

# A new player in the Panamanian fig tree – fig wasp mutualism; a study on the effect of gall midges on *Ficus citrifolia*

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## ABSTRACT

The mutualism between the fig tree and the pollinating fig wasps is a keystone interaction in tropical forests. However, many antagonistic interactions also occur in the system, taking advantage of the fig trees and the pollinator. One such example is an antagonistic gall midge (Cecidomyiidae) that develops inside figs. Gall midges inside figs have been documented in a few *Ficus* species around the world, but to our knowledge they have not previously been observed in Panama. In this study the newly observed Panamanian fig gall midge is documented, together with its parasitoid wasp. The fig gall midge was only found in *Ficus citrifolia* figs. We investigated the effect of fig gall midge presence on the number of seeds and the number of pollinating wasps (*Pegoscapus tonduzi*) in a fig and aimed to identify the species based on morphology and barcoding of the COI region. We found that the fig gall midge had no, or a negligible effect, on the reproduction of the fig tree – fig wasp mutualism. The fig gall midge most likely belongs to the genus *Ficiomyia*, close to *Ficiomyia perarticulata*. The parasitoid belongs to the genus *Physothorax*, close to *Physothorax russelli*. This study suggests that the potentially newly arrived fig gall midge currently has no major effect on the fig tree – fig wasp mutualism. However, should infestation rates increase, it is likely that the fig gall midge would affect the mutualism negatively as it has in other parts of the world. More studies on the fig gall midge species distributions in this region would be valuable and would connect these newly observed species to a larger community, adding yet another species to this complex but classic example of a mutualism.

## 1. Introduction

Parasitism is one of the most prevalent forms of life, with at least one parasite species found for each free-living metazoan species (Poulin and Morand 2004). Parasites have a variety of direct negative impacts on their hosts and can even indirectly influence ecosystem dynamics and alter biodiversity (Marcogliese 2004; Lafferty and Kuris 2012; Preston et al. 2016; Frainer et al. 2018). To determine the effect of a parasite on an ecosystem one must consider (1) the ecological importance of the host, (2) the parasite's fitness effect on the host, and (3) the frequency of infestation (Lafferty and Kuris 2012). Parasitism is often derived from mutualistic systems, where one or more species alter their mutualistic interactions and start taking advantage without providing any benefits back to their partner in the mutualism (Boucher et al. 1982). Mutualisms are fundamental to the majority of ecosystems (Boucher et al. 1982; Chomicki et al. 2019; Biedermann and Vega 2020). For instance, most land plants interact with mycorrhizal fungi that aid plants with nutrient uptake, and the majority of flowering plants need pollinators to

reproduce (Ollerton et al. 2011; Antoine et al. 2021). A mutualism in which fitness effects on both partners can be quantified is the one between fig trees (*Ficus*, Moraceae) and their pollinating fig wasps (Agaonidae) (Herre 1989; Herre et al. 2008).

Fig trees are keystone species in tropical ecosystems and are often a crucial food source for frugivores, providing a stable food supply by producing fruits throughout the year (Shanahan et al. 2001). Both parties of the fig tree – fig wasp mutualism fully rely on each other for reproduction, making them highly dependent on each other (Wiebes 1979; Weiblen 2002). The fig (formally syconium, hereafter fig) is an enclosed inflorescence that contains hundreds or thousands of uniovulate flowers (Cook and Rasplus 2003). Fig trees flower asynchronously throughout the year and thereby provide the wasps with constant reproductive opportunities. The pollinating female fig wasps are very short lived: around two days outside the fig (Machado et al. 2005; Kjellberg et al. 2005; van Kolfshoten et al. 2022) during which they have to find a receptive fig to lay eggs in within their flying range. The average flight distance for Panamanian pollinator fig wasps is estimated

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to be between 5.8 and 14.2 km (Nason et al. 1998). When a receptive fig is reached, the pollinating female fig wasp enters the fig where she pollinates, oviposits, and dies. The fig wasp offspring develop in galled flowers for several weeks before hatching. In the Neotropical region, only the pollinating fig wasps access flowers from inside while other non-pollinating wasps oviposit from outside the fig (Cook and Rasplus 2003). Fig trees and their pollinators (Agaonidae) are closely co-evolved (Herre 1989; Cruaud et al. 2012). In addition, many antagonistic species are associated with this mutualism.

The fig can be seen as a microcosm containing many different mutualistic and antagonistic organisms. The different fig tree species are often associated with specific compositions of mutualistic and antagonistic species (Jackson 2004; Costa and Gracioli 2010; Palmieri and Pereira 2018). Apart from the pollinator (Agaonidae), a diversity of antagonistic non-pollinating wasp species (e.g., Torymidae and Pteromalidae) can be found, as well as other invertebrates (Bronstein 1991; West and Herre 1998; Elias et al. 2008, 2012; Farache et al. 2018; Palmieri and Pereira 2018). Some of these organisms have seemingly no negative effect on the fig host but others definitely do. Host defence against antagonists is complicated due to the intertwined symbiont communities within a fig where the mutualistic pollinator might be harmed by drastic actions such as fig abortion. It is therefore argued that the fig tree host cannot easily exclude antagonists which consequently remain (Cook and Rasplus 2003), although antagonist fitness might be reduced by selective resource allocation to more beneficial figs (Jandér and Herre 2016) or by ant predation (Janzen 1966; Compton and Robertson 1988; Schatz et al. 2006; Bain et al., 2014; Wang et al., 2014; Jandér 2015). Whereas some antagonists like non-pollinating fig wasp genera are fairly well documented (Bronstein 1991; West and Herre 1998; West et al. 1996; Elias et al. 2008, 2012; Farache et al. 2018), much less is known about other invertebrates that develop inside figs (Bai et al. 2008; Palmieri and Pereira 2018; Piatscheck et al. 2018; van Kolschoten et al., submitted). One such less studied invertebrate are the fig-associated gall midges (Diptera: Cecidomyiidae).

Cecidomyiidae can damage cultivated plants and cause economic losses in forestry and agriculture, but they can also function as biocontrol agents against invasive plants and other pests (Kolesik and Gagné 2020). Additionally, they can function as pollinators: plant species from at least seven families (including Moraceae) have Cecidomyiidae pollinators, but no Cecidomyiidae are known to pollinate *Ficus* (Gan et al. 2022). Cecidomyiidae induce galls that are usually species-specific growths often developing on leaves, vegetative and floral meristems, flowers, stems, and rarely roots (Kolesik and Gagné 2020). The galls are induced from phytohormonal changes manipulated by effectors secreted from Cecidomyiidae larvae (Tanaka et al. 2013; Zhao et al. 2015; Kolesik and Gagné 2020), similar to other galling insects (Tooker and Helms 2014; Oates et al. 2016; Hearn et al. 2019; Krogaonkar et al. 2021). Fig-associated Cecidomyiidae are specific to *Ficus* and have larvae that develop in galls inside figs (Felt 1922; Roskam and Nadel 1990; Bai et al. 2008; Miao et al. 2011). Fig-associated Cecidomyiidae have been observed since the beginning of the 20th century (Felt 1922), but only one species has been fully described: *Ficiomyia perarticulata* in figs from *Ficus citrifolia* found in Florida, USA (Felt 1922; Roskam and Nadel 1990; Gagné 2018). Apart from *Ficiomyia perarticulata*, other fig-associated Cecidomyiidae have been studied in China on *Ficus benjamina* and Japan on *Ficus macrocarpa*, but no formal species descriptions of those are yet published (Bai et al. 2008; Miao et al. 2011; Yafuso et al. 2013; Yukawa and Kim 2021). There have been sporadic reports of fig-associated Cecidomyiidae in Taiwan, Malaysia, India, Philippines, Dominica, and Cambodia (Roskam and Nadel 1990; Hsieh et al. 2021; Yukawa and Kim 2021), but not in Panama and direct neighbouring countries.

