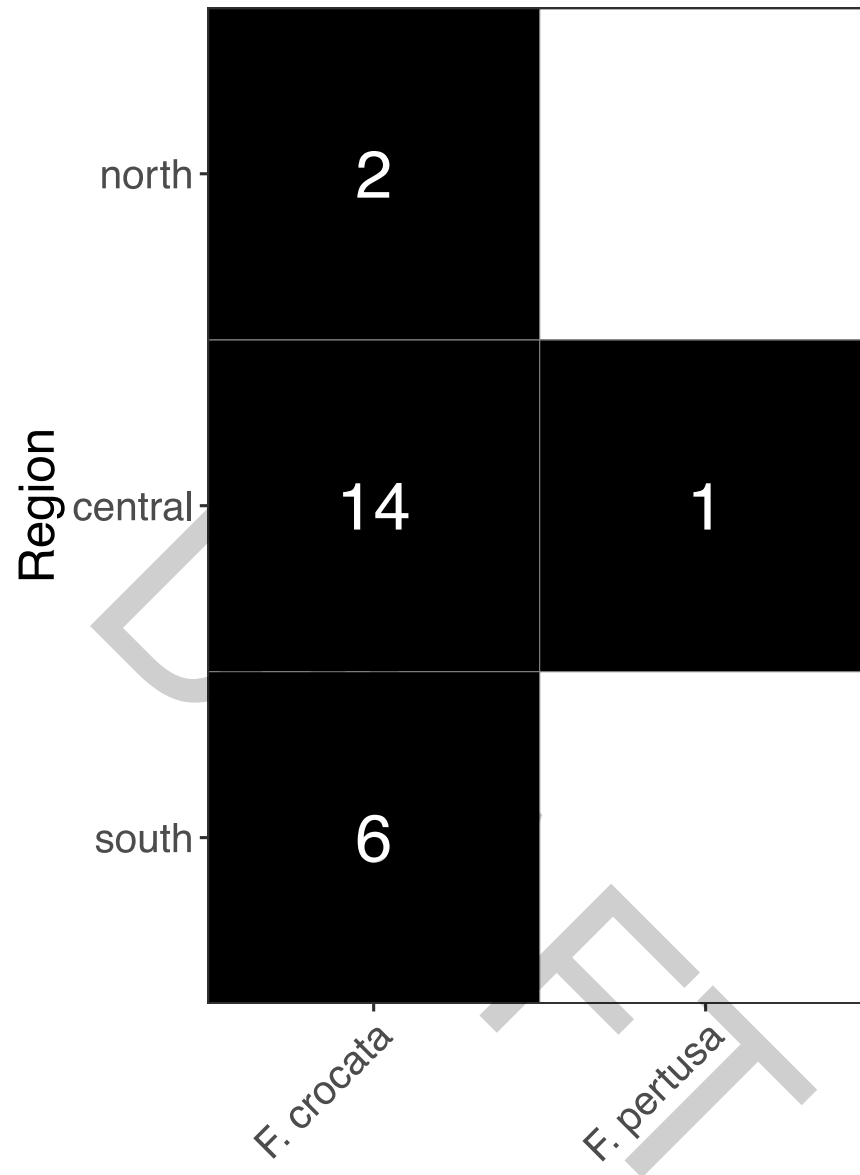


Figure 9: Fig hosts of *Pegoscapus sp.5* arranged by sampling region. A filled in square between region and fig species represents the region in which the fig species was sampled. The count of pollinator samples are written in each square. North represents the northern Mexican states of Sonora and Sinaloa. Central represents the central Mexican states of Colima and Jalisco. South represents the southern Mexican states of Morelos, Oaxaca and Chiapas.