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# Figs, pollinators, and parasites: A longitudinal study of the effects of nematode infection on fig wasp fitness

Justin Van Goor

*Iowa State University*, [jvangoor@iastate.edu](mailto:jvangoor@iastate.edu)

Finn Piatscheck

*Iowa State University*, [finnp@iastate.edu](mailto:finnp@iastate.edu)

Derek D. Houston

*Iowa State University*

John D. Nason

*Iowa State University*, [jnason@iastate.edu](mailto:jnason@iastate.edu)

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## Abstract

Mutualisms are interactions between two species in which the fitnesses of both symbionts benefit from the relationship. Although examples of mutualism are ubiquitous in nature, the ecology, evolution, and stability of mutualism has rarely been studied in the broader, multispecies community context in which they occur. The pollination mutualism between figs and fig wasps provides an excellent model system for investigating interactions between obligate mutualists and antagonists. Compared to the community of non-pollinating fig wasps that develop within figs inflorescences at the expense of fig seeds and pollinators, consequences of interactions between female pollinating wasps and their host-specialist nematode parasites is much less well understood. Here we focus on a tri-partite system comprised of a fig (*Ficus petiolaris*), pollinating wasp (*Pegoscapus* sp.), and nematode (*Parasitodiplogaster* sp.), investigating geographical variation in the incidence of attack and mechanisms through which nematodes may limit the fitness of their wasp hosts at successive life history stages. Observational data reveals that nematodes are ubiquitous across their host range in Baja California, Mexico; that the incidence of nematode infection varies across seasons within- and between locations, and that infected pollinators are sometimes associated with fitness declines through reduced offspring production. We find that moderate levels of infection (1-9 juvenile nematodes per host) are well tolerated by pollinator wasps whereas higher infection levels ( $\geq 10$  nematodes per host) are correlated with a significant reduction in wasp lifespan and dispersal success. This overexploitation, however, is estimated to occur in only 2.8% of wasps in each generation. The result that nematode infection appears to be largely benign – and the unexpected finding that nematodes frequently infect non-pollinating wasps – highlight gaps in our knowledge of pollinator-*Parasitodiplogaster* interactions and suggest previously unappreciated ways in which this nematode may influence fig and pollinator fitness, mutualism persistence, and non-pollinator community dynamics.

## Keywords

*Ficus*, *Pegoscapus*, *Parasitodiplogaster*, dispersal, longevity, antagonism

## Disciplines

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## Comments

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**Figs, pollinators, and parasites: a longitudinal study of the effects of nematode infection on  
fig wasp fitness**

**Justin Van Goor, Finn Piatscheck, Derek D. Houston, and John D. Nason\***

Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames,  
Iowa, 50011, USA

\*Corresponding Author.

Email Addresses: [jvangoor@iastate.edu](mailto:jvangoor@iastate.edu) (J. Van Goor), [finnp@iastate.edu](mailto:finnp@iastate.edu) (F. Piatscheck),  
[derek.d.houston@gmail.com](mailto:derek.d.houston@gmail.com) (D. Houston), [jnason@iastate.edu](mailto:jnason@iastate.edu) (J. Nason)\*

## Abstract

Mutualisms are interactions between two species in which the fitnesses of both symbionts benefit from the relationship. Although examples of mutualism are ubiquitous in nature, the ecology, evolution, and stability of mutualism has rarely been studied in the broader, multi-species community context in which they occur. The pollination mutualism between figs and fig wasps provides an excellent model system for investigating interactions between obligate mutualists and antagonists. Compared to the community of non-pollinating fig wasps that develop within figs inflorescences at the expense of fig seeds and pollinators, consequences of interactions between female pollinating wasps and their host-specialist nematode parasites is much less well understood. Here we focus on a tri-partite system comprised of a fig (*Ficus petiolaris*), pollinating wasp (*Pegoscapus* sp.), and nematode (*Parasitodiplogaster* sp.), investigating geographical variation in the incidence of attack and mechanisms through which nematodes may limit the fitness of their wasp hosts at successive life history stages.

Observational data reveals that nematodes are ubiquitous across their host range in Baja California, Mexico; that the incidence of nematode infection varies across seasons within- and between locations, and that infected pollinators are sometimes associated with fitness declines through reduced offspring production. We find that moderate levels of infection (1-9 juvenile nematodes per host) are well tolerated by pollinator wasps whereas higher infection levels ( $\geq 10$  nematodes per host) are correlated with a significant reduction in wasp lifespan and dispersal success. This overexploitation, however, is estimated to occur in only 2.8% of wasps in each generation. The result that nematode infection appears to be largely benign – and the unexpected finding that nematodes frequently infect non-pollinating wasps – highlight gaps in our knowledge of pollinator-*Parasitodiplogaster* interactions and suggest previously unappreciated

ways in which this nematode may influence fig and pollinator fitness, mutualism persistence, and non-pollinator community dynamics.

*Keywords: Ficus, Pegoscapus, Parasitodiplogaster, dispersal, longevity, antagonism*

## **1. Introduction**

Mutualistic interactions that benefit partner organisms are ubiquitous in nature and are associated with many ecological processes that underlie ecosystem function. Much is understood regarding the formation (Axelrod and Hamilton 1981, Leung and Poulin 2008) and regulation (Herre *et al* 1999, Lee 2015) of mutualistic associations over evolutionary time. Likewise, a broad range of mutualistic lifestyles, including context-dependent mutualisms and “cheater” partnerships, have been explored (Bronstein 2001, Holland *et al* 2004, Thompson and Fernandez 2006), and many theoretical (Soberon and Martinez del Rio 1985, Ferriere *et al* 2002) and empirical (Boucher *et al* 1982, Margulis and Fester 1991, Maynard Smith and Szathmary 1995, Heil and McKey 2003) studies of mutualism have focused on the fitness and stability of pairwise species interactions. Virtually all mutualistic species pairs, however, are members of more complex communities and networks of organismal interactions. This context too can be important for a clear understanding of the ecological and evolutionary dynamics of mutualistic systems (Herrera *et al* 2002, Thomson 2003).

Mutualisms are almost universally targets for exploiter species that benefit from the products of mutualistic interactions but do not offer any benefits in return (Bronstein 2001). Multiple species can antagonize a given mutualistic partnership through a variety of ecological roles, including predation, parasitism, or competition, and are likely to have profound effects on the stability of mutualistic partnerships. Over time, antagonists may coexist with their mutualist symbionts, destabilize mutualism through over-exploitation and drive the extinction of one or both mutualists, or enhance the stability of mutualism by aligning the fitness interests of mutualist partners. These concepts have been explored theoretically with contrasting results. Depending on model assumptions, antagonists have been found to influence mutualist and

mutualist-antagonist stability negatively in some cases (Ferriere *et al* 2002, Mougi and Kondoh 2014) or positively in others (Morris *et al* 2003, Jones *et al* 2009, Lee 2015). Similarly, models have found the effect of antagonism on mutualism fitness to be negative (Sakata 1994, Stanton *et al* 1999), positive (Klinkhamer and de Jong 1993, Maloof and Inouye 2000), or neutral (Arizmendi *et al* 1996, Bronstein 2001). In contrast to theoretical studies, empirical studies of exploiter effects on mutualism have been relatively few (West and Herre 1994, Thompson and Fernandez 2006, Althoff 2008, Mushegian and Ebert 2015, Duthie and Nason 2016). One impediment to investigating the effects of antagonism on mutualism fitness is that lifetime fitness in many systems is difficult to quantify (West *et al* 1996, Bronstein 2001). This impediment can be alleviated by focusing on model systems in which all strongly interacting species are known, ecological roles as mutualists and exploiters are well understood, and key components of lifetime fitness are easily estimated.

One such model system useful for addressing the effects of antagonists on the fitness of mutualists is the fig-fig wasp pollination-nursery mutualism. Figs (family Moraceae, genus *Ficus*) are represented by more than 750 species worldwide, with approximately 120 species in the New World (Berg 1989). Figs are important components of tropical and subtropical ecosystems because their aseasonal fruit production serves as keystone food resource for a diversity of animal consumers (Terborgh 1986, Lambert and Marshall 1991, Shanahan *et al* 2001). Figs are entirely reliant on typically host species-specific fig wasps (superfamily Chalcioidea, family Agaonidae) for the pollination of fig inflorescences, while the pollinator wasp larvae develop within a subset of the fig female ovules (Janzen 1979). This interaction is one of the most extensively examined and well-understood examples of mutualism, and has been the focus of many studies investigating ecological and evolutionary processes (Herre 1989,

Jousselin *et al* 2003, Molbo *et al* 2003, Jandér and Herre 2010, Cruaud *et al* 2012, McLeish and Van Noort 2012). Associated with each fig-pollinator species pair is a number of typically host-specific (but see Marussich and Machado 2007; Farrache current volume) non-pollinating wasps (superfamily Chalcidoidea, multiple families) that exploit the mutualism as parasites of developing wasps, ovule-gallers, and less frequently fig-wall gallers (Compton and Hawkins 1992, Weiblen 2002, Cook and Rasplus 2003, Borges 2015). Figs and their associates thus provide an excellent system for testing hypotheses concerning the influence of antagonists on mutualism fitness and stability.

To date, the vast majority of research investigating antagonist effects on the fig-fig pollinator mutualism has focused on non-pollinating fig wasps (West and Herre 1994, West *et al* 1996, Elias *et al* 2012, Duthie and Nason 2016). Equally ubiquitous, but much less studied, are entomopathogenic nematodes that parasitize fig pollinators (Martin *et al* 1973). Nematodes of the genus *Parasitodiplogaster* (family Diplogastridae) are pan tropical parasites that specialize on fig wasp pollinators. These nematodes have been investigated in terms of morphology and taxonomy (Poinar 1979, Poinar and Herre 1991, Giblin-Davis *et al* 2006, Kanzaki *et al* 2016), infection rates (Giblin-Davis *et al* 1995, Jauharlina *et al* 2012), and virulence evolution based on pollinator population dynamics (Herre 1993, Herre 1995). Research on Barro Colorado Island, Panama, indicates that *Parasitodiplogaster* infection reduces offspring production in most, but not all, fig pollinator species (Herre 1993, Herre 1995).