In Panama in November 2021, fig-associated Cecidomyiidae were observed in figs of *Ficus citrifolia* for the first time (field observations by LD, HRH, LvK, and F. Piatscheck (personal communication)). Fig-associated Cecidomyiidae had not previously been observed by fig

researchers working in the area (LD (since 2015), KCJ (since 2003), A. Gomez (since 1999 (personal communication Jan 2023)), E. A. Herre (since 1982 (personal communication Jan 2023))). We therefore believe that the fig-associated Cecidomyiidae could be a recent arrival to the local fig community or a previously unnoticed endemic species. Furthermore, a *Physothorax* (Hymenoptera: Torymidae) presumed to be a parasitoid of the Cecidomyiidae was first observed in March 2022 (HRH).

In this study we describe our findings of a newly observed fig-associated Cecidomyiidae and its *Physothorax* parasitoid and their potential fitness effects on the fig tree – fig wasp mutualism. To determine the potential fitness effect, the number of seeds and the number of pollinator offspring were counted in figs with and without the studied Cecidomyiidae (from now on referred to as gall midge or Panamanian gall midge) and its parasitoid (from now on referred to as *Physothorax*). The gall midge and the *Physothorax* were barcoded, and we present their Barcode Index Number (BIN) IDs.

## 2. Materials & methods

### 2.1. Study species

*Ficus citrifolia* is a monoecious New World fig species (subgenus *Urostigma*, section *Americanae*) that grows in a wide range from Florida, USA, to southern Brazil (Dunn et al. 2014). *F. citrifolia* flowering is generally asynchronous among trees and synchronous within a tree. In Panama *F. citrifolia* is pollinated by *Pegoscapus tonduzi* (Hymenoptera: Agaonidae) (Wiebes 1995). The most common invertebrates observed in figs of *Ficus citrifolia* across its full distribution range are the following: *Pegoscapus* spp. (Agaonidae; pollinator), *Idarnes* group *flavicollis* spp. (Hymenoptera: Pteromalidae; non-pollinating gall-inducing wasp), *Idarnes* group *carne* spp. (Hymenoptera: Pteromalidae; non-pollinating cleptoparasitic wasps), *Idarnes* group *incertus* spp. (Hymenoptera: Pteromalidae; non-pollinating wasp), caterpillars of Crambidae and Pyralidae, nematodes *Parasitodiplogaster* sp. (Nematoda: Diplogasteridae), mites (Arachnida), and larvae of *Charoxus spinifer* (Coleoptera: Staphylinidae) and *Ceratopus* sp. (Coleoptera: Curculionidae) (Herre 1993; Giblin-Davis et al. 1995; West et al. 1996; West and Herre 1998; Marussich and Machado 2007; Elias et al. 2012; Frank and Nadel 2012; Farache et al. 2017; Palmieri and Pereira 2018; van Kolschoten et al., submitted). The non-pollinating wasps (Hymenoptera) that are most commonly observed for *F. citrifolia* at BCNM in Panama are *Idarnes* group *carne* and *Idarnes* group *incertus* (Marussich and Machado 2007), and rare non-pollinating wasps are: *Aepocerus* (Agaonidae), *Heterandrium* (Agaonidae), *Physothorax*, (Torymidae) and the subfamily Doryctina (Braconidae) (West et al. 1996).

### 2.2. Fig-associated gall midges

Fig-associated gall midge larvae develop inside figs in individual galls (Felt 1922; Roskam and Nadel 1990; Bai et al. 2008; Miao et al. 2011; Yafuso et al. 2013). The midge galls in *Ficus macrocarpa* and *Ficus benjamina* figs are induced within female flowers (Bai et al. 2008; Yafuso et al. 2013), while *Ficiomyia perarticulata* galls are described as pocket-shaped outgrowths from the receptacle (fig wall) (Roskam and Nadel 1990). All gall midges described in the literature leave emergence holes on the fig surface from where they exit the fig, but the emergence mechanism and characteristics vary. Sexual dimorphism is present for all described gall midges. Females are generally larger with a pink/red-orange abdomen and with a retractable ovipositor, while males are yellowish and grey (Roskam and Nadel 1990; Bai et al. 2008).

### 2.3. Study site

The field work was conducted in Barro Colorado Nature Monument (BCNM) around the Smithsonian Tropical Research Institute field station

of Barro Colorado Island (BCI) in the Panama Canal in Panama. Data were collected 17 Feb – Apr 13, 2022 and 25 Jan – Feb 19, 2023. The data collected 2022 was used to estimate the gall midge effect on the fig tree-fig wasp mutualism. Data was also collected for gall midge observation and identification, *Physothorax* observation and identification, gall midge prevalence, and gall midge infestation rates. Data collected 2023 was used to study the gall midge ability to prevent abortion of unpollinated figs, and to estimate gall midge prevalence and infestation rates.

*Ficus citrifolia* trees that were approaching the flowering stage were visited and their flowering status, together with any presence of gall midges, were observed. Trees were revisited and surveyed for gall midge presence if they would get figs with emerging pollinators (D phase figs; Galil and Eisikowitch 1968) within the study period. A total of twelve *F. citrifolia* trees were at the correct fig maturation stage and could be included in this study (field season 2022). In 2023, we continued monitoring gall midge prevalence together with fig abortion: a total of eight *F. citrifolia* trees were screened in 2023.

#### 2.4. Field collection

Figs were collected in the field if there were signs indicative of gall midge presence on the fig surface (Fig. 1). As soon as gall midge pre-emergence spots occurred, 30 figs without pre-emergence spots (control) and 30 figs with pre-emergence spots (gall midge figs) were haphazardly collected from each tree. If there were fewer than 30 gall midge figs available on a tree, all gall midge figs that could be reached were collected. In total, we collected figs for dissection from eight trees in 2022.

The ability of gall midges to prevent abortion of unpollinated figs (Jandér and Herre 2010) was estimated in 2023 on one tree. We placed mesh bags over twigs with pre-receptive figs (B phase figs; Galil and Eisikowitch 1968) to prevent pollinator foundresses from entering figs. The tree was re-visited after 16 days when pollinator wasps were soon to emerge from figs that had not been covered by mesh bags. All figs in the mesh bags were collected and the number of midge galls inside aborted and un-aborted figs was counted.

In 2023 we also surveyed eight *F. citrifolia* trees in the area with figs at the correct maturation stage (late C-phase: early D-phase) for presence of gall midges. From each tree we haphazardly collected fifty figs from accessible parts, then counted the number of midge galls inside each fig.

#### 2.5. Laboratory procedure

Each fig was cut in half and sealed in a Petri dish at room temperature to allow all pollinators (*P. tonduzi*) and gall midges to emerge before being frozen. Presence of other invertebrates in the fig was recorded.

