MORPHOLOGICAL VARIATIONS OF EPIPHYTIC *Ficus deltoidea* (Moraceae) AND ITS AGAONID POLLINATORS, *Blastophaga* spp.

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ABSTRACT

The interaction between fig trees (*Ficus*) and their fig wasp pollinators (Agaonidae) is usually described as obligate mutualism. *Ficus deltoidea* (Moraceae) is distributed across southern Southeast Asia. Currently, *F. deltoidea* is regarded as only one species with 13 recognised varieties, and seven of them are native to Peninsular Malaysia. In this study, the morphological variation and relationship between epiphytic *F. deltoidea* var. *angustifolia*, var. *deltoidea*, and var. *trengganuensis* and their pollinating fig wasp in selected oil palm plantations from January 2017 to September 2017 were investigated. The ostiole diameter, gall width, and thickness of fig wall as well as fig wasp morphology were measured using image analyser. The female fig wasp associated with var. *trengganuensis* recorded the biggest value for wing length, mandible length, hind tibia length, and overall body length, whereas fig wasp associated with var. *deltoidea* recorded the highest value for head length, wing width, and ovipositor length. Fig wasp associated with var. *angustifolia* recorded the smallest value for all morphological parameters measured. Similarly, the male fig wasp associated with var. *trengganuensis* recorded the biggest value for mandible length, hind tibia length, and overall body length. There was a significant relationship between mandible size of male fig wasps and fig wall thickness (F=15.92, $P<0.05$), female fig wasp head width and ostiole diameter (F=9.02, $P<0.05$), and the male and female fig wasp with gall size (male: F=164.34, $P<0.05$; female: F=47.39, $P<0.05$) across the varieties. The results from this research explain that the morphological adaptations towards the structure of the figs occurred in the fig wasp's evolution. The mutualism between the fig and fig wasp is also extremely specific across varieties in which even the same species of fig tree can have different pollinating fig wasps associated with different varieties.

Keywords: Fig, fig wasp, mutualism, sister species

ABSTRAK

Interaksi antara pokok ara (*Ficus)* dan penyengat ara (Agaonidae) dikenali sebagai mutualisme obligat. *Ficus deltoidea* (Moraceae) mempunyai taburan di seluruh selatan Asia Tenggara dan sehingga kini *F. deltoidea* diklasifikasikan sebagai satu spesies dengan 13 jenis varieti dan tujuh daripadanya berasal dari Semenanjung Malaysia. Dalam kajian ini, variasi morfologi dan hubungan antara *F. deltoidea* var. *angustifolia*, var. *deltoidea* dan var. *trengganuensis* dan penyengat aranya dari ladang kelapa sawit terpilih telah dikaji dari Januari 2017 hingga September 2017. Pengukuran diameter ostiol, lebar puru dan ketebalan dinding buah ara serta morfologi penyengat ara diukur menggunakan "Image Analyser". Penyengat ara betina dari var. *trengganuensis* mencatatkan nilai terbesar untuk ukuran panjang sayap, panjang mandibel, panjang tibia kaki belakang dan panjang keseluruhan badan manakala penyengat ara dari var. *deltoidea* mencatatkan nilai tertinggi untuk panjang kepala, lebar sayap dan panjang ovipositor. Penyengat ara dari var. *angustifolia* mencatatkan nilai terkecil untuk semua parameter morfologi yang diukur. Sementara itu, penyengat ara jantan dari dengan var. *trengganuensis* mencatatkan nilai terbesar untuk panjang mandibel, panjang tibia kaki belakang dan panjang keseluruhan badan. Terdapat hubungan yang ketara antara ukuran saiz mandibel penyengat ara jantan dengan ketebalan dinding ara (F=15.92, $P<0.05$), lebar kepala penyengat ara betina dan diameter ostiol (F=9.02, P<0.05), dan saiz puru penyengat ara jantan dan betina (jantan: F=164.34, $P<0.05$; betina: F=47.39, $P<0.05$) merentasi varieti. Hasil yang diperoleh dari penyelidikan ini menunjukkan bahawa penyesuaian morfologi terhadap struktur buah ara berlaku dalam evolusi penyengat. Mutualisme antara buah ara dan penyengat ara juga sangat spesifik di mana spesies pokok ara di bawah spesies yang sama boleh mempunyai penyengat ara yang berbeza yang mengikut varieti.