To gain a deeper understanding of the impacts of *Parasitodiplogaster* on the fitness and stability of the fig-pollinator mutualism, we investigate inter-site variation in nematode-pollinator interactions, the effects of non-pollinating figs wasps on these interactions, and the fitness impacts of nematode infection at all stages in the pollinator life cycle. Specifically, this



paper evaluates the mutualism consequences of inter-specific interactions between *Parasitodiplogaster* nematodes and wasp pollinators (genus *Pegoscapus*) associated with the Sonoran Desert rock fig, *Ficus petiolaris*. We determine how pollinator offspring production is influenced by nematode infection in the context of non-pollinator wasp antagonists across nine locations in Baja California, Mexico. We also analyze the effects of nematode infection on the longevity of female pollinator wasps that have exited their natal fig. Further, we investigate the impacts of nematode infection on the dispersal ability of pollinator wasps searching for new, receptive figs. The results of these analyses are considered with respect to the fitness of the *Pegoscapus* pollinator and, more generally, of its mutualism with *F. petiolaris*. Unexpectedly, we observed nematodes infecting male pollinators and males and females of several non-pollinator wasp species, the implications of which we also consider with respect to fig-fig wasp population dynamics and mutualism stability.

## **2. Materials and Methods**

### *2.1 Background: Figs, Fig Wasps, and Parasitodiplogaster Nematodes*

All *Ficus* are characterized by their production of a unique, nearly closed urn-shaped inflorescence commonly referred to as a fig. Neotropical *Ficus* are all monoecious and, depending on the species, their figs may contain tens to thousands of female and male flowers within the same enclosed inflorescence (Janzen 1979, Herre 1989). Figs containing receptive female flowers produce species-specific blends of volatiles (Chen *et al* 2009, Wang *et al* 2016) to attract pollen-bearing female pollinators, most of which are host-species specific. The

successful pollinator enters the fig through a small terminal pore (the ostiole), pollinates the female flowers, and lays her eggs in a subset of these flowers before dying inside the fig. The foundress wasp thus initiates seed development for the plant and larval development for her own offspring, which gall the seeds in which they occur (Janzen 1979). After approximately four to six weeks, seeds and larvae mature, and adult male wasps (unwinged) emerge from their galls to release and inseminate females. Females then collect pollen from male flowers while males chew an exit hole out of the fig. Prior to consumption of the mature fig by vertebrate frugivores (Shanahan *et al* 2001), females exit via this hole to seek out new receptive figs in which to reproduce. Female pollinators have short adult lifecycles (<60 hours; Kjellberg *et al* 1988, Dunn *et al* 2008) but excellent dispersal capabilities, employing wind currents to reach receptive, host-specific *Ficus* trees that are often located many kilometers from their natal trees (Nason *et al* 1998, Harrison and Rasplus 2006, Ahmed *et al* 2009).

Neotropical *Ficus* are subject to exploitation by a diversity of non-pollinating fig wasp genera (Bouček 1993, West *et al* 1996). Each fig typically supports at least one and often several non-pollinator species, most of which, like the pollinator, are host specific. The majority of non-pollinators are attracted to receptive figs by the same volatile blends produced to attract pollinators (Proffitt *et al* 2007). They have also evolved life history characteristics similar to pollinating wasps in order to utilize resources within the developing fig, and to successfully time their emergence, mating, and departure from the mature fig. In contrast to the pollinator, which oviposits from inside the fig, all Neotropical (and most Old World) non-pollinators oviposit from the outside by inserting their ovipositors through the fig wall. Depending upon the species, non-pollinators parasitize developing ovules, pollinators, or non-pollinators, or induce galls within the fig wall. Thus, the non-pollinators may parasitize or be in direct or indirect competition for

important components of the fig-pollinator mutualism (West and Herre 1994, Weiblen 2002, Jansen-González *et al.* 2014, Borges 2015).

The life history of *Parasitodiplogaster* nematodes is tightly coupled with that of their fig pollinator hosts, which they rely upon for energy, transport to a new fig, and reproductive success. These nematodes are internal parasites that enter receptive figs inside the body of their host wasp, consume host tissue, mate (Figure 1), and then disperse throughout the fig to reproduce (Giblin-Davis *et al.* 1995, Kanzaki *et al.* 2014, Ramirez-Benavides and Salazar-Figueroa 2015). Nematode development is synchronized with fig and wasp development. Infective juvenile-stage nematodes are waiting inside the fig when pollinator females emerge from their galls. These juvenile nematodes perform nictation behavior until they contact a host, which they then quickly enter through openings in the thoracic or abdominal cavities (Poinar and Herre 1991). *Parasitodiplogaster* nematodes require transport to a new fig in each generation, and it is thus necessary that their impacts on female pollinator wasp survival are not so great as to prohibit her from successfully dispersing to trees bearing receptive stage figs. Despite this constraint, the virulence of nematode infection varies across species as a function of host-wasp population density (Herre 1993) and can range from avirulent or commensal (Herre 1995, Ramirez-Benavides and Salazar-Figueroa 2015) to virulent (Herre 1995), though not so virulent as to prohibit the host from successfully dispersing to a receptive fig and producing the next generation of hosts.

## 2.2 Study Community: The *Ficus petiolaris* System of Northwestern Mexico

*Ficus petiolaris* (subgenus *Urostigma*, section *Americana*) is a monoecious rock-strangling fig species that is widespread in Sonoran Desert habitats of Baja California and mainland Mexico. The nine census sites used in this study are located in the states of Baja California and Baja California Sur, where *F. petiolaris* is the only native fig species. *Ficus petiolaris* is obligately pollinated by an unclassified *Pegoscapus* wasp, which appears to be a single species based on sequencing of the mitochondrial gene cytochrome oxidase I (COI; Su *et al* 2008) and 2500 ultraconserved element (UCE) loci (J. Satler unpublished data). Because this pollinator dies within the fig in which it lays its eggs, the number of foundresses contributing to the brood of offspring within a fig can be determined by count of foundress corpses.

*Ficus petiolaris* is also host to seven chalcidoid non-pollinator wasp species, all of which are found throughout the range of *F. petiolaris* in Baja California. This non-pollinator wasp community is comprised of three species of *Idarnes* (Family Agaonidae, subfamily Sycophaginae [placement within the Agaonidae disputed, Munro *et al* 2011, Cruaud *et al* 2011]); one from species group *flavicollis* (ovule-gallers) and two from species group *carme* (cleptoparasites or parasitoids) (Elias *et al* 2012, Jansen-González *et al* 2014). Additionally, there are two species of *Heterandrium* (family Pteromalidae), both of which parasitize fig ovules (Cardona *et al* 2013). These five species are the most commonly observed non-pollinator wasps associated with *F. petiolaris* and as ovule-gallers, cleptoparasites, or seed parasites either compete directly with *Pegoscapus* pollinators for reproductive resources or utilize them for their own development (Cardona *et al* 2013, Duthie *et al* 2015). *Ficus petiolaris* is also host to one species of *Ficicola* (family Pteromalidae) that generates large galls protruding from the receptacle into the interior of the fig and which may spatially impact developing seeds or larvae.

One species of *Physothorax* (family Torymidae) is a parasitoid that develops within *Ficicola* larvae.

The *Pegoscapus* pollinator associated with *F. petiolaris* is subject to parasitism by a single species of *Parasitodiplogaster* nematode (family Diplogastridae), whose 28S rDNA sequences form a single, well-supported clade that clusters with other publicly available Neotropical *Parasitodiplogaster* sequences (Supplementary Figure 1). No other fig-associated nematode genera (*Schistonchus*, *Pristionchus*, *Ficophagus*; Vovlas and Larizza 1996, Susoy *et al* 2016, Davies *et al* 2017) have been observed in *F. petiolaris* figs. As adult wasp hosts emerge from their galls, they may be infected by infective-stage juvenile nematodes, which molt into consumptive juveniles once wasps have arrived at a receptive fig, molt again into adults after wasp hosts have died (though sometimes before), and then mate before dispersing through the fig to reproduce (Giblin-Davis 1995, Van Goor personal observation, see Figure 1).

### 2.3 Study Sites and Seasons

*Ficus petiolaris* trees were geo-referenced at nine sites along a latitudinal gradient spanning 741 km of the Baja California peninsula (Table 1, Figure 2) in Mexico. Several of these sites were also investigated by Gates and Nason (2012), Duthie *et al* (2015), Duthie and Nason (2016), and Piatscheck *et al* (current volume). Mature figs were sampled from trees at all nine sites in order to quantify the numbers of pollinating and non-pollinating wasps produced per fig, as well as the incidence of nematode infestation of individual figs. These study sites were visited at four time points (November-December 2012, May-July 2013, November-December 2013, and May-July 2014) to ensure adequate sample sizes of wasp producing figs in both wet (October-

December) and dry (May-July) seasons. Wasps reared from individual mature figs were preserved in 95% ethanol and transported to Iowa State University, where counts of pollinating and non-pollinating wasp species male and female offspring were obtained.

#### *2.4 Geographical and Seasonal Variation in Host Availability and Nematode Infection*

Before evaluating the effect of nematode infection on the offspring production, longevity, and dispersal ability of pollinating wasps, we characterized geographical and seasonal variation in pollinating wasp population structure and nematode infection dynamics. Pollinator foundress number is positively associated with the likelihood of nematode infection within a fig and the number of host lineages available to parasites, both of which are factors influencing the evolution of nematode virulence (Herre 1993, 1995). Geographical variation in *Parasitodiplogaster* infection dynamics, however, has only rarely been studied (Giblin-Davis *et al* 1995, Jauharlina *et al* 2012). In the *F. petiolaris* system, we evaluate the how host foundress counts are influenced by variation in study site and sample season (wet versus dry). Subsequently, we investigate how the incidence of nematode infection is influenced by these predictors as well as variation in foundress counts.