Figs that contained invertebrates other than pollinators (*P. tonduzi*), gall midges (Cecidomyiidae) and the *Physothorax* were excluded from the study (i.e., figs that contained *Parasitodiplogaster* sp., Lepidoptera larvae, Coleoptera larvae, *Idarnes* group *incertus*, and/or mites). Figs that had more than one pollinator foundress were also excluded, due to the resulting likely increase in the total number of offspring (Herre 1987, 1989; West and Herre 1998). The number of foundresses per fig was recorded directly or estimated from the number of *P. tonduzi* males inside a fig, figs with more than 15 males were highly likely to have had two or more *P. tonduzi* foundresses and were therefore excluded from this study (Herre 1987; West and Herre 1998). However, foundress numbers could not be assessed for trees 1 & 6 because the *P. tonduzi* offspring had already emerged in the field. Sampled figs were stored in a freezer at  $-25^{\circ}\text{C}$ .

Each fig was divided into quarters and dissected. The following structures were counted: empty pollinator *P. tonduzi* galls from which a *P. tonduzi* had emerged, galls with *P. tonduzi* females still inside, galls with male *P. tonduzi* still inside, bladders (*P. tonduzi* galls whose development failed), undeveloped pistillate (female) flowers, staminate (male) flowers, fig seeds, midge galls, and gall midges inside midge galls (Figure A1). Total number of *P. tonduzi* offspring was calculated by adding together galls with *P. tonduzi* still inside and empty *P. tonduzi* galls where *P. tonduzi* had emerged. Number of gall midge emergence holes on the fig surface were also counted (Fig. 1). An unidentified parasitoid species, morphologically keyed to *Physothorax* (Hymenoptera: Torymidae) was repeatedly found inside midge galls or emerged into the sealed petri dishes. Figs that had *Physothorax* in midge galls or emerged adults were counted and ordered as a separate group (Gall midge + *Physothorax*) and collected for identification.

In 2022, gall midges were found in eight out of the twelve *Ficus citrifolia* trees that had figs at the stage when pollinators emerged (D phase; Galil and Eisikowitch 1968). Two of these eight trees were not used in the analyses because for these trees fewer than five figs remained in each treatment group (control and gall midge figs) after figs with other invertebrates were excluded. In total, 53 gall midge figs, 55 control figs, and 14 figs with both gall midges and *Physothorax* were analysed (Table A1). All collected figs were dissected; the uneven sample sizes were due to many figs having to be omitted from the study due to the presence of other invertebrates.

#### 2.6. Barcoding

For the barcoding, in 2022 we collected seven midge pupae and three *Physothorax* pupae from their galls and stored them at  $-20^{\circ}\text{C}$  in absolute ethanol. For the complete DNA extraction, an adapted high salt method was used, based on the original method from Aljanabi and Martinez (1997). The region cytochrome oxidase subunit I (COI) gene of mtDNA was amplified in a Polymerase chain reaction with 35 cycles of



**Fig. 1.** Figs with gall midge emergence holes. A: three gall midge pre-emergence spots (white colour) where the gall midge has not yet emerged and five emergence holes with some protruding pupal exuviae (brown colour). All emergences took place within 1–2 days ( $n = 99$ ). B: close up of gall midge emergence hole where pupal exuviae from a gall midge can be seen (photo taken the same day as emergence occurred). C: fig with gall midge emergence hole (after emergence).

30 s 95 °C, 30 s of 50 °C and 90 s of 72 °C, using the primer pairs COIS forward (5'-GGATCACCTGATATAGCATTC-3') COIA reverse (5'-CCCGGTAAAATTAATAAATTAACCTTC-3') (300-402bp) and LCO1490 forward (5'-GGTCAACAAATCATAAAGATATTGG-3') HCO2198 reverse (5'-TAAACTTCAGGTGACCAAAAATCA-3') (645bp) for both the gall midge and the parasitoid wasp. The product was cleaned with EXO1 clean-up (Thermo Fisher Scientific). Samples were sent to Eurofins for sanger DNA sequencing and the obtained sequences were uploaded to BOLD in the dataset "DS-KOLFI" (DOI requested) to get Barcoding Index Numbers (BINs) assigned.

## 2.7. Statistical analysis

To test if the different treatment groups differed in their number of seeds, number of offspring, total number of flowers, undeveloped pistillate flowers, or bladders, multiple Linear Mixed-Effect Models (LMER) were built. ANOVA (Analysis of variance) were used for group comparisons for the three treatment groups: control, gall midge figs, and figs with both gall midges and *Physothorax*. Group category was treated as the independent variable and Tree ID as a random factor to avoid pseudoreplication.

LMER models were also used to test if the degree of gall midge infestation influenced the number of seeds, number of offspring, total number of flowers, or number of undeveloped pistillate flowers. Degree of infestation rate was recorded as the number of midge galls inside figs (independent variable) and tested against the different response variables measured. Tree ID was treated as a random factor and presence of *Physothorax* as a fixed effect. Models with both normal and poisson distributions and without the interaction factor were compared using AIC (Akaike information criterion). In all cases the LMER with a normal distribution had the lowest AIC value. The statistical analyses were performed in RStudio (RStudio Team, 2020) using the lme4 package (v1.1-29, Bates et al. 2015). Significance for all statistical analyses was set at  $p < 0.05$ .

## 3. Results

The gall midge was only found in figs of *Ficus citrifolia*. Other *Ficus* species in the study area had no visible signs of gall midges on figs; *Ficus americana* subsp. *americana eugeniifolia*-form (Berg 2007; also referred to in publications from the study site as *Ficus perforata* (Croft 1978)), *Ficus bullenei*, *Ficus* aff. *crocata* (undescribed; also referred to in publications from the study site as *Ficus triangle* or *Ficus near trigonata*), *Ficus costaricana*, *Ficus insipida*, *Ficus maxima*, *Ficus nymphaeifolia*, *Ficus obtusifolia*, and *Ficus popenoi*. Eight out of the twelve *F. citrifolia* trees that had D phase figs (Galil and Eisikowitch 1968) showed presence of gall midges in 2022, and five out of eight trees showed presence of gall midges in 2023. Based on this, a rough estimation of the gall midge infestation rate in the study area around the time of the study would be that two thirds of the *F. citrifolia* trees were affected, keeping in mind that infestation could be higher because not all parts of tree canopies could be properly examined due to restrictions in tree and branch accessibility. The mean number of midge galls in figs with gall midges was  $2.64 \pm 2.24$  galls (Mean  $\pm$  SD,  $n = 67$ ) in 2022 and  $3.35 \pm 3.15$  galls (Mean  $\pm$  SD,  $n = 156$ ) in 2023. In 2023, 35% (156 of 452) of collected figs contained midge galls. On the five trees with gall midges present, 56% (156 of 277) of figs were infested with gall midges. The highest number of midge galls we observed in a single fig was 12 in 2022 and 26 in 2023. In the dissected figs of 2022 the *Physothorax* was found in 19.4 percent of the figs with gall midge galls ( $n = 67$ ), and seven out of the eight trees with gall midges also harboured *Physothorax*. No more than two *Physothorax* was found in a single fig ( $n = 67$ ).

The presence of gall midge galls could prevent abortion of unpollinated figs (unentered by pollinator foundresses) if the number of midge galls reached a certain threshold, seemingly around four midge galls. All unpollinated figs that did not contain any gall midge galls aborted. Of

figs containing midge galls, unpollinated figs that remained on the tree contained a higher number of gall midge galls (4,7,7,7,7,8,8,10) than did unpollinated figs that aborted (1,1,1,4), ( $t$ -test  $t = -5.992$ ,  $df = 6.066$ ,  $p = 0.000933$ ).