Kata kunci: Buah ara, penyengat ara, mutualisme, spesies beradik

INTRODUCTION

Ficus is one of the largest genera of flowering plants as it comprises more than 800 species from all over the world (Frodin 2004). These species can be found either as shrubs, trees, epiphytes, or hemiepiphytes (Harrison 2005). *Ficus* is possibly the most important food source to frugivores in lowland tropical rainforests (Harrison 2005) and more generally (Shanahan et al. 2001). *Ficus* produces an enclosed, urn-shaped inflorescence known as a fig (Harrison 2005) that acts as a platform for mutualistic relationship with its pollinators (Cook & Rasplus 2003). The mutualism between the fig and fig wasp pollinators is widely known and studied (Cook & Rasplus 2003) as they show an obligate mutualism, where neither of them can live without the other. The mutualism between fig and fig wasp pollinators is said to be species-specific, in which one kind of fig is pollinated only by a specific fig wasp (Hossaert-McKey et al. 2016: Ma et al. 2009) and vice versa. The growth and development of each *Ficus* species is highly dependent on its specific wasp pollinator (Harrison 2005). The fig wasps and figs are believed to coevolve in regard to their physical traits which maximise their mutualism, and the interaction has frequently been used to study the costs and advantages of reproductive successes to both parties (Herre 1989).

Ficus species exhibits two breeding systems, namely dioecy and monoecy. Roughly half of all fig species are monoecious, with individual inflorescences providing both female (seed production and dispersal) and male (pollen production and dispersal) reproductive functions (Herre et al. 2008). The remaining *Ficus* species are functionally dioecious. In these species, there are two types of trees: female trees that produce only seed-bearing figs and male trees with figs that produce only pollen and pollinator wasp progeny to transport the pollen (Patel & Hossaert-McKey 2000).

Fig wasps from the family Agaonidae need brood sites in figs for the development of their young. They can only reproduce within the figs they pollinate as they are definite pollinators of *Ficus* species (Kjellberg et al. 2001). This sole pollinator of fig trees has a length ranging from less than 1 mm to around 2 mm with different features for adult male and female fig wasps based on their role for the pollination, where the females have wings and the males are wingless (van Noort 2003). With these specific features, only the female fig wasps are capable of flying and pollinating the flowers. The pollination in *Ficus* starts when specific volatile compounds are released from receptive figs to attract the specific fig wasps associated with each species of fig tree (Hossaert-McKey et al. 2016). The female fig wasp (known as a foundress when she enters a receptive fig) detects the volatiles and flies towards the receptive figs (Molbo et al. 2003). The foundresses can be seen hovering around receptive figs (Ware & Compton 1992) before penetrating through a narrow (usually) bract-lined tunnel ostiole (Liu et al. 2013).

The ostiole is located on top of the fig, which leads to a bract-lined tunnel and eventually into the fig cavity, where the flowers are found (Eisikowitch & Ghara 2015). The female fig wasp loses its wings and antennae on the way in, so it cannot fly away to other figs (Mohd Hatta et al. 2021). Once inside, it pollinates the flowers (Nefdt & Compton 1996) using the pollen from its natal fig (Herre et al. 2008) and at the same time lays eggs in some of the flowers (Kjellberg et al. 2005). Only one egg is laid per flower (Ghana et al. 2012). A single ovule then provides the site for larval development and turns into a seed-sized gall (Kjellberg et al. 2005). The larva feeds on the endosperm (Deng et al. 2016) that developed from either double fertilisation or parthenogenesis (Borges & Kjellberg 2014).