At each study site, mature figs were collected, partially cut open, and placed in plastic vials to allow time for wasp emergence. After 12-24 hours, the figs were removed from the vials and presence or absence of juvenile nematodes within the fig (infestation) was determined by light microscopy. Because pollinators die within the fig after laying eggs, we were also able to count the number of foundress wasps per fig. The *foundress count per fig* was analyzed using a Generalized Linear Mixed Model (GLMM) with Poisson errors and a log-link function with *site*,

*season*, *tree*, *site by season*, and *tree by season* interaction terms as categorical predictor variables, and *latitude* as a continuous predictor variable. The predictor variable *tree* was treated as a random effect because of the over dispersion of foundress counts observed between trees within sites. We included in the model the *tree by season* interaction term (a random effect) because some of the same trees were sampled across seasonal trips and *site by season* to evaluate the consistency of site effects on foundress counts across seasons. Unless otherwise noted, all analyses were performed using JMP® Pro 12 (SAS Institute Inc., Cary, CN, 1989-2007), which does not report *p*-values for random effect variables or associated interaction terms.

Additionally, we used a GLMM with binomial errors and a logit link function (logistic regression) to investigate the relationship between the odds of *nematode infestation per fig* (presence/absence) as a function of main effects *tree*, *site*, *season*, *tree by season*, *site by season*, *latitude*, and *foundress count per fig*. The predictor variable *tree* was treated as a random effect because of the over dispersion of infestation levels observed between trees within sites. We included in the model the *tree by season* interaction term (a random effect) because some of the same trees were sampled across seasonal trips and *site by season* to evaluate the consistency of site effects on nematode infestation across seasons.

## 2.5 Nematode Impacts on Pollinator Offspring Production in the Context of Non-Pollinator Wasps

*Parasitodiplogaster* infection can negatively influence offspring production in pollinators, particularly in systems characterized by high mean foundress counts per fig (Herre 1993, 1995). In evaluating nematode impacts on host fitness, previous studies have not, however,

accounted for potential impacts of non-pollinating fig wasps on pollinator production. The pollinator and non-pollinator offspring counts and the incidence of nematode infection were obtained for a subset of the mature fig collection described above (see Section 2.3). Specifically, to investigate the effects of nematode infection on the offspring production of *individual* pollinators while accounting for non-pollinators, only single foundress figs were used. For this analysis, we used a GLMM with Poisson errors and a log link function with *pollinator offspring per fig* as the response variable and predictor variables *tree*, *site*, *season*, *tree by season*, *site by season*, *fig volume (mm<sup>3</sup>)*, *nematode infestation* (presence or absence), *site by nematode infestation*, *season by nematode infestation*, and the *number of non-pollinator offspring* produced by each non-pollinating wasp species (*Idarnes* species 1-3, *Heterandrium* species 1 and 2, *Ficicola*, and *Physothorax*). As above, *tree* and *tree by season* were treated as random effects. The *site by nematode infestation* and *season by nematode infestation* interaction effects were included to evaluate the consistency of nematode infection across geographic space and time. If *site by nematode infestation* effects were found to be significant, we then conducted site-specific GLMM analyses as above, but excluded *site*-associated effects.

## 2.6 Nematode Infection Effects on Pollinator Longevity

*Parasitodiplogaster* infection has been shown to limit the reproductive success of pollinating fig wasps (Herre 1993, 1995) and has also been predicted to negatively affect host longevity (Herre 1995, Giblin-Davis *et al* 1995), though this latter hypothesis has yet to be tested. We tested this hypothesis in our study system by conducting a series of controlled longevity trials using female pollinator wasps reared from mature *F. petiolaris* figs collected



from Site 96 (for location see Figure 2 and Table 1). A total of three replicate longevity trials were conducted in July 2013, December 2014, and August 2016. In the first two trials (July 2013 and December 2014), the figs were partially cut open placed in plastic vials with breathable mesh tops. During the third longevity trial (August 2016), to more accurately simulate ‘natural’ wasp emergence conditions, figs were placed in similar plastic vials with breathable mesh tops, but were not cut open. In all three trials, wasps were allowed to emerge from the figs for 24 hours before the fig was removed and examined for the presence or absence of nematodes. Figs were selected for inclusion in the longevity trials based on adequate wasp emergence (at least 50 individuals observed in the vial after the 24 hour period) and to provide a relatively equal numbers of nematode infested and uninfested figs. After figs were removed, cotton balls dipped in 10% sugar solution were placed on the top of the vials to provide potential nourishment to wasps and to prevent their desiccation.

Beginning at hour 24, each vial was censused every 12 hours and dead wasps removed and preserved in 95% ethanol until no wasps remained. A survival analysis was conducted to evaluate the longevity of pollinator wasps from infested and uninfested figs, with differences in survivorship curves analyzed using a log-rank test (Schoenfeld 1981). Further, the abdominal and thoracic cavities of wasps from nematode infested figs were dissected using 0.25mm diameter tungsten needles (Fine Science Tools®) to determine the presence and number of juvenile nematodes within each pollinating wasp. A Generalized Linear Model (GLM) with Poisson errors and a log-link function was used to analyze the relationship between the *number of nematodes extracted per wasp host* and effects of the *longevity study, hour* in which that host died (a measure of host longevity) and the *individual fig* in which the wasp originated. A similar

ANOVA was used to analyze the *hour* in which the wasp host died as a function of predictor variables *longevity study*, *individual fig*, and the *number of nematodes extracted per wasp host*.

### *2.7 Nematode Effects on Pollinator Dispersal Ability*

To estimate the effect of nematode infection on host dispersal ability, the numbers of nematodes infecting pollinating wasps that emerged in the controlled longevity trial was compared to the number of nematodes observed infecting pollinators that successfully dispersed to reach receptive figs. If nematode infection decreases the likelihood of successful host dispersal, then we predict that successfully dispersing wasps will contain fewer nematodes than wasps emerging from nematode infested figs. Successfully dispersed pollinators were sampled from single foundress, interfloral phase figs. Pollinators entering receptive figs may contain juvenile nematodes, but it is not until days later, during the early interfloral stage of fig development, that the nematodes molt into adults, and emerge from the host to mate, at which time they are easily counted (J. Van Goor personal observation). We compared these counts of nematodes from successfully dispersed infected wasps against the counts of nematodes found in infected hosts from the controlled longevity trials (Section 2.6) to detect differences in infection rate. This two-sample test was conducted using the `poisson.test` function in R (R Core Team 2014).

## **3. Results**

### *3.1 Geographical and Seasonal Variation in Host Availability and Nematode Infection*

The four field collections conducted from 2012 to 2014 yielded a total of 2077 mature, wasp producing figs. Although most study sites yielded mature figs for all four collections, in a few cases mature figs could not be located at the time of collection. Excluding Site 250 (which was sampled only once), we obtained an average of 248.1 figs (range 157-351) per study site, with a mean foundress count per fig of 1.437 (range 1.08-1.938). Our GLMM model ( $n = 2077$ ,  $df = 4$ , Chi-Square = 38.114) found *foundress count per fig* to vary significantly in response to *site* ( $p = 0.013$ ), *site by season*, and *latitude* (both  $p < 0.001$ ), but not *season* ( $p = 0.538$ ). Despite the nonsignificant difference between wet and dry seasons, foundress counts varied substantially within geographic locations over time (Figure 3A).

Over all collections, nematode infestation was observed in 39% of all sampled figs and ranged from 12-80% depending on the site and collection trip. As per foundress counts, our GLMM model ( $n = 2077$ ,  $df = 17$ , Chi-Square = 262.995) of the *nematode infestation per fig* was significantly associated with *site*, *season*, and *site by season* interaction (all  $p < 0.001$ ), was higher in wet seasons than in dry (Figure 3B), and negatively associated with *latitude* ( $p < 0.001$ ). Nematode infection was also positively associated with *foundress count per fig* ( $p < 0.001$ ). Interestingly, the *Parasitodiplogaster* nematodes associated with *F. petiolaris* were also frequently observed infecting pollinator males as well as non-pollinator male and female wasps. This was unexpected because only pollinator females enter receptive figs where nematode offspring have access to the next generation of hosts. The ecological and evolutionary implications of this variation in host specificity are considered in the Discussion section.

### 3.2 Nematode Impacts on Pollinator Offspring Production in the Context of Non-Pollinator Wasps

Overall, we observed an average of 38.9 pollinator and 53.9 total non-pollinator offspring produced per each of the 2077 mature figs collected from 2012-2014. Of these, 1379 were single foundress figs and included in the GLMM analysis of factors affecting pollinator offspring production. The number of single foundress figs averaged 153.22 per site (range 26-230) and individual pollinators produced an average of 36.34 offspring per fig. The mean numbers of pollinators and non-pollinators produced per fig varied substantially across sites (Figure 4).