### 3.1. Description of the gall midge in Panama

The gall midge larvae developed inside the fig in individual galls. The midge galls varied in size but were 2–3 times larger ( $n = 177$ , Fig. 2E) than *P. tonduzi* galls. The midge galls were hard and thick pocket-shaped broad-based outgrowths from the receptacle (fig wall), often with flower remnants on its exterior (Fig. 2). Unlike *Ficiomyia perarticulata* (Roskam and Nadel 1990) there was no observable dimorphism between male and female midge galls ( $n = 36$ ). Midge gall colouration varied from orange/yellow to pale yellow ( $n = 177$ ). Colour dimorphism was observed between adult male and female gall midges ( $n = 36$ ). The abdomen of adult females was pink/red and the thorax and ovipositor were yellow ( $n = 21$ , Fig. 3E). Adult males were pale yellow with spots of grey ( $n = 14$ , Fig. 3E). Both females and males had grey hair sporadically covering their whole body ( $n = 36$ ). The abdominal hair grew in a spotted pattern. Males had fewer antenna flagellomeres (32–33 stalked flagellomere) than females (42–47 stalked flagellomere) ( $n = 5$ ). The general shape of the antennae can be seen in Fig. 3. The gall midge most likely belongs to the *Ficiomyia* family, close to *Ficiomyia perarticulata* (Felt 1922; Roskam and Nadel 1990).

When the gall midge reached the adult stage, it emerged from its gall directly through the receptacle, without passing the fig lumen. White pre-emergence spots were seen developing prior to emergence and the spots remained after emergence on the fig surface ( $n = 99$ , Fig. 1). The white tissue appears to be derived from plant tissue, but further analysis is needed. The gall midge pre-emergence spots started to appear within a week before *P. tonduzi* emerged. The earliest signs noticed of gall midge emergence was 22 days after the receptive stage, i.e. just a few days before pollinator emergence. Pupal exuviae often remained protruding from the emergence hole after adult emergence (Fig. 1: A & B). Each midge gall had an individual emergence hole ( $n = 99$ ).

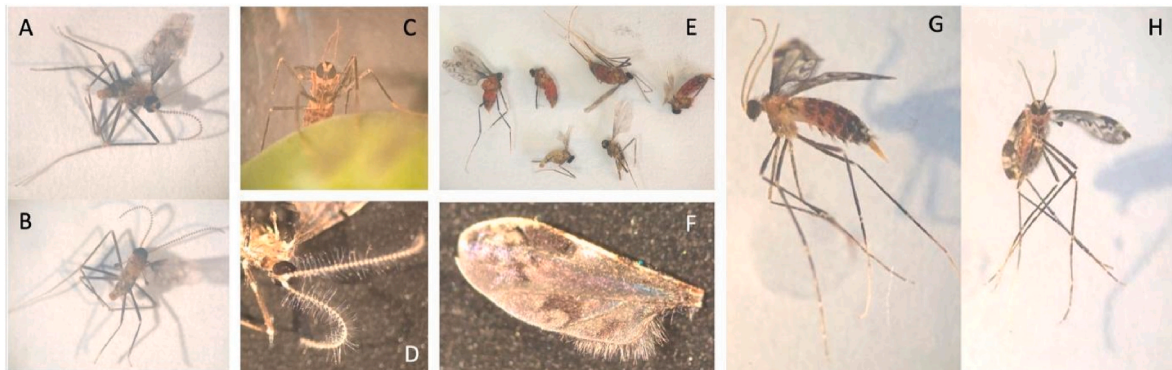
### 3.2. Description of the gall midge parasitoid in Panama

We believe that the *Physothorax* repeatedly found inside midge galls is a parasitoid of the gall midge. *Physothorax* larvae developed inside the fig in individual galls ( $n = 64$ ). The *Physothorax* galls did not differ conspicuously from galls with midges inside, and *Physothorax* occurrence was only established after opening a supposed midge gall or by examining emergence holes. The emergence hole of the *Physothorax* lacked both exuviae and the white tissue found around a gall midge emergence hole ( $n = 64$ , Figs. 1, Figure 4: A-D). Instead, the *Physothorax* emergence hole was similar in appearance (but different in location) to the chewed emergence hole created by *P. tonduzi* males. However, an emergence hole connected to a *Physothorax* gall cannot be chewed by *P. tonduzi* because the gall's walls prevent *P. tonduzi* in the fig lumen from entering the gall ( $n = 64$ , Fig. 4: E – G). It is therefore likely that the *Physothorax* chews itself out with their mandibles, instead of manipulating plant tissue as does the gall midge. A mixed emergence hole was occasionally found on figs where a gall midge pre-emergence spot could be seen together with a chewed *Physothorax* emergence hole (Fig. 4: C & D). We interpret the mixed emergence hole as a *Physothorax* emergence, where the *Physothorax* incapacitated its host only after the gall midge had started to induce its emergence process.

Based on morphology, we identified the parasitoid up to the genus *Physothorax* (Hymenoptera: Torymidae), close to *Physothorax russelli* Crawford (Bouce 1993). Jean-Yves Rasplus kindly confirmed this identification using photos (personal communication June 2023). The *Physothorax* was bright metallic green with large red compound eyes and was 3 mm from head capsule to the abdomen and 6 mm in total from antennae to ovipositor ( $n = 16$ , Fig. 4: G-H, Fig. 5). The ovipositor was



**Fig. 2.** Photos of gall midge pupae and midge galls. A: a midge gall separated from a fig, B: a midge gall cut open showing the internal walls, C: a midge pupa in a cut open gall, D: gall midge pupae from different perspectives, E: fig cut in half where a midge gall can be seen, inside the white circle, as an outgrowth from the receptacle (fig wall).



**Fig. 3.** Photos of the gall midge. A–B: male gall midge, C: newly emerged adult female gall midge resting on a fig, D: head and antenna of male gall midge, E: four females in the upper row and two males in the lower row, F: gall midge wing, G–H: female gall midge. The terminal antennal segments are broken in H.

approximately 3 mm long and protruded from the abdomen at an almost 90-degree angle (Fig. 5B). The red eye colour appeared early on in the pupal stage while the rest of the pupal body was transparent in colour (Fig. 5C).

### 3.3. Barcoding

Seven gall midges and three *Physothorax* individuals were barcoded; sequences are deposited in BOLD, dataset “DS-KOLFMI” ([dx.doi.org/10.5883/DS-KOLFMI](https://dx.doi.org/10.5883/DS-KOLFMI)). BOLD assigned the gall midge a unique BIN (AEZ0215), the *Physothorax* has not yet been assigned a BIN. Within BOLD, the gall midge had a distance to the nearest neighbour of 14.3%; there was a 86% overlap with non-fig gall midges of the family Cecidomyiidae like *Asteromyia carbonifera* and *Asteromyia modesta*, both known from North America (Gagné 1989). The *Physothorax* in our study had 88% overlap with the known gall midge parasitoid *Physothorax bidentulus* from Florida (U.S.A.), associated with *Ficus citrifolia* (Burks 1969; Roskam and Nadel 1990). The relatively low percentage of overlap suggests that there are no previous records of the two species of our study within the BOLD system. Blasting the sequences in the NCBI system did not give different results.

The gall midge effect on the fig tree - fig wasp mutualism.