The wingless adult male fig wasps hatch first from their galls and search for galls containing females (Yang et al. 2002). The males bite holes into the female galls and insert their genitalia in order to mate (Cook &Segar 2010). Female wasps then emerge into the fig cavity, and at this time, the male fig flowers are mature and have mature pollen. After the females collect some pollen either actively or passively, the male fig wasps bore a hole to let the pollen-bearing female fig wasps get out from the natal fig (Weiblen 2002). The females then search for other receptive figs to deposit their eggs and pollinate the flowers (Nefdt & Compton 1996).

Ficus deltoidea Jack. (Moraceae) is currently placed in subgenus *Ficus*, section *Ficus*, subsection *Frutescentiae* (Berg & Corner 2005). Its distribution includes Thailand, Indonesia, and Malaysia (Cardellina 2012). Corner (1969) classified 13 varieties in *F. deltoidea*, and seven of them are found in Peninsular Malaysia. Subsequent treatments have been based on this work, and the taxonomy of the group has remained essentially unchanged since 1969, except that many of his varieties were merged by Berg & Corner (2005). This study is concordant with the studies by Mat et al. (2012) and Nur Fatihah et al. (2014), which found that those seven varieties are native to Peninsular Malaysia. The varieties are var. *angustifolia* (Miq.), var. *deltoidea* Corner, var. *trengganuensis* Corner, var. *kunstleri* King, var. *bilobata* Corner, var. *motleyana*

(Miq.), and var. *intermedia* Corner. Leaf morphology and anatomy are often used for the identification of *F. deltoidea* varieties because they are particularly discriminative (Nur Fatihah et al. 2014), but leaf shape often varies greatly between young and mature plants.

Ficus deltoidea is dioecious, with male and female plants that either supports the development of fig wasp pollinator offspring or produce seeds, respectively. It is unusual among *Ficus* species in that the figs produced by female plants contain only small numbers of flowers and sometimes just a single flower (Corner 1969). Its seeds are also unusually large, a feature that may be linked to the plant's ability to grow as a true epiphyte (Corner 1969). The growth form of *F. deltoidea* varies between varieties and includes true epiphytes (rather than hemiepiphytic stranglers), terrestrial bushes, and small trees (Starr et al. 2003). They commonly occupy coastal, heathland, and montane habitats (Starr et al. 2003). Several varieties, including *F. deltoidea* var. *angustifolia*, also occur regularly as epiphytes in oil palm plantations in Peninsular Malaysia (Mohd Hatta 2019).

Information on the pollination process of *F. deltoidea* and its fig wasp pollinator is still lacking. A general pattern regarding the subgenus and section *Ficus* has been established, where the dioecious subgenus *Ficus* section *Ficus* is usually pollinated by a fig wasp from the genus *Blastophaga* (Wiebes 1979). *Blastophaga quadrupes* Mayr is the only recorded pollinator of *F. deltoidea*. It probably pollinates var. *lutescens* Desf. as it was collected in Java and Sumatra (Wiebes 1993). Whether varieties have different species of host-specific associated pollinators provides an indication of likely barriers to gene flow between them. Among the factors that maintain specificity in the fig and fig wasp mutualism are fig wasp behavioural responses and morphological adaptations towards the structure of the figs (Liu et al. 2013). In closely related fig tree species, pollinator host specificity is maintained\ by combinations of long-range cues from floral scent, short-range contact cues, and physical matching between the fig wasp and its host (Wang et al. 2013). This host specificity between fig and fig wasps ensures that genetic integrity is maintained (van Noort 2003).

Previously, the classification of *F. deltoidea* varieties was based solely on plant morphology. Biological features, including the identity of pollinators (the behaviour of which largely determines gene flow in *Ficus*), were not taken into account when describing *F. deltoidea*. Consequently, sympatric biologically distinct taxa (with gene flow not taking place between them) are not always recognised as being distinct species using the current morphological species concept.

We sought to understand whether some of the Malaysian varieties of *F. deltoidea* are likely to be distinct species morphologically. Specifically, the questions are as follows: (1) Are there morphological differences between the figs and fig wasps of several *F. deltoidea* varieties? (2) Are any morphological differences in the fig wasps related to variations in their host figs? Our study can give additional information to update the classification of this complex relationship and explore their evolution.