If all other predictor variables are ignored, pollinator offspring production appears to be reduced by 21% in nematode infested relative to uninfested figs (Figure 5), a difference that largely disappears when other sources of variation are taken into account. Indeed, in the GLMM model ( $n = 1379$ ,  $df = 27$ , Chi-Square = 756.472), *pollinator offspring per fig* was not significantly reduced by *nematode infestation* ( $p = 0.224$ ), though *site* and *fig volume* ( $mm^3$ ) were both highly significant ( $p < 0.001$ ). The effect of *season* was also found to be highly significant ( $p < 0.001$ ) with pollinator offspring production per fig found to be 18% higher in wet seasons than in dry. The interaction terms *site by season* and *season by nematode infestation* were found to be non-significant and were thus removed from the analysis above. Interestingly, however, the interaction term *site by nematode infestation* was found to be highly significant ( $p < 0.001$ ). Also, while most non-pollinator wasp species were found to negatively impact pollinator offspring production, not all species impacted production significantly. *Idarnes* species 1 (*Flavicollis*) ( $p = 0.022$ ), *Idarnes* species 3 (*Carme*) ( $p < 0.001$ ), *Heterandrium* species 1 ( $p < 0.001$ ), and *Physothorax* ( $p = 0.032$ ) were found to be significantly associated with reduced

pollinator offspring production whereas *Idarnes* species 2 (*Carme*) ( $p = 0.080$ ), *Heterandrium* species 2 ( $p = 0.290$ ), and *Ficicola* ( $p = 0.962$ ) were not.

Because we observed a significant *site by nematode infestation* interaction effect, we conducted *additional*, site-specific GLMM analyses, as above. Employing Holm's (1979) Sequential Bonferroni Procedure for multiple tests, we identified three sites in which, after accounting for non-pollinators, pollinator offspring reduction was reduced significantly due to nematode infection (Figure 6). At the remaining sites, differences in offspring production between infested and uninfested figs were not significant.

### 3.3 Nematode Infection Effects on Pollinator Longevity

The July 2013, December 2014, and August 2016 controlled longevity trials produced a total of 50 mature figs, 29 infested with nematodes and 21 uninfested (14 and 6, 6 and 5, and 9 and 10 respectively). In total, 1986 female pollinator wasps were reared from these trials, 1104 from infested figs and 882 from uninfested figs. Dissections were performed on a total of 766 individuals from infested figs (276, 373, and 127 individuals in each trial respectively). Dissections revealed that across the three trials 63% ( $n = 486$ ) of pollinator wasps emerging from infested figs were infected by at least one juvenile nematode. Observed wasp infection rates were higher in the third trial (82%) where pollinators were allowed to emerge naturally compared to the first two trials (59% and 60% respectively) where the figs were cut open. Wasps that successfully emerged from the natal fig survived from less than 24 hours to up to 84 hours, regardless of treatment group. In uninfested figs, four individual wasps survived until hour 96. As indicated below, the number of nematodes per infected host varied only marginally

significantly between longevity trials, and in pooling across trials a survival analysis comparing wasps from infested and uninfested figs suggests that there is not a significant difference between the longevity of either group (log-rank test,  $n = 1104$ ,  $df = 1$ , Chi-Square = 0.110,  $p = 0.740$ ).

The number of juvenile *Parasitodiplogaster* nematodes found in infected individual female pollinating wasps averaged 4.61 (median of 3), with a range of 0-50 individuals per wasp host. The *number of nematodes extracted per wasp host* varied significantly with host longevity *hour* (GLM,  $n = 482$ ,  $df = 3$ , Chi-Square = 43.69,  $p < 0.001$ ) (Figure 7A). However, the *number of nematodes extracted per wasp host* was not found to vary significantly due to *individual fig* ( $p = 0.366$ ) and was only found to be marginally significant due to *longevity study* ( $p = 0.056$ ). Wasps that died by hour 24 contained an average of 8.22 (median of 4, range of 1-50) juvenile nematodes whereas wasps that survived to hour 36 or longer contained an average of only 3.81 (median of 3, range of 1-36) juvenile nematodes. The effect of host longevity *hour* was found to vary significantly due to the *number of nematodes extracted per wasp host* (ANOVA,  $n = 482$ ,  $df = 3$ ,  $F = 10.918$ , model  $r^2 = 0.064$ ,  $p < 0.001$ ) (Figure 7B), but not due to the *individual fig* ( $p = 0.424$ ) or *longevity study* ( $p = 0.576$ ). Of note, every time 15 or more nematodes were found in a wasp host, at least one nematode was found attached to the external surface of the host abdomen or thorax (Figure 8), suggesting high levels of infection can exceed the space available within the host. Non-pollinator wasps from nematode infested figs were also dissected to note the incidence of nematode infection and mean infection loads (Table 2).

### 3.4 Nematode Effects on Pollinator Dispersal Ability

A total of 81 single foundress, nematode infected, early interfloral phase figs were collected from the *F. petiolaris* sites surveyed. Adult nematodes within the fig were often located in close proximity to the dead foundress wasp and were occasionally observed mating in large aggregates (see Figure 1). Successfully dispersed infected pollinators ( $n = 81$ ) were infected by a mean of 3.69 nematodes per host (range of 1-12, median of 3), with 98% of hosts containing 2 or more nematodes. This level of infection was significantly lower than for wasps emerged from nematode infested fruit in the longevity trial (Figure 9; mean 4.61 nematodes per host,  $n = 486$ , rate ratio = 0.800, poisson.test  $p < 0.001$ ). Details of the longevity trials (Figure 7B) indicate that wasps surviving 24 hours or less are often infected with more nematodes (mean 8.22 nematodes per host) than wasps surviving 24 hours or longer (mean 3.81 nematodes per host). We did not find successfully dispersed wasps to differ in infection level from these longer-lived (>24 hrs) wasps ( $n = 398$ , rate ratio = 0.969, poisson.test  $p = 0.639$ ), whereas both the dispersed and longer-lived wasps had substantially lower infection levels than the shorter-lived ( $\leq 24$  hrs) wasps ( $n = 88$ , rate ratio = 0.449 and 0.464 respectively, and poisson.test  $p < 0.001$  in both tests). Additionally, while the number of nematode infested figs was significantly greater in the wet than the dry season ( $p < 0.001$ , see Section 3.1), there was no significant difference between seasons in the number of nematodes infecting individual wasps (GLM,  $n = 81$ ,  $df = 1$ , Chi-Square = 2.3273,  $p = 0.127$ ).

#### **4. Discussion**

Figs, fig wasp pollinators, their nematode parasites, and the associated non-pollinator fig wasps provide a useful model system for analyzing the influence of antagonists on the fitness of

a mutualism. Each fig provides a directly observable community of known mutualists and antagonists that can be easily identified and quantified, permitting analyses that account for community-level interspecific interactions. Nematodes that infect pollinating wasps have been shown to reduce host reproduction, and thus the fitness of the fig-pollinator mutualism as a whole, however, the mechanisms and degree to which nematodes limit total lifetime fitness of wasps is not well understood. Further, the influence of nematode parasitism on mutualism fitness in the context of co-occurring non-pollinating fig wasps has not been explored. In this paper we investigate the fitness impacts of *Parasitodiplogaster* nematode infection at multiple stages in the life history of a pollinating fig wasp, taking into account interactions with the broader non-pollinating wasp community. Specifically, we examine landscape-level spatial and temporal variation in infection rates, as well as impacts of infection on the reproductive success, longevity, and dispersal ability of pollinating wasp hosts.

#### *4.1 Geographical and Seasonal Variation in Host Availability and Nematode Infection*

Herre (1989) surveyed populations of 12 *Ficus* species on Barro Colorado Island, Panama, finding a mean of 1.87 pollinator foundresses per mature fig (range 1.01 to 4.53), which is similar to the grand mean of 1.44 foundresses we observed per *F. petiolaris* fig in Baja California, Mexico. Unlike Herre's study, however, we surveyed foundress counts at multiple geographical locations, as well as through time within these sites (both across collecting trips and seasons), finding substantial spatial and temporal variation in the arrival of pollinators at receptive figs (mean foundress count per site/visit ranging from 1.08 to 1.94). This variation



indicates that foundress counts obtained at any one location or time point, or that are averaged over time, may not accurately represent the species as a whole.

Like foundress counts, nematode infection rates varied significantly with respect to site and season, and also exhibited substantial unexplained variation. Figs surveyed at individual census sites experienced between 12% and 80% nematode infection, consistent with the widespread presence of these antagonists in other New World (Giblin-Davis *et al* 1995) and African (Martin *et al* 1973, Jauharlina *et al* 2012) *Ficus* systems. Unexpectedly, Figure 3B reveals a downward trend in nematode infection rates over the two years of this study. This trend transcends wet and dry season effects and is not readily understood. In general, however, with a grand mean of 39% of all figs infested with nematodes across nine geographical locations and four census periods, it is clear that *Parasitodiplogaster* nematodes are an ecologically relevant member of the *F. petiolaris* community.

The effect of season was found to be very influential for pollinating wasp population dynamics as well as the incidence of their associated nematodes. In the *F. petiolaris* system, both foundress counts and the incidence of fig infestation by nematodes were found to be generally lower in dry seasons than in wet (though not significantly so for foundresses). The lower density of foundresses in the dry season is likely related to the sensitivity of dispersing wasps to desiccation (Ramirez and Malavasi 1997, Warren *et al* 2010). Nematode infestation of figs was found to be positively associated with foundress count so that the dry season reduction in host abundance is likely largely responsible for the associated reduction in nematode abundance. However, even after accounting for variation in foundress count, figs were significantly more likely to be infested with nematodes in wet seasons than in dry. This suggests that nematode infected fig wasps are better able to successfully disperse and locate receptive figs when

conditions are milder and more humid and, conversely, that nematode infection has a greater negative impact on host dispersal when conditions are hotter and dryer. Because nematode infected fig wasps are often successful in dispersing and founding broods there is an indication of a certain tolerance to nematode infection. However, this infection may vary in response to environmental conditions and, as discussed below, variation in nematode virulence and levels of infection.

#### *4.2 Nematode Impacts on Pollinator Offspring Production in the Context of Non-Pollinator Wasps*

Much like the substantial variation observed in foundress counts and nematode infection rates, the mean number of offspring produced per *pollinator* wasp varied significantly across site and season. Interestingly, offspring production per pollinator foundress was significantly greater in wet than in dry seasons, while the opposite was true for the non-pollinating fig wasps (unpublished data). Regardless of season, however, the abundance of pollinators and most non-pollinators was negatively correlated, and the numbers of non-pollinators produced per fig typically outnumbered that of pollinators across sites (Figure 4). This contrasts with most *Ficus* species (Compton and Hawkins 1992, Bouček 1993, West and Herre 1994, Marussich and Machado 2007, Conchou *et al* 2013, Borges 2015, Castro *et al* 2015) and reveals an unusually high level of exploitation in the *F. petiolaris* pollination mutualism by non-pollinators.