We first tested whether the presence of midge galls in a fig had any effect on the reproduction of the tree or the pollinator (*P. tonduzi*). The treatment groups (control figs, gall midge figs, & gall midge + *Physothorax* figs) did not significantly differ from each other in the number of seeds ( $F_{2,120} = 0.169$ ,  $p = 0.85$ ; Fig. 6A) or the number of *P. tonduzi*

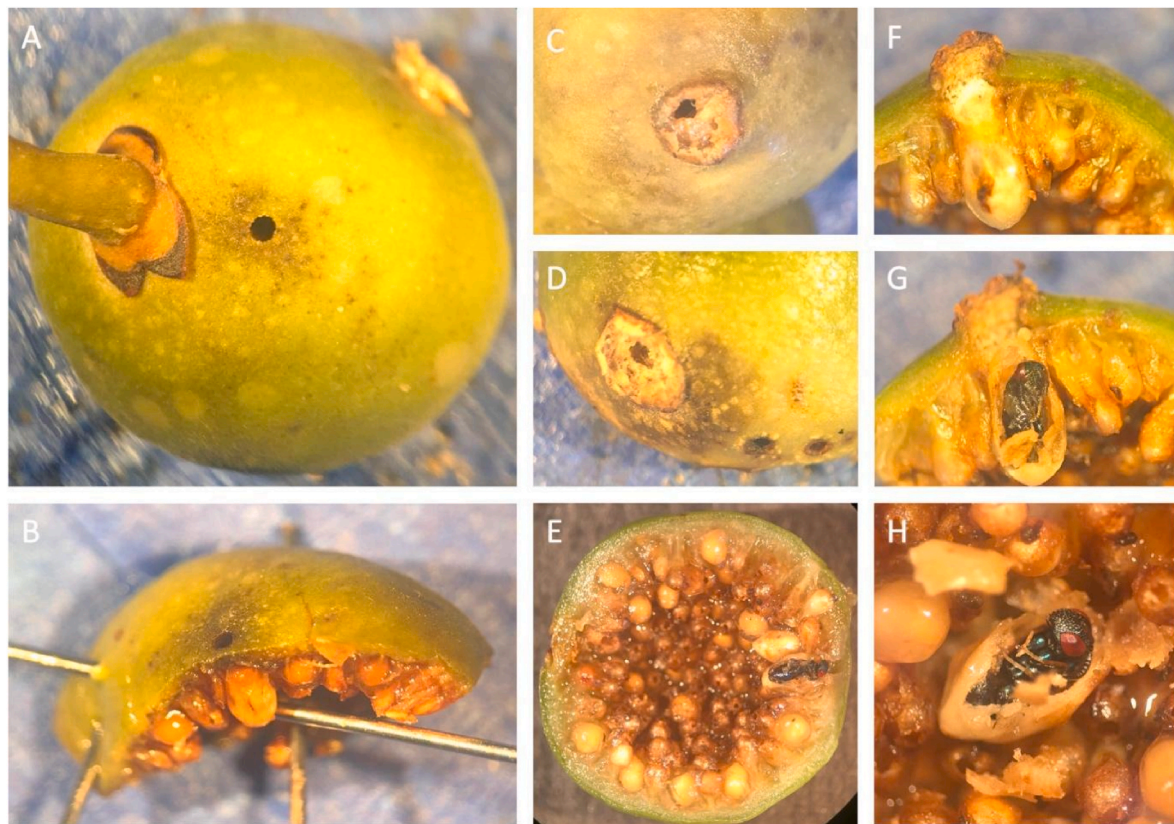
offspring ( $F_{2,120} = 1.85$ ,  $p = 0.16$ ; Fig. 6B). Furthermore, the treatment groups did not significantly differ in total number of flowers ( $F_{2,120} = 2.39$ ,  $p = 0.097$ ), undeveloped pistillate flowers ( $F_{2,120} = 1.09$ ,  $p = 0.34$ ), or bladders ( $F_{2,120} = 2.44$ ,  $p = 0.092$ ).

We then tested whether the number of midge galls in a fig had an effect on the reproduction of the tree or the pollinators. The number of midge galls did not significantly affect the number of seeds in a fig ( $t_{121} = -0.440$ ,  $p = 0.66$ ; Fig. 7A), the number of fig wasp (*P. tonduzi*) offspring ( $t_{121} = -0.446$ ,  $p = 0.66$ ; Fig. 7B), total number of flowers ( $t_{121} = -1.48$ ,  $p = 0.14$ ), or undeveloped pistillate flowers ( $t_{121} = -1.55$ ,  $p = 0.12$ ) (Table A2).

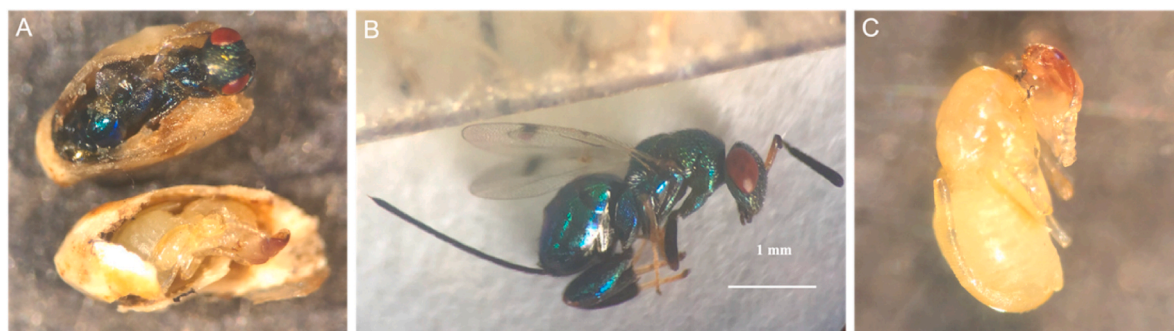
## 4. Discussion

The observed gall midge in this study is either an endemic species previously unnoticed, an infrequent visitor, or a new arrival to central Panama. The level of gall midges inside figs was low during the study period, and the gall midge had no significant effect on the reproduction of the pollinator (*P. tonduzi*) and fig tree. The gall midge most likely belongs to the *Ficiomyia* genus due to its close resemblance to *Ficiomyia perarticulata*.

We only found the gall midge in figs of *Ficus citrifolia*. Other sympatric fig species that produced figs during the study showed no signs of gall midges. This suggests that the gall midge specialises on *F. citrifolia* which is a very common fig species in the area. Similarly, *Ficiomyia perarticulata* was only found in *Ficus citrifolia* figs in Florida and not in the more abundant species *Ficus aurea* (Roskam and Nadel 1990). Both



**Fig. 4.** Photos of the gall midge parasitoid (*Physiothorax*). A: *Physiothorax* emergence hole, B: side view of fig quarter showing *Physiothorax* emergence hole and gall, C–D: mixed emergence holes where a gall midge pre-emergence spot can be seen together with a *Physiothorax* emergence hole, E: fig half displaying *Physiothorax* in its gall, F–G: *Physiothorax* in midge gall before and after dissection, H: close up of *Physiothorax* in midge gall.