MATERIALS AND METHOD

Morphology of *Blastophaga* spp.

The morphological differences of the pollinators for three varieties of *F. deltoidea* were examined (var. *angustifolia*, var. *deltoidea*, and var. *trengganuensis*). Morphological parameters of head width, head length, wing width, wing length, mandible width, mandible length, tibia length, ovipositor length, and overall body length (Figure 1) were measured from female fig wasps.

Figure 1. Morphological parameters for female fig wasp: (a) head length and width at 150× magnification, (b) wing length and width at 70× magnification, (c) mandible width and length at 10× magnification, (d) overall body size at 70× magnification, (e) tibia length at 150× magnification, and (f) ovipositor length at 150× magnification

Similar parameters were measured in the male wasps with the exclusion of wing width, wing length, and ovipositor length (Figure 2). Pollinators for var. *angustifolia* figs were collected at four oil palm plantations in Bagan Serai (Perak), Banting and Dengkil (Selangor) as well as Batu Pahat (Johor). The Batu Pahat plantation also provided the figs for var. *deltoidea*, and Tembila plantation provided the figs for var. *trengganuensis*. The study sites were chosen because they had *F. deltoidea* growing as epiphytes on the oil palm trunks. Measurements were made on 30 fig wasps (15 females and 15 males) from mature male figs (*sensu* Galil & Eisikowitch 1968) collected from five different trees of each variety (n = 90). During the mature phase, the next generation of fig wasp offspring emerge from their galls. Usually, figs at this phase have a softish feel when squeezed, though no clearly defined central cavity develops (Mohd Hatta 2019). The colour of mature figs is usually yellow-green, and the anthers of the male flowers start to dehisce.