Theory indicates that parasite virulence should evolve in response to the rate of transmission between hosts, with vertical transmission favoring benign parasites and horizontal transmission favoring more virulent ones (Anderson and May 1981, 1982). The greater the

number of fig wasp foundresses per fig, the greater the opportunity for transmission of nematode lineages among wasp-host lineages, and foundress number can vary substantially among species (Herre 1989). These observations led Poinar and Herre (1991) to hypothesize that the foundress number characteristic of a species should be positively associated with the virulence of its associated nematode. Herre (1993) empirically tested this hypothesis in an investigation of 11 *Ficus* species on Barro Colorado Island, Panama. The hypothesis is supported by his results, showing that nematode infection is consistent with the reduced reproductive success of an infected pollinator species when that species was characterized by a higher foundress count (and lower proportion of single foundress broods). In the Panamanian figs, foundress count ranged from 1.01 to 4.53 across species and the reduction in host wasp fitness was most prevalent in species with foundress counts exceeding approximately 1.5 per fig. Herre's (1993) study focused on how differences in transmission rate between host species influence evolution of parasitic virulence, but the same theory can be extended to predict how parasite virulence evolves in response to variation in transmission rate across populations *within* a host species.

We tested this population/site-level prediction in the *F. petiolaris* system. We observed a grand mean of 1.44 foundress wasps per *F. petiolaris* fig in Baja California, Mexico, which is close to the foundress count at which Panamanian fig wasps were associated with more virulent nematodes. Further, depending on the census period, individual sites of *F. petiolaris* varied substantially in mean foundress count (range 1.08 to 1.94), suggesting that at sites with higher foundress counts and opportunities for parasite transmission, natural selection may favor the evolution of increased nematode virulence. While we found mean offspring production to be lower in nematode infected than uninfected foundresses (Figure 5), statistical models accounting for non-pollinators and other predictor variables failed to reveal a significant relationship

between nematode virulence (reduction in reproductive success of infected wasps) and site-level foundress counts. While uninfected wasps did exhibit significantly higher reproductive success than infected wasps at three sites, at five sites reproductive success was actually higher in infected wasps, though not significantly so (Figure 6). The explanation for this lack of a consistent nematode effect on host fitness likely rests on the observation that although sites differed significantly in foundress count, individual sites also varied substantially in foundress count through time (across collecting trips and seasons). Further, there was substantial variation in foundress count that was unexplained by the spatial and temporal variables in our models. Substantial temporal and stochastic variation in foundress count naturally limits the ability of natural selection to optimize nematode virulence with respect to host transmission rate. It will be interesting to see if future studies reveal population-level evolutionary optimization for nematode virulence in fig-pollinator mutualisms with locally stable transmission rates.

In sum, fig-pollination mutualisms are subject to antagonism from both nematode parasites and non-pollinating fig wasps. Our results are in line with numerous other studies showing that non-pollinators can strongly and consistently negatively impact the production of pollinating wasps and *Ficus* seeds. While we find that nematodes can also have strong negative impacts on the reproductive success of pollinators (Figure 6), these effects are more site-specific and less consistent through time. The abundance of non-pollinators associated with *F. petiolaris* is higher than reported for many other Neotropical figs (West and Herre 1994); however, other *Ficus* systems are associated with more highly virulent nematodes (Herre 1993, 1995). Given this variation, the relative impacts of non-pollinators and nematodes on the fitness and stability of fig-pollination mutualisms are likely to be system dependent.

### 4.3 Nematode Infection Effects on Pollinator Longevity

Our controlled longevity trials provide interesting insights into the lifespan of pollinating fig wasps and to their interactions with nematode parasites. The longevity of *F. petiolaris* pollinators was found to be up to 84 hours, regardless of nematode infection (and up to 96 hours in uninfested figs). With few exceptions (Warren *et al* 2010) this lifespan is much longer than has been previously reported for pollinating fig wasps (Kjellberg *et al* 1988, Dunn *et al* 2008) and may be linked to the environment in which they occur. As a desert rock strangler, *F. petiolaris* has a very patchy spatial distribution that may often require their pollinators to disperse long distances to reach receptive trees, a factor potentially favoring the evolution of longer wasp lifespans. Also, fig wasps in desert environments may have greater desiccation tolerance (Warren *et al* 2010), which may allow for an unusually long lifespan under our relatively benign observational conditions.

As noted previously, 39% of the 2077 mature figs we sampled across sites and censuses were infested with *Parasitodiplogaster* nematodes. In turn, in our longevity trial we found 63% of pollinator wasps emerging from nematode infested figs to be infected by at least one juvenile nematode; a level of infection consistent with previous studies (Giblin-Davis *et al* 1995, Jauharlina *et al* 2012). However, wasp infection rates were found to be higher (by over 20%) in the August 2016 observational study where the fig was not cut open and wasps were allowed to freely emerge, indicating that natural infection rates may generally be higher as well (although the number of individuals involved in an infective event may not be different). Taken together, these trials indicate that a substantial proportion of dispersing *F. petiolaris* wasps will be parasitized by nematodes. However, infection does not influence each wasp equally. Most

infected wasps had longevities similar to those that were uninfected. This suggests, as discussed in Ramirez-Benavides and Salazar-Figueroa (2015), that nematode parasitism may have relatively benign impacts on fitness at least at certain life history stages.

Our survival analysis of infected and uninfected wasps did not reveal a significant difference in survivorship curves, indicating that moderate levels of nematode exploitation can be tolerated by wasp pollinators. Examination of nematode infection level as a function of host longevity (Figure 7), however, reveals that wasps subject to unusually high levels of nematode infection (mean 8.22 nematodes per host) are more likely to die within 24 hours post emergence than are wasps surviving 24 to 84 hours (mean 3.81 nematodes per host). High numbers of juvenile nematodes within the host abdominal and thoracic cavities may lead to lethal mechanical or chemical virulence, as has been described in other entomopathogenic nematode species (Bashey *et al* 2013, O'Callaghan *et al* 2014). Of note, when infection was unusually high, presumably resulting in competition for space and resources within host-wasp cavities, we observed juvenile nematodes on external wasp surfaces (Figure 8). In addition to an association with reducing longevity, high parasite loads may also thus mechanically decrease a wasp's ability to fly and to successfully disperse to a receptive fig, as suggested by Herre (1995). It is in a nematode's best interest for its host wasp to successfully reach a receptive fig and to lay her eggs, providing new hosts for that nematode's offspring (Ramirez-Benavides and Salazar-Figueroa 2015). By decreasing the longevity of the host and, importantly, its capacity as a dispersal vector to new figs (see below), overexploitation by nematodes is likely a maladaptive behavior with strong negative consequences for both host and nematode fitness.

Although pollinating wasps were correlated with a sensitivity to overexploitation by infective stage nematodes, we found that when nematodes do occur within a pollinator, they do

not typically occur alone (Figures 7-9). Given that *Parasitodiplogaster* has separate sexes (Poinar 1979, Poinar and Herre 1991), multiple infection of the same host may be an adaptation enhancing their reproductive success, with trade-offs arising from overexploitation. Interestingly, in ongoing research, we have found that male and female nematodes co-occur in individual Panamanian fig wasp hosts significantly more often than expected by chance; suggesting that colonization of individual hosts involves an assessment of potential mates or, possibly, an as yet undescribed process of delayed sex determination influenced by the sex of co-occurring infective nematodes.

#### *4.4 Nematode Effects on Wasp Dispersal Ability*

We found the mean number of nematodes infecting pollinators that successfully arrived at receptive *F. petiolaris* figs (3.6 nematodes per host) to be similar to the numbers of nematodes found in the wasps that survived longer (post-24 hours) in the controlled longevity trials (Figure 9). This further supports our previous conclusion that relatively moderate levels of nematode infection are well tolerated by pollinating wasps. In contrast, the high mean number of nematodes in wasps that died within the first 24 hours of the longevity trial (Figure 7) was rarely observed in successfully dispersed hosts. This observation further supports our previous conclusion that high infection levels (overexploitation) is associated with a reduction of the longevity and dispersal ability of pollinating fig wasps.

How frequently does it occur that overexploitation of pollinating wasps by nematodes is sufficient to prohibit hosts from successfully dispersing to new figs? To define a cut-off value for overexploitation, we compared the frequency with which a wasp was infected by  $n$  nematodes

between the longevity trials and successfully dispersed wasps. While we found similar frequencies for  $n = 1-9$  nematodes per host, the frequency of infection by ten or more nematodes was significantly lower in dispersed wasps ( $n_{\text{longevity}} = 486$ ,  $n_{\text{dispersed}} = 81$ , rate ratio = 0.969, poisson.test  $p < 0.001$ ), leading us to define infection by 10 or more nematodes as overexploitation. Taking into account the global frequency of infested figs (0.39), the probability that a wasp emerging from an infested fig is in fact infected by a nematode (0.63), and the frequency with which an infected wasp contains ten or more nematodes (0.12), we estimate that only 2.8% of all pollinator wasps are overexploited by nematodes to the extent that the odds of successful dispersal is significantly reduced. Stated another way, only 2.8% of all pollinator wasps per generation are expected to be effectively eliminated from the population due to infection by *Parasitodiplogaster* nematodes. Ultimately, the effect of nematode infection on fig wasp fitness depends on the number of infective nematodes per host: the effect is mostly benign and only occasionally reaches essentially lethal levels.