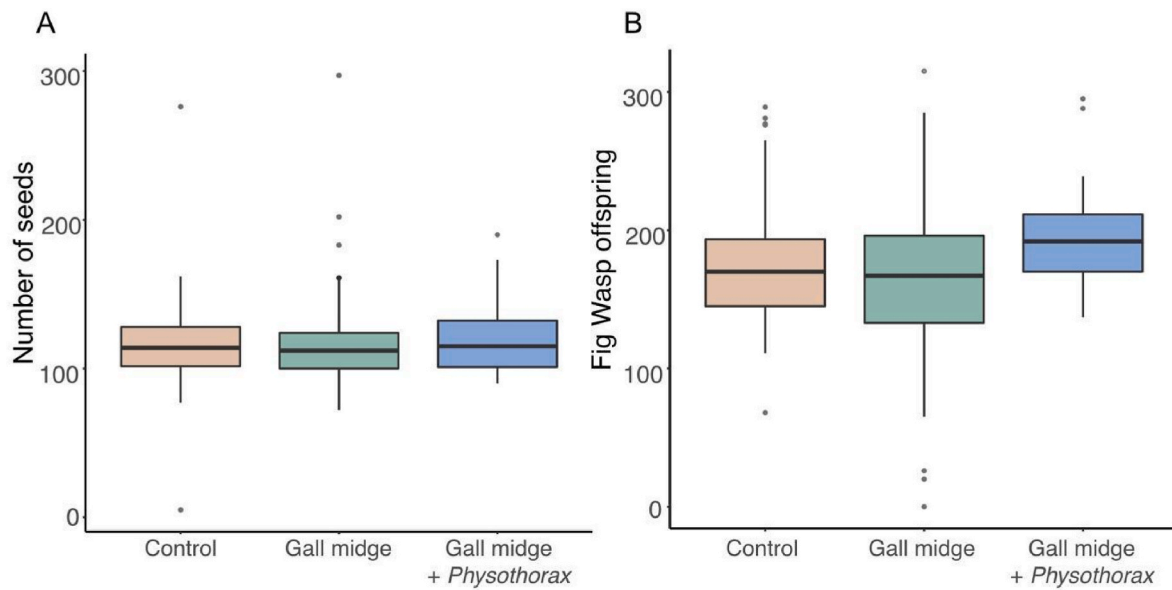
**Fig. 5.** Photos of the gall midge parasitoid (*Physiothorax*). A: *Physiothorax* adult ready for emergence and *Physiothorax* pupa in midge galls, B: adult *Physiothorax*, D: *Physiothorax* pupa.

the gall midge and the pollinator (*P. tonduzi*) are dependent on receptive figs within flying range. The gall midge is therefore thought to reproduce throughout the year similar to *P. tonduzi*.

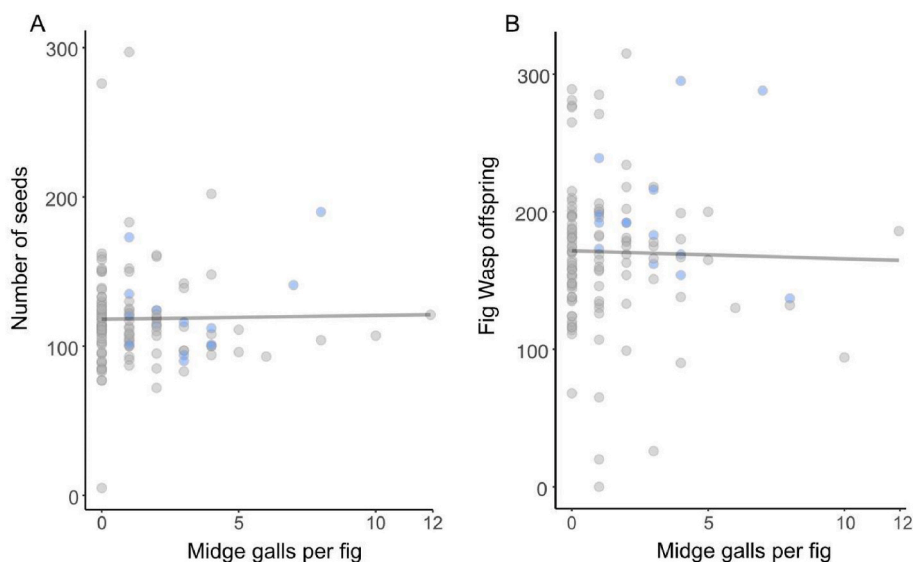
The mean number of midge galls in figs with gall midges was  $2.64 \pm 2.24$  galls (Mean  $\pm$  SD,  $n = 67$ ) in 2022 and  $3.35 \pm 3.15$  galls (Mean  $\pm$  SD,  $n = 156$ ) in 2023. The highest amount of midge galls in a fig was twelve in 2022 and 26 in 2023. Higher levels of gall midge infestation have been observed in China on *Ficus benjamina* where over 250 Cecidomyiidae were found in one fig (Bai et al. 2008). However, local conditions might alter the level of infestation. It should be added that *Ficus benjamina* has a much higher number of female flowers per fig ( $1128 \pm 42.6$  (Mean  $\pm$  SE,  $n = 30$ ) (Bai et al. 2008);) compared to *Ficus citrifolia* ( $325 \pm 38$  (Mean  $\pm$  SD,  $n = 55$ ) (Herre 1989)), which potentially could facilitate increased Cecidomyiidae infestation. However, we expect that the number of flowers does not limit the Panamanian gall midge:

because the Panamanian gall midge induce galls from the receptacle, whereas the *Ficus benjamina* Cecidomyiidae induce galls from female flowers. For receptacle galls, fig size could instead be a limiting factor. However, these two fig species are fairly similar in size (*Ficus benjamina*: mature Fi gs. 12–25 mm in diameter (Bai et al. 2008), *Ficus citrifolia*: 14.1–16.5 mm in diameter (Korine et al. 2000)). The differences in infestation rate might instead be explained by the different Cecidomyiidae taxonomic groups, that the Panamanian gall midge population is likely still increasing, and/or *Physiothorax* abundance. Presence of ants in trees might also reduce the infestation of Cecidomyiidae like it does for parasitic wasps (Jandér 2015) and fig consuming moth larvae (van Kolschoten et al., submitted).

In our data set about two thirds of *F. citrifolia* trees were infested with gall midges both in 2022 and 2023. On trees with gall midges, over 50% of figs were infested in 2023. This relatively high infestation rate could



**Fig. 6.** Boxplots comparing the (A) number of seeds and (B) number of fig wasp (*P. tonduzi*) offspring between three different treatment groups. There was no significant difference between the groups when tree ID was accounted for as a random factor (A:  $F_{2,120} = 0.169$ ,  $p = 0.85$ ; B:  $F_{2,120} = 1.85$ ,  $p = 0.16$ ).



**Fig. 7.** Regression graphs showing no significant relation between the number of midge galls in a fig and (A) the number of seeds and (B) the number of fig wasp (*P. tonduzi*) offspring (A:  $t_{121} = -0.440$ ,  $p = 0.66$ ; B:  $t_{121} = -0.446$ ,  $p = 0.66$ ). The blue dots represent figs in which the *Physiothorax* was present.

hypothetically have negative impact on the *F. citrifolia* population if the number of midge galls per fig increases. We would like to point out that the infestation rates mentioned here are estimates based on the branches that were accessible for inspection, but there could be considerable variation among branches even within the same tree. Some trees were uniformly infested by gall midges while other trees had some branches with no visible signs of gall midge emergence spots and other branches with clusters of gall midge figs.