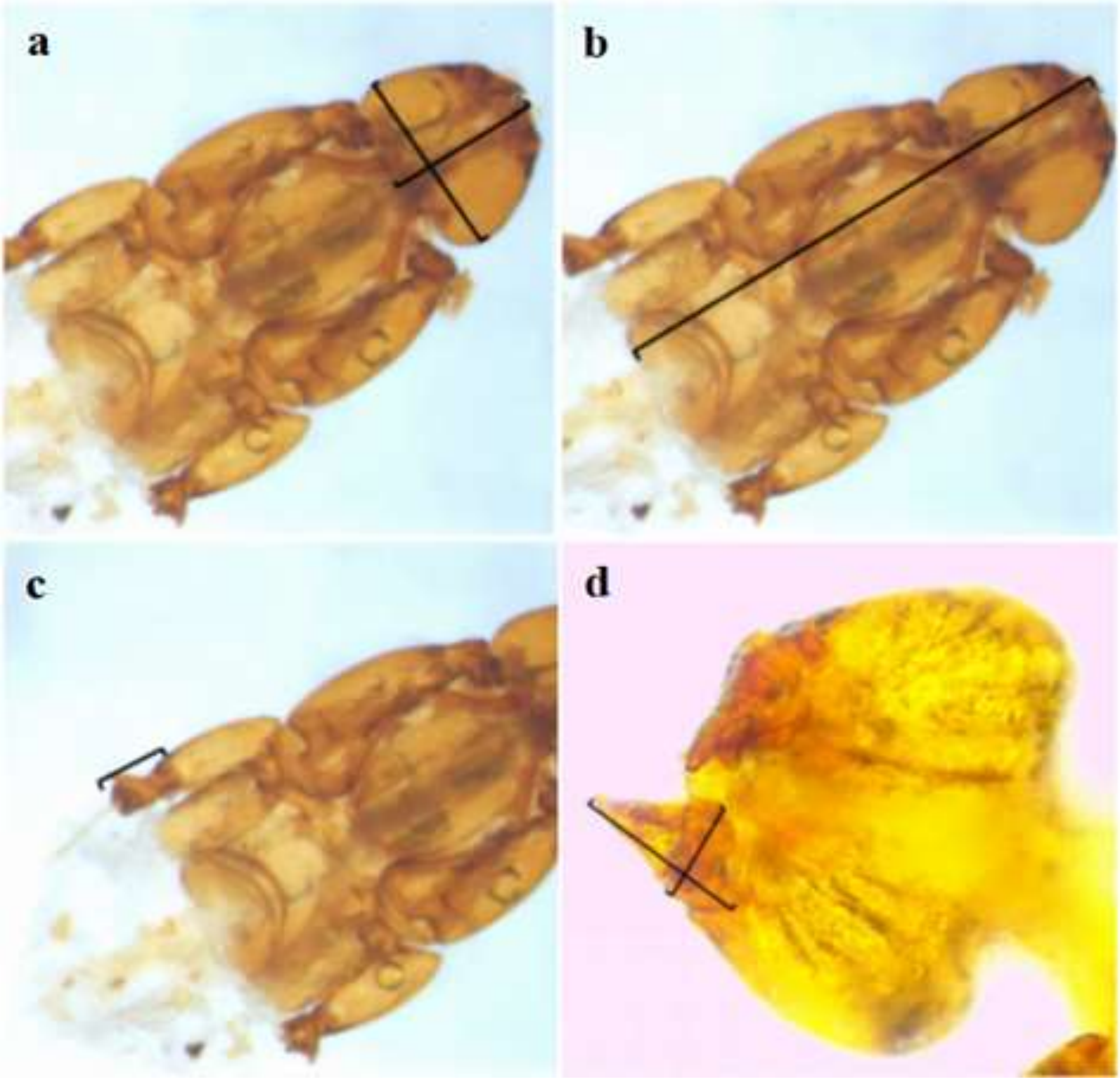

Figure 2. Morphological parameters for male fig wasp: (a) head length and width at 150× magnification, (b) head length and thorax at 100× magnification, (c) tibia length at 150× magnification, and (d) mandible width and length at 10× magnification.

The figs from the same tree were placed together in a container covered by fine mesh to allow the pollinators to emerge naturally. The container was kept at room temperature. The fig wasps emerged a few hours after collection. Individual fig wasps were selected at random from those that emerged from the figs. Morphological parameters were measured by placing the females in a drop of water on a glass slide under a coverslip. The females were squashed with moderate pressure to displace the ovipositor sheaths for ovipositor measurement without breaking the ovipositor. All parameters were measured using an image analysis software (Carl Zeiss V12), while the mandibles were measured using MT image analysis microscope (i-Solution IMTcamCCD5).

Fig wasp head length was measured at maximum length, which includes the protrusion of the clypeus edge, while the head width was measured at the largest width across the compound eyes (*sensu* van Noort & Compton 1996). The overall body size of a female fig wasp is the length of the fig wasp which takes into account the length of the head, thorax, and gaster. The body size of a male fig wasp is the length of the head and thorax (*sensu* Wiebes 1993). The mandible size is taken from length times width of the mandible measurements.

Morphology of Figs from Three Different *F. deltoidea* Varieties

A total of 15 receptive male figs from five different trees were collected for each variety of *F. deltoidea* (total of three varieties) and placed in a plastic bag (15 × 13 cm). The figs from the same tree were placed together (*n* = 45). All figs were taken to the laboratory for the measurement of the ostiole diameter and fig wall thickness (Figure 3). The measurements of gall width, fig wall thickness, and ostiole diameter were taken using a Carl Zeiss V12 analyser microscope at 25× magnification while the fig was in fresh condition (IPGRI & CIHEAM 2003; *sensu* Storey 1975).