#### 4.5 Implications of Nematode Infection on Non-Pollinating Wasps

Although the life history of *Parasitodiplogaster* nematodes is tied to that of pollinating fig wasp females, in the course of this study we also observed them infecting male pollinators, male non-pollinators, and female non-pollinating wasps. Dissections of non-pollinating wasps from the longevity trial verified the presence of juvenile nematodes within host abdominal and thoracic cavities. While *Parasitodiplogaster* nematodes have been previously observed infecting a non-pollinator wasp female (Giblin-Davis *et al* 1995), this infection was dismissed as a maladaptive and rare behavior. This conclusion follows from the fact that non-pollinating wasps



oviposit from outside the fig and do not provide nematodes access to the brood of wasp offspring developing within a fig. In contrast, our preliminary data indicate that *Parasitodiplogaster* commonly infects the entire community of fig wasps associated with *F. petiolaris*, pollinators and non-pollinators alike (Table 2).

*Parasitodiplogaster* exploitation of non-pollinating fig wasps likely has benefits for fig-pollinator mutualisms that have not previously been appreciated. If nematode infection limits the fitness of pollinating wasps through reduced offspring production, longevity, and dispersal ability, they may exact similar fitness limitations on non-pollinators. This, in turn, may suppress non-pollinator densities and reduce their net exploitation of fig seeds and pollinators. If this is the case, then taking into account broader, community-level interactions can provide a new perspective on *Parasitodiplogaster* and its functional relationship to the fig-pollinator mutualism. While these nematodes can be antagonists of fig pollinators with potentially strongly correlated negative effects on their fitness, through their suppression of non-pollinators they may provide important indirect benefits to both pollinators and host figs. Future research will provide a better understanding of *Parasitodiplogaster* infection of non-pollinators and its ecological and evolutionary implications.

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**Tables**

<b>Site Number</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Number of GPS Mapped Trees</b>
158	29°26'27.0"N	114°02'09.0"W	86
172	28°29'06.9"N	113°11'19.7"W	67
112	27°56'04.3"N	113°06'71.9"W	59
113	27°14'85.2"N	112°43'55.4"W	80
95	26°35'80.8"N	111°80'34.6"W	65
201	25°22'33.6"N	111°19'01.2"W	34
96	24°03'38.0"N	110°12'57.0"W	291
250	24°04'91.0"N	109°98'90.2"W	3
70	23°73'76.9"N	109°82'88.7"W	87

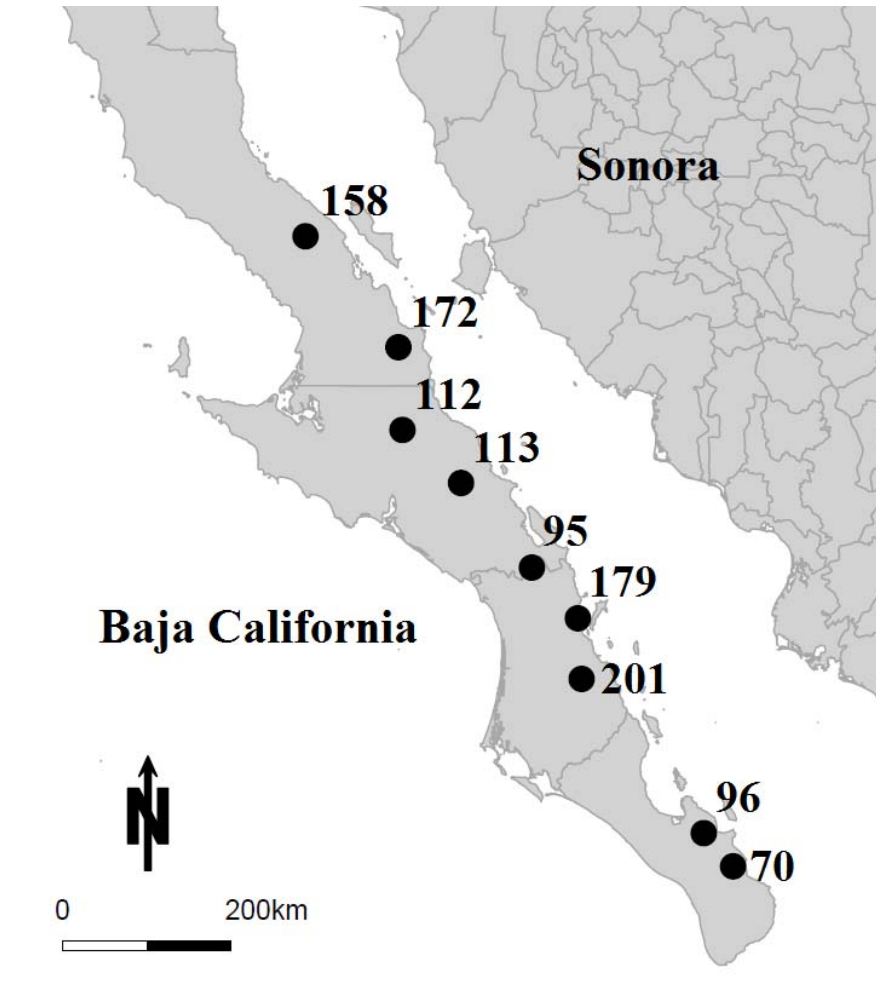
**Table 1.** *Ficus petiolaris* study site identification number, latitude and longitude coordinates, and number of GPS-mapped trees.

<b>Non-Pollinator</b>	<b>Individuals</b>	<b>Percent Infected</b>	<b>Nematodes Per Host</b>
<b>Species</b>	<b>Dissected</b>		
<i>Idarnes</i> sp. 1	534	37	2.50
<i>Idarnes</i> sp. 2	410	7	1.20
<i>Idarnes</i> sp. 3	88	6	1.20
<i>Heterandrium</i> sp. 1	45	31	3.37
<i>Heterandrium</i> sp. 2	69	11	1.75
<i>Ficicola</i> sp.	76	1	2.00
<i>Physothorax</i> sp.	30	7	2.50

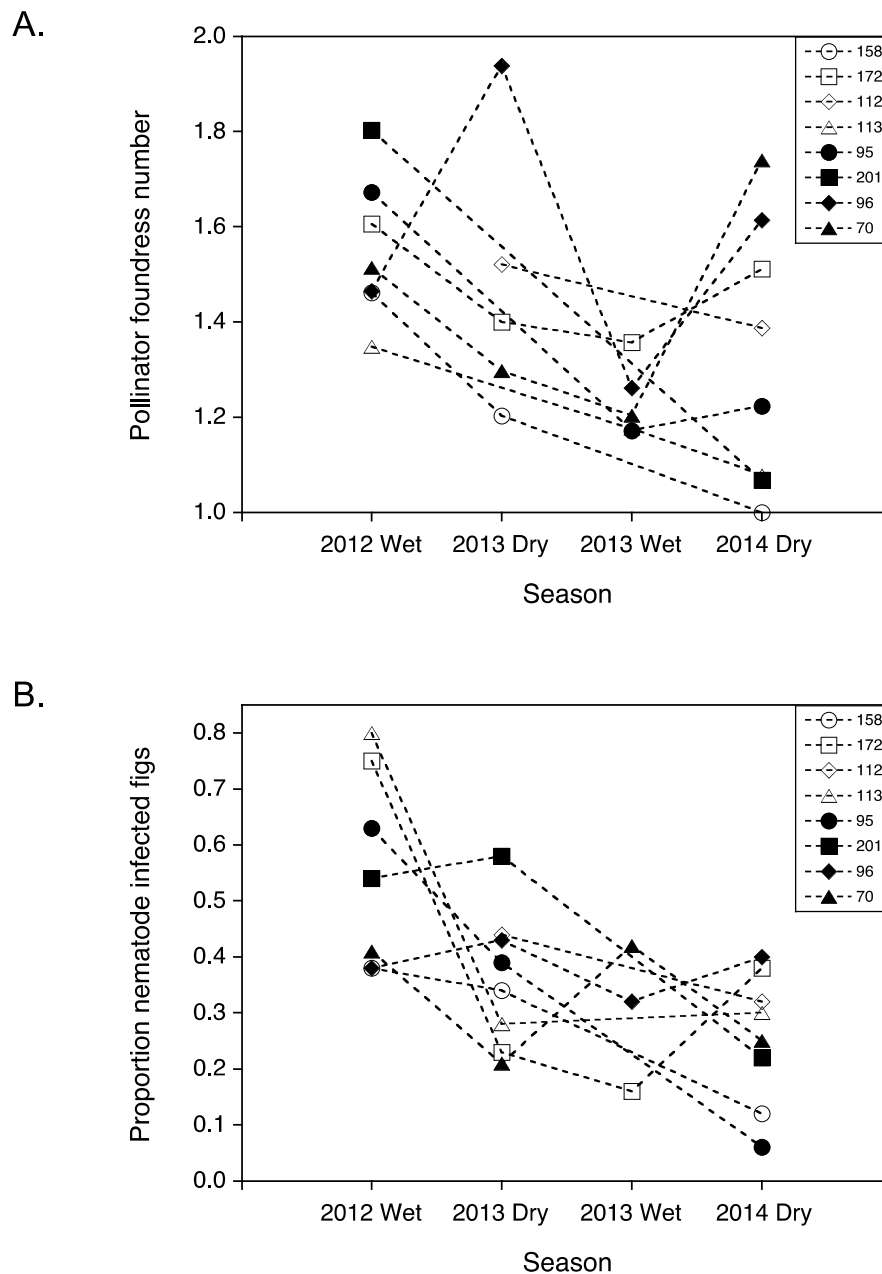
**Table 2.** All *F. petiolaris* non-pollinator wasp species present in the 2014 controlled longevity trial were found to be targets of juvenile *Parasitodiplogaster* nematode infection. Only a subset of non-pollinators emerging from nematode infested figs in the longevity trial were dissected to quantify nematode infection. Indicated here are the non-pollinator species, the number of individuals dissected, the percentage of individuals found to be infected with juvenile nematodes, and the mean number of nematodes per host.