The gall midge had no significant effect on seed number or number of pollinator (*P. tonduzi*) offspring in this study (Figs. 6 and 7, Table A2). However, if the gall midge effect on *P. tonduzi* offspring number was significant, the effect we found was so small that we suggest it could be neglected with this level of infestation, especially compared to other sources of variation in reproductive success (Herre 1989; Herre 1993, West et al. 1996, Jandér and Herre 2010; van Kolschoten et al., submitted). Although we expect that the gall midges might have a larger

impact on the fig tree - fig wasp mutualism if the infestation rate increases. High levels of fig-associated Cecidomyiidae infestations have severe negative effects on the fig tree - fig wasp mutualism on *Ficus benjamina* in China due to fig abortion, lower number of seeds (20–50% decrease in Cecidomyiidae infested figs) and pollinator offspring (90–100% decrease in Cecidomyiidae infested figs) (Bai et al. 2008; Miao et al. 2011). Another reason why seed number and *P. tonduzi* offspring were not significantly affected in our study might be because we only studied single foundress figs. One foundress might not be capable of saturating all available flowers in a fig and the fig might therefore have sufficient resources for both the developing flowers and the gall midge development.

The gall midge might also have a negative effect on the fig tree by preventing fig abortion. Fig trees can save resources by aborting unpollinated figs; *F. citrifolia* is known to do this (Jandér & Herre 2010, 2016). If abortion is hindered the fig tree will continue to allocate

resources to unpollinated figs without gaining any reproductive benefits. We found that fig abortion of unpollinated figs could be prevented when a threshold of four or more gall midges inside one fig was reached, presumably by the midge galls acting as a strong nutrient sink (Jandér and Herre 2016). Our observation also suggests that gall midge oviposition can occur before the pollinator *P. tonduzi* arrives.

The effect of the *Ficus benjamina* Cecidomyiidae in Bai et al.'s (2008) study was more severe for pollinator offspring than for seeds. Bai et al. hypothesized that the negative effect from Cecidomyiidae is caused by diversion of nutrient resources from other contents of the fig to Cecidomyiidae. Pollinators appeared to be more susceptible to nutrient diversion in comparison to fig seeds. However, Miao et al. (2011) showed that seed weight and quality, measured by germination success and root growth rates, was greatly reduced whenever Cecidomyiidae were present, while seed number was only affected at high Cecidomyiidae densities. The germination success of *Ficus benjamina* in Miao et al.'s study was close to 100% for seeds from control figs, but only 23% for seeds from figs containing Cecidomyiidae. A future study of nutrient diversion and germination success in Panamanian gall midge figs would be of importance to further understand how the gall midge affects the fig tree - fig wasp mutualism. A first step might be to check if seed size and weight are altered in figs with gall midges.

Because *Physothorax* use the same gall as a gall midge, we expect the effect of *Physothorax* on the fig tree - fig wasp mutualism to be similar to a gall midge's effect. However, some other genera of non-pollinating fig wasps (*Idarnes*, *Critogaster*, and *Aepocerus*) have negative effects on the pollinator and seed production (West et al. 1996). *Physothorax* as a genus are parasitoids of fig gallers with multiple hosts. Apart from fig-associated Cecidomyiidae (this study, Roskam and Nadel 1990), *Physothorax* have also been found consuming both *Aepocerus* (West et al. 1996; Conchou et al. 2014) and possibly *Ficicola* sp. (Conchou et al. 2014). *Physothorax* may depress populations of their *Aepocerus* hosts in *Ficus dugandi* (West et al. 1996). Lower infestation rates of *Physothorax* hosts, for example *Aepocerus* or Cecidomyiidae, may benefit the fig tree - fig wasp mutualism.

The gall midge of this study shares more similarities in both morphology and other aspects of its biology with *Ficiomyia perarticulata* (Roskam and Nadel 1990) than with the old-world Cecidomyiidae of *Ficus macrocarpa* (Yafuso et al. 2013) and *Ficus benjamina* (Bai et al. 2008; Miao et al. 2011). Our specimens appear morphologically related to *Ficiomyia perarticulata* (Roskam and Nadel 1990), but we have not yet been able to send specimens to an expert for identification. However, we believe the gall midge in this study to be in the *Ficiomyia* genus because it has more than 25 antennal flagellomeres and the females had more antennal flagellomeres than did the males (Gagné 2018). The only species described so far in the *Ficiomyia* genus is *Ficiomyia perarticulata* which have ♂ 29–31 and ♀ 37–39 stalked flagellomeres. Although the Panamanian gall midge has more antennal flagellomeres for both sexes (♂ 32–33 and ♀ 42–47), the number of flagellomeres are known to be a variable trait in the Lasipteridi, the supertribe of *Ficiomyia* (R. J. Gagné (personal communication 2022)). The infestation rate of the gall midge of this study ( $\bar{x} = 2.64$ ) is also more similar to *F. perarticulata* (a couple to several midge galls per fig (Roskam and Nadel 1990)), compared to the considerably higher rates of the *Ficus macrocarpa* Cecidomyiidae ( $\bar{x} = 25.5$  (Yafuso et al. 2013)) and *Ficus benjamina* ( $\bar{x} = 67.8$  (Bai et al. 2008)). The gall midge of this study induces galls from the receptacle like *F. perarticulata* (Roskam and Nadel 1990) while the Cecidomyiidae of *Ficus benjamina* (Miao et al. 2011) and *Ficus macrocarpa* (Yafuso et al. 2013) develop inside galled female flowers. Furthermore, the emergence holes of the Panamanian gall midge (Fig. 1) and *F. perarticulata* share similarities in their crown-like appearance, pupae exuviae, and neither emerges from host plant tissue projections like *Ficus macrocarpa* Cecidomyiidae (Yafuso et al. 2013). However, *F. perarticulata* galls have clear sexual dimorphism and shared emergence holes (Roskam and Nadel 1990). Gall dimorphism and shared emergence holes were not observed for the Panamanian gall midge.

Within the BOLD database our submitted sequence of the gall midge was assigned a unique BIN, which can be seen as a proxy taxonomic unit (Ratnasingham & Hebert 2007, 2013), and had a distance to the nearest neighbour of 14.3%. This suggests that this is the first sequence of this species to be uploaded to the BOLD system. The closest match that was found in the BOLD database was an 86% overlap with a non-fig-associated Cecidomyiidae. *Physothorax* has, to date, been assigned a BIN. This might be because the uploaded sequences for the *Physothorax*, were no longer than 400 bp. The nearest neighbour of the *Physothorax* in the BOLD database had an 88% overlap, suggesting it not to be the same species. Future taxonomic work and molecular phylogenetic analyses will hopefully clarify the species identities of both the Panamanian gall midge and the *Physothorax* and might link *Physothorax* to a future BIN.