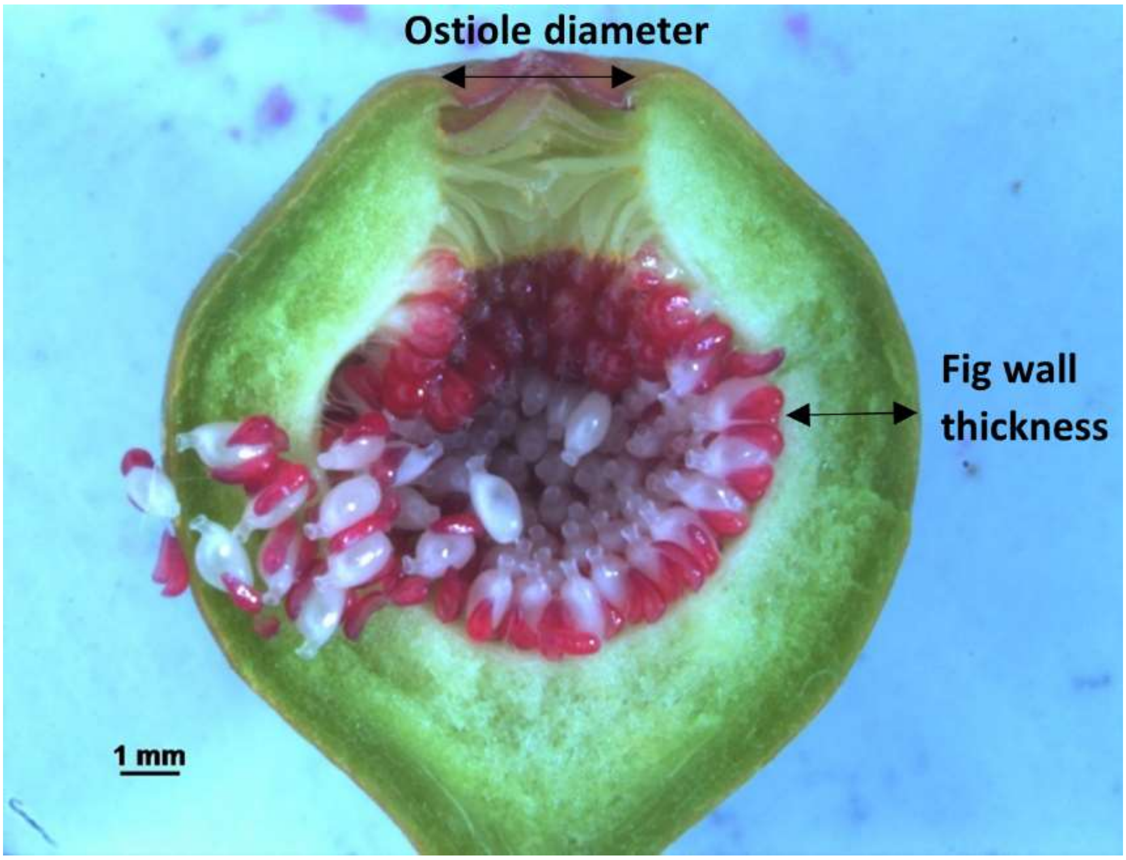

Figure 3. Male fig morphological parameters at 25× magnification

Data Analysis

Statistical analyses were performed by using SPSS Statistics 20. The morphological variations within and across *F. deltoidea* varieties were analysed using one-way ANOVA followed by Tukey tests. Pearson correlation and linear regression were used in examining the relationships between the morphology of the figs and the fig wasps across and within varieties of *F. deltoidea*.

RESULTS

Variation Between Fig Wasps from Three *F. deltoidea* Varieties

Measurements of the head length, head width, wing length, wing width, mandible length, mandible width, tibia length, ovipositor length, and overall body length of female fig wasps from three different varieties of *F. deltoidea* showed that the var. *angustifolia* wasps recorded the lowest measurement for all parameters (Table 1). Fig wasps from var. *deltoidea* recorded the highest value for head length (0.30±0.00mm), wing width (0.49±0.01 mm), and ovipositor length (0.54±0.01mm). On the other hand, fig wasps from var. *trengganuensis* recorded the highest value for wing length (1.01±0.08mm), tibia length (0.17±0.01 mm), and overall body length (1.43±0.03 mm). For head and mandible width, var. *deltoidea* and var. *trengganuensis* shared the highest value with 0.31±0.02 mm and 0.10±0.01 mm, respectively.