**Figures**

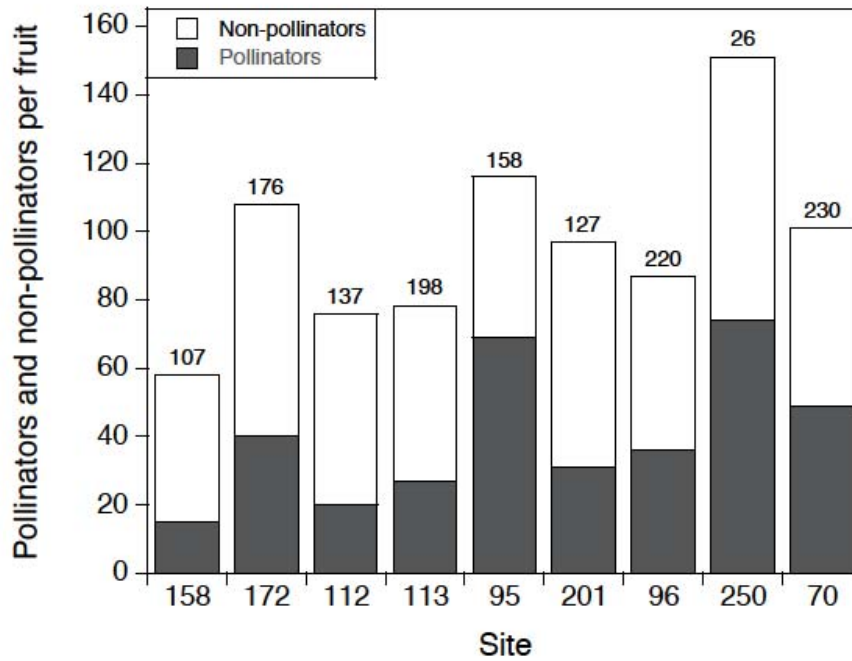
**Figure 1.** An aggregate of adult *Parasitodiplogaster* nematodes mating within an interfloral phase *Ficus petiolaris* fig.



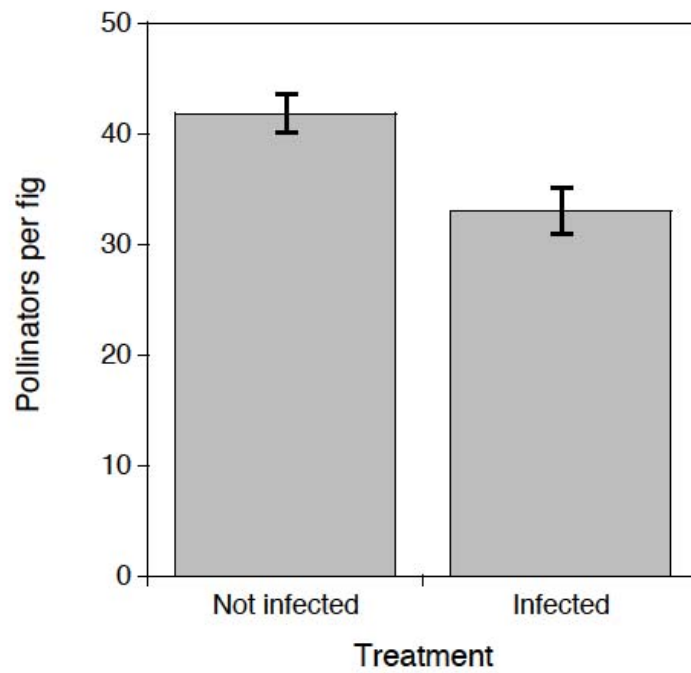
**Figure 2.** Map of *Ficus petiolaris* study sites in Baja California and Baja California Sur, Mexico.



**Figure 3.** (A) The mean *Pegoscapus* pollinator foundress wasps observed at eight *Ficus petiolaris* sites over four collection trips. (B) The mean proportion of mature, wasp rearing, *F. petiolaris* figs found to be infested with *Parasitodiplogaster* nematodes by sample site across four collection trips. Not all sample sites yielded wasp rearing figs for every collection trip.

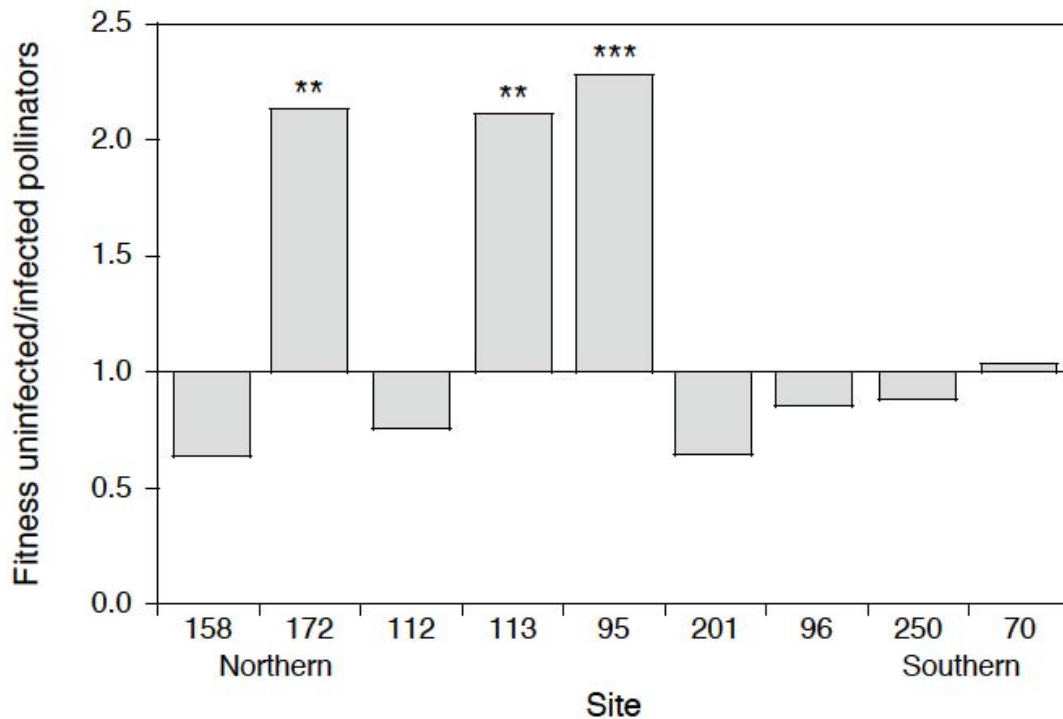


**Figure 4.** The mean number of pollinator (*Pegoscapus*) and total non-pollinator (*Idarnes*, *Heterandrium*, *Ficicola*, and *Physothorax*) offspring from single foundress figs for each sample site. The total number of figs collected across the four collection trips (2012-2014) is indicated above each sample site bar. Sample sites are arranged by latitude, with the northernmost site (158) on the left and the southernmost (70) on the right.

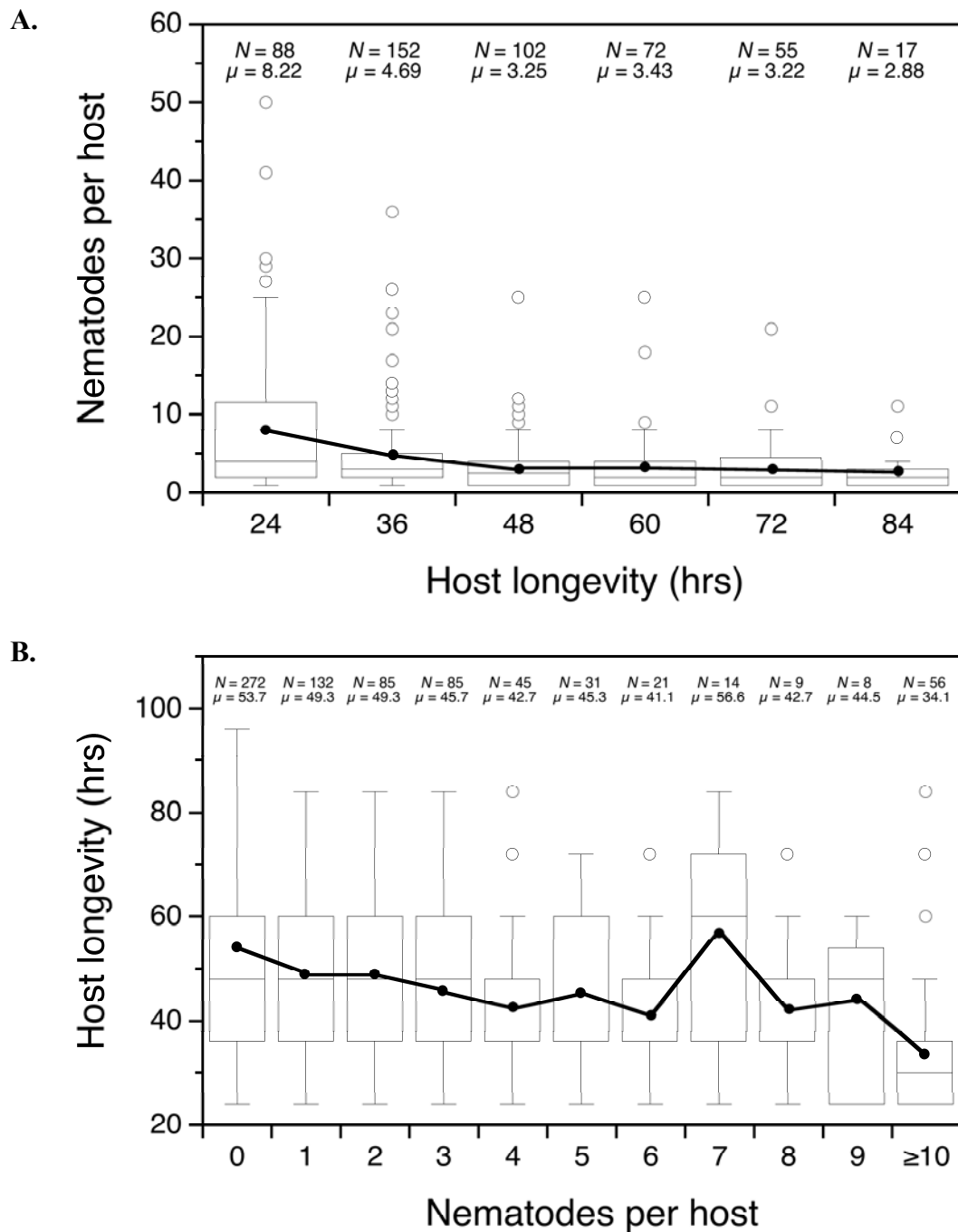


**Figure 5.** Grand mean offspring production is lower in nematode infected than uninfected *Pegoscopus* pollinators for samples collected between 2012 and 2014 (GLM,  $p < 0.001$ ). Error bars indicate standard error.