We do not currently know if the gall midge has recently migrated to Panama or if it previously existed in the study area without being noticed. Gall midges are generally difficult to notice by non-specialists due to their small size. Cecidomyiidae presently contains less than 7000 species (Gagné and Jaschof 2021), but recent estimates suggest that there could be 1.8 million taxa (Hebert et al. 2016). The fig gall midge could therefore be an undescribed native species. However, we believe that the gall midge might be a recent arrival to the local fig community because the Panama Canal area is intensely studied by fig researchers, and fig gall midges have not been observed previously inside figs (LD, KCJ, A. Gomez (personal communication), E. A. Herre (personal communication)). Further argument for a recent arrival is the low infestation rate of the gall midge in 2022, followed by a seeming increase in infestation rate in 2023. If the gall midge has recently arrived, and if it can continue to survive and reproduce in Panama, we might in the future see a further increased population with higher infestation rates. The increase would continue until it reaches carrying capacity or gets further stabilised by parasitoids. The *Physothorax* found inside midge galls could have arrived simultaneously with the gall midge, or later. Roskam and Nadel (1990) mentions *Physothorax* parasitoids for *Ficiomyia perarticulata* in Florida. They observed galls parasitised by *Physothorax bidentulus* (Burks 1969) and *Physothorax russelli* in the same samples examined with *Ficiomyia perarticulata*. No fig-associated Cecidomyiidae parasitoids are mentioned for *Ficus microcarpa* nor *Ficus benjamina* (Bai et al. 2008; Miao et al. 2011; Yafuso et al. 2013). Another alternative is that *Physothorax* could be an opportunistic generalist that existed in the area before the gall midge. *Physothorax* species are present in the study area as parasitoids of non-pollinating fig wasps that produce large galls within figs (West et al. 1996; Marussich and Machado 2007; Conchou et al. 2014; Jandér 2015). We would like to see further taxonomic and molecular phylogenetic studies for the entire Neotropics to better understand prevalence and movement of the gall midge and *Physothorax*.

## 5. Conclusion

The gall midge found inside figs of *Ficus citrifolia* in central Panama is presumably either a recent arrival to the area, an infrequent visitor, or an endemic species previously unnoticed. Our species identification and barcoding suggest that (1) the gall midge most likely belongs to the genus *Ficiomyia*, close to *Ficiomyia perarticulata*, and (2) the parasitoid wasp belongs to the genus *Physothorax*, close to *Physothorax russelli*. In this study infestation rates were low, and the gall midge had no significant effect on the reproduction of the fig tree - fig wasp mutualism. However, should infestation rates increase, it is likely that the gall midge would affect the mutualism negatively.

## Data accessibility

Data and R code is available on FigShare (DOI:10.6084/m9.figshare.24910983)

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Authors contributions

Lovisa Dück: Conceptualization (lead), Methodology (supporting), Investigation (supporting), Writing – review and editing (supporting), supervision (supporting). K. Charlotte Jandér: Conceptualization (supporting), Methodology (supporting), Writing – original draft (supporting); Writing – review and editing (supporting), Supervision (supporting). H. Roberta Hedberg: Conceptualization (lead), Methodology (lead), Formal analysis (equal), Investigation (lead), Writing – original draft (lead); Writing – review and editing (equal). Lisette van Kolfsooten: Conceptualization (supporting), Methodology (supporting), Formal analysis (equal), Investigation (supporting), Writing – original draft (supporting); Writing – review and editing (equal), Supervision (lead).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

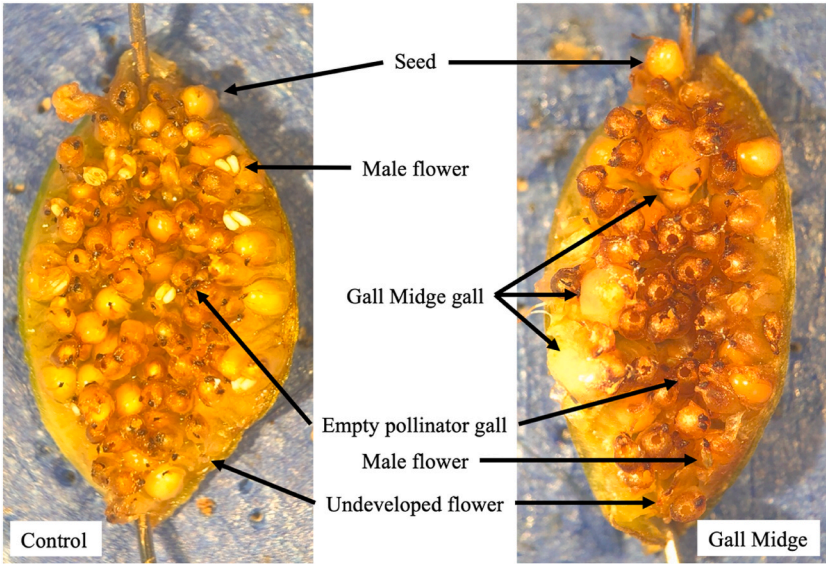
Data availability

Data will be made available on request.

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Appendix



**Fig. A1.** Structures of dissected figs, here split into quarters and pinned for dissection. Control fig (left) and Gall midge fig (right). Fig structures are described by arrows.

**Table A1**  
Number of dissected figs (2022) included in the study per sampled tree.

| Tree ID | Control figs | Gall midge figs | Gall midge + <i>Physothorax</i> | collection Date |
|---------|--------------|-----------------|---------------------------------|-----------------|
| 1       | 10           | 10              | 5                               | 17 Feb 22       |
| 2       | 16           | 16              | –                               | 22 Feb 22       |
| 3       | 14           | 14              | 1                               | 24 Feb 22       |
| 4       | –            | –               | –                               | 25 Feb 22       |
| 5       | –            | –               | –                               | 08 Mar 22       |
| 6       | 5            | 5               | 5                               | 08 Mar 22       |
| 7       | 5            | 3               | 3                               | 16 Mar 22       |
| 8       | 5            | 5               | –                               | 09 Apr 22       |

**Table A2**  
The results of the regressions (LMER). Degree of syconia gall midge infestation (independent variable) against the different measured response variables. Presence of the *Physothorax* parasitoid was set as a fixed effect and tree ID as random factor.

| Response variables              | Fixed effect             | Estimate | SE     | df   | t-value | Pr (> t ) |
|---------------------------------|--------------------------|----------|--------|------|---------|-----------|
| Fig seeds                       | Intercept                | 119      | 4.00   | 3.19 | 29.7    | <0.001    |
|                                 | Gall midge               | −0.696   | 1.58   | 118  | −0.440  | 0.661     |
|                                 | Physothorax              | −8.00    | 10.1   | 117  | −0.791  | 0.430     |
|                                 | Gall midge + Physothorax | 3.22     | 2.23   | 115  | 1.45    | 0.151     |
| Total NO. of fig wasp offspring | Intercept                | 171      | 10.2   | 6.61 | 16.7    | <0.001    |
|                                 | Gall midge               | −0.980   | 2.20   | 117  | −0.446  | 0.657     |
|                                 | Physothorax              | 10.1     | 8.73   | 119  | 1.15    | 0.252     |
|                                 | Intercept                | 155      | 14.0   | 5.93 | 11.1    | <0.001    |
| Empty fig wasp galls            | Gall midge               | −0.427   | 2.35   | 116  | −0.181  | 0.856     |
|                                 | Physothorax              | 5.56     | 9.40   | 119  | 0.591   | 0.556     |
| Total no. of flowers            | Intercept                | 418      | 15.176 | 5.64 | 27.5    | <0.001    |
|                                 | Gall midge               | −3.28    | 2.22   | 115  | −1.48   | 0.141     |
|                                 | Physothorax              | 12.0     | 8.88   | 118  | 1.35    | 0.180     |
|                                 | Intercept                | 84.6     | 15.4   | 5.50 | 5.48    | 0.00204   |
| Undeveloped flowers             | Gall midge               | −2.80    | 1.80   | 115  | −1.55   | 0.123     |
|                                 | Physothorax              | −2.45    | 7.25   | 117  | −0.339  | 0.736     |

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