Table 1. Morphological parameters (mean±SD) of female fig wasps from three *F. deltoidea* varieties (var. *angustifolia*, var. *deltoidea*, dan var. *trengganuensis*). Values with different superscripts differ significantly (Tukey test, $P<0.05$)

Morphological Parameters	**Female Fig Wasps (*Blastophaga* spp.)** var. *angustifolia*	var. *deltoidea*	var. *trengganuensis*
Head length (mm)	0.26 ± 0.02^{a}	0.30 ± 0.01^{b}	0.28 ± 0.02^{c}
Head width (mm)	0.29 ± 0.03^{a}	0.31 ± 0.02^{b}	0.31 ± 0.02^{b}
Wing length (mm)	0.84 ± 0.04^{a}	0.98 ± 0.22^{ab}	1.01 ± 0.08^{c}
Wing width (mm)	0.41 ± 0.02^{a}	0.49 ± 0.02^{b}	0.48 ± 0.34^{b}
Mandible length (mm)	0.14 ± 0.02^{a}	0.14 ± 0.04^{a}	0.15 ± 0.01^{a}
Mandible width (mm)	0.09 ± 0.01^{a}	0.10 ± 0.01^{b}	0.10 ± 0.00^{b}
Tibia length (mm)	0.14 ± 0.01^{a}	0.16 ± 0.23^{a}	0.17 ± 0.01^{a}
Overall body length (mm)	1.11 ± 0.06^{a}	1.29 ± 0.05^{b}	1.43 ± 0.03^{c}
Ovipositor length (mm)	0.44 ± 0.02^{a}	0.54 ± 0.02^{b}	0.51 ± 0.01^{b}

Fewer morphological parameters were measured for male fig wasps due to the lack of wings and ovipositor. Male fig wasps from *F. deltoidea* var. *trengganuensis* showed the highest values for all parameters. The highest values of head length and head width were the same for *F. deltoidea* var. *trengganuensis* with var. *angustifolia* and var. *deltoidea* respectively. Those three varieties shared the same mean values for mandible width (Table 2). Apart from having significant differences between all three varieties in most parameters, the mean values for mandible width showed great similarities between the varieties.

Table 2. Morphological parameters (mean±SD) of male fig wasps from three *F. deltoidea* varieties (var. *angustifolia*, var. *deltoidea*, and var. *trengganuensis*). Values with different superscripts differ significantly (Tukey test, $P<0.05$)

Morphological parameters	**Male fig wasps (*Blastophaga* spp.)** var. *angustifolia*	var. *deltoidea*	var. *trengganuensis*
Head length (mm)	0.18 ± 0.02^{a}	0.16 ± 0.02^{b}	0.18 ± 0.02^{a}
Head width (mm)	0.24 ± 0.01^{a}	0.27 ± 0.01^{b}	0.27 ± 0.01^{b}
Mandible length (mm)	0.09 ± 0.00^{a}	0.08 ± 0.01^{b}	0.10 ± 0.00^{c}
Mandible width (mm)	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}
Tibia length (mm)	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}	0.11 ± 0.01^{b}
Overall body length (mm)	0.63 ± 0.04^{a}	0.63 ± 0.03^{a}	0.81 ± 0.03^{b}

Fig and Fig Wasp Inter-Relationships

The ostiole diameter and the head width for all three varieties of *F. deltoidea* were negatively correlated (Figure 4). A significant negative correlation was found for *F. deltoidea* var. *angustifolia* (Pearson correlation, $r=-0.63$, $F=8.55$, $P<0.05$) and *F. deltoidea* var. *trengganuensis* (Pearson correlation, $r=-0.51$, $F=4.66$, $P<0.05$), while a nonsignificant relationship was found for *F. deltoidea* var. *deltoidea* (Pearson correlation, $r=-0.31$, $F=1.36$, $P>0.05$). Linear regressions were used to examine relationships across and within variables. There was a strong relationship between head width and ostiole pedicel length in female fig wasps (linear regression, $R^2=0.32$, $F=9.02$, $df=44$, $P<0.05$). Within varieties, there was no relationship between head width and ostiole pedicel line of the female pollinator from var. *deltoidea* (linear regression, $R^2=0.09$, $F=1.36$, $df=14$, $P>0.05$), while the other two varieties showed a strong relationship with $P<0.05$. Linear regression was also used together to examine relationships across and within variables in the male fig wasps, and no relationship between head width and ostiole diameter was observed across ($R^2=0.49$, $F=1.32$, $df=44$, $P>0.05$) and within varieties.