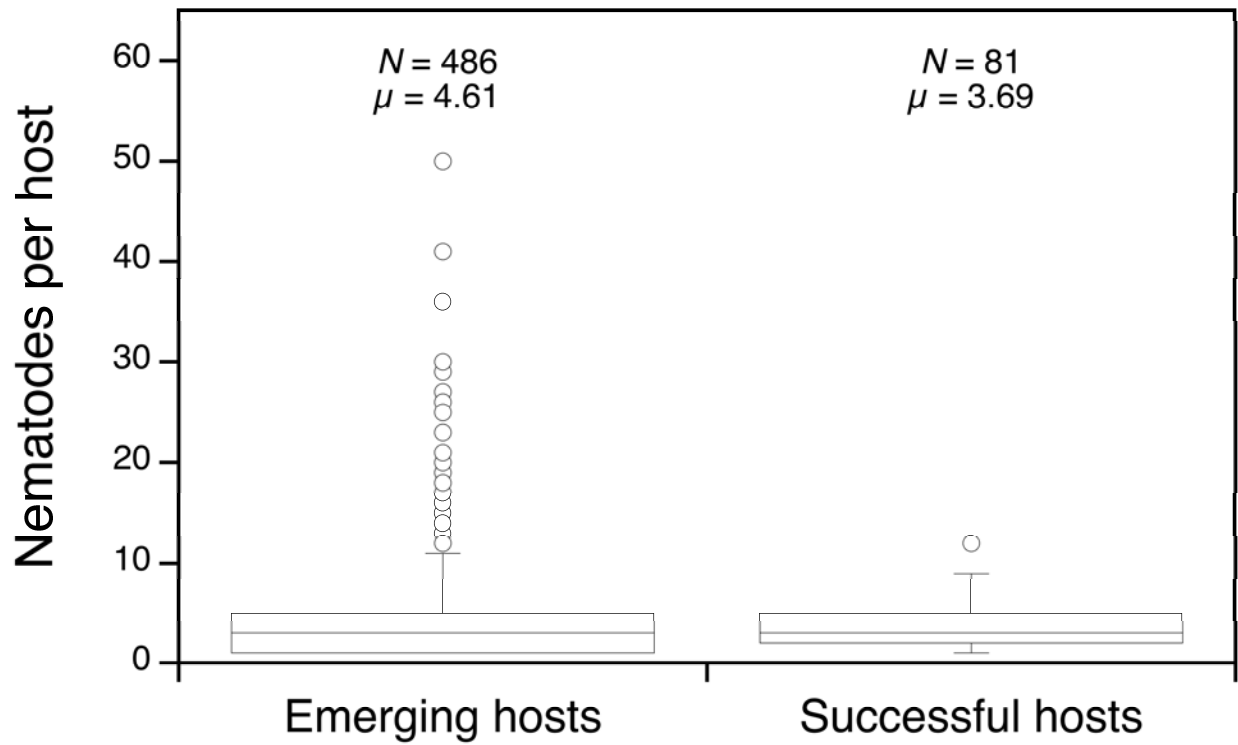
**Figure 6.** The relationship between mean *Pegoscapus* pollinator offspring production in single foundress figs uninfected and infected with *Parasitodiplogaster* nematodes by sample site. A positive value indicates more pollinator offspring produced in uninfested figs versus infested figs, indicating a relative fitness cost due to this infection. Sites are arranged by latitude. Site-specific significant differences between mean pollinator offspring production in uninfested and infested figs were obtained using Holm's Sequential Bonferroni Procedure for multiple tests (\*= $p < 0.05$ , \*\*= $p < 0.01$ , \*\*\*= $p < 0.001$ ).



**Figure 7.** The relationship between the *Parasitodiplogaster* nematode infection level and *Pegoscapus* host wasp longevity. Infection level was determined from the number of juvenile nematodes infecting individual hosts, and host longevity was measured from the 24-hour time period in which an infected wasp died in the controlled longevity trials. (A) Nematodes per host as a function of host longevity, and (B) host longevity as a function of nematodes per host. Mid-box lines indicate hourly median values and closed circles indicate means. Above each box is the sample size ( $N$ ) and mean ( $\mu$ ).

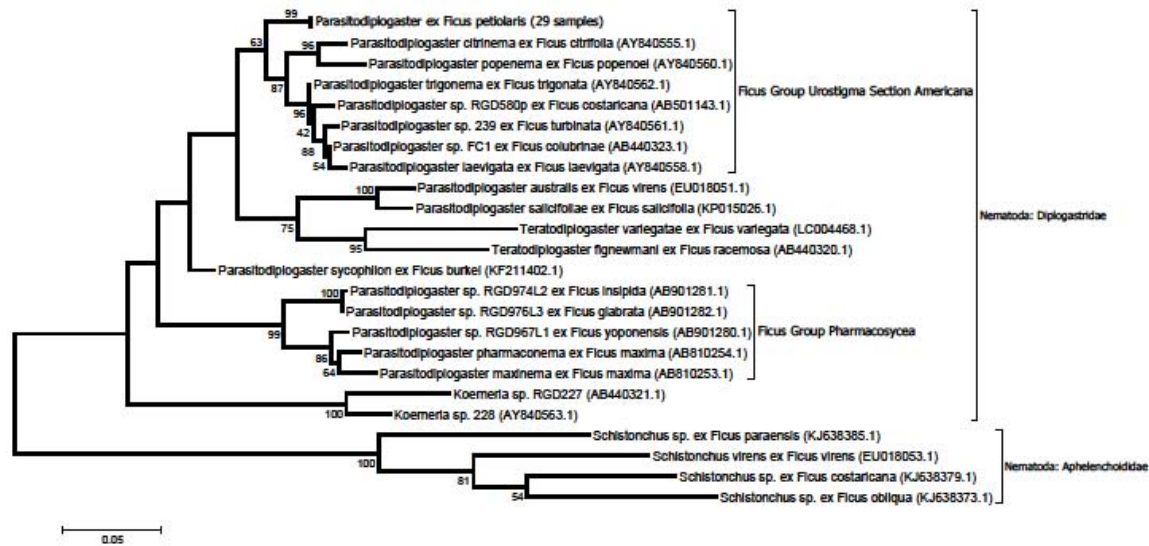


**Figure 8.** A female *Pegoscapus* pollinator of *Ficus petiolaris* infected with 21 juvenile *Parasitodiplogaster* nematodes. In cases where there were more than 15 nematodes infecting a wasp, nematodes were commonly observed in both the abdominal and thoracic cavities and on other external host surfaces



**Figure 9.** Numbers of *Parasitodiplogaster* nematodes per emerged *F. petiolaris* pollinator in the longevity trials are greater than infection levels in successfully-dispersed pollinators. The middle boxplot lines indicate median values and the open circles above the plots indicate outlier values. Above each boxplot is the sample size ( $N$ ) and mean ( $\mu$ ).

## Supplementary Information



**Supplementary Fig 1.** Maximum Likelihood tree constructed from *Parasitodiplogaster* 28S LSU rRNA sequences. 29 *Parasitodiplogaster* samples were taken from *Ficus petiolaris* figs across the sampled species range in Baja California, Mexico. DNA extraction, PCR amplification, and sequencing were conducted at Iowa State University using the protocol and primers in Nunn (1992) and Giblin-Davis *et al* (2006). The resulting 29 *F. petiolaris* associated sequences shared >99% sequence identity. These sequences were then compared to other *Parasitodiplogaster*, *Teratodiplogaster*, *Koerneria*, and *Schistonchus* 28S LSU rRNA sequences publically available through the National Center for Biotechnology Information (NCBI). All sequences were aligned using MUSCLE (Edgar 2004). Maximum Likelihood reconstruction was conducted using MEGA Version 6.06 (Tamura *et al* 2013) with 500 bootstrap replicates

following a Tamura-Nei Model. All *F. petiolaris Parasitodiplogaster* sequences clustered together and nested among other New World *Parasitodiplogaster* sequences originating from *Ficus* hosts in the subgenus *Urostigma* section *Americana*. All bootstrap values <50 on basal branches have been omitted.

### **Author Contribution Statement**

1. **Justin Van Goor** developed conceptual aspects of the project, conducted data collection and field trials, analyzed data, provided interpretations, and wrote the manuscript.
2. **Finn Piatscheck** conducted data collection and field trials, assisted with interpretations, provided a figure, and provided comments for the manuscript.
3. **Derek Houston** conducted data collection and field trials, assisted with data analysis, and provided comments for the manuscript.
4. **John Nason** contributed to the conceptual development for the project, conducted data collection and field trials, assisted with data analysis, provided interpretations, and co-wrote the manuscript.

All of the authors have approved the final manuscript and have provided true contributions to this manuscript.