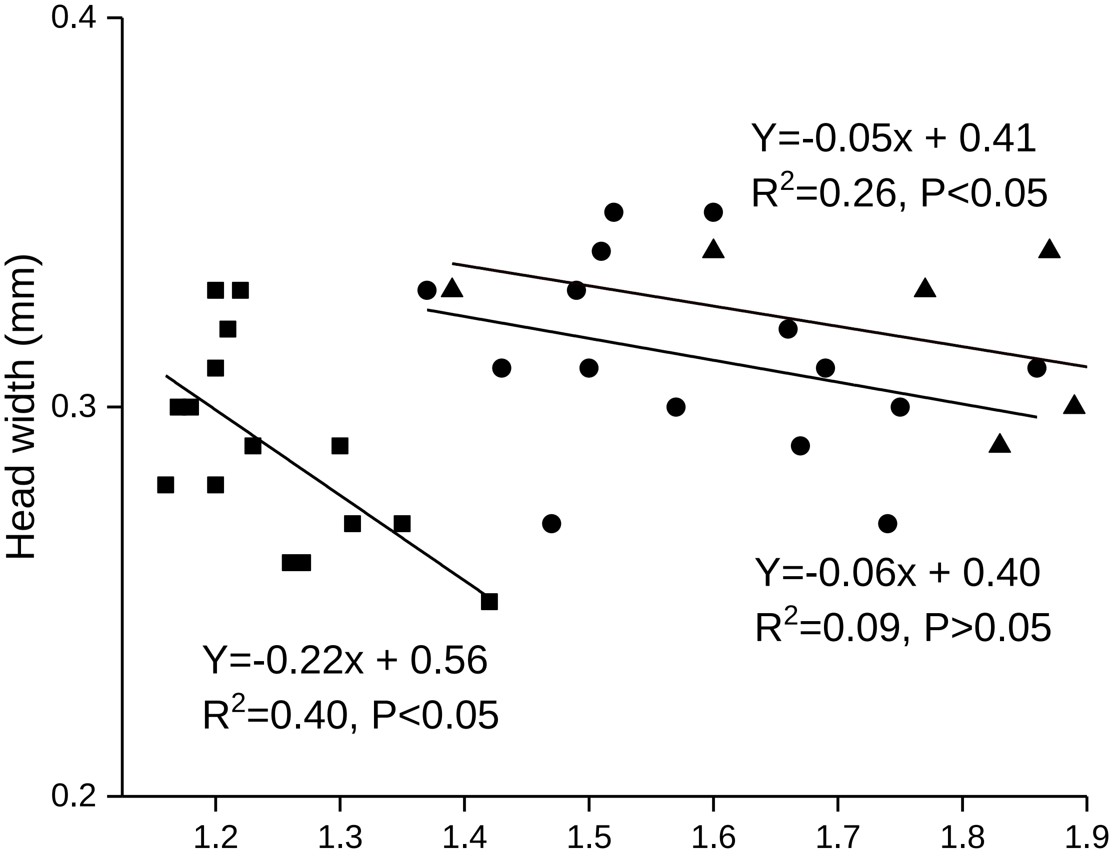

Figure 4. Relationship between head width of female fig wasps and size of ostiole pedicel line in three *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*

There was a nonsignificant negative correlation between the overall body length of male fig wasps and gall width for var. *angustifolia* (Pearson correlation, $r=-0.17$, $F=0.38$, $P>0.05$) and var. *trengganuensis* (Pearson correlation, $r=-0.25$, $F=0.86$, $P>0.05$), while the relationship was positively correlated in var. *deltoidea* but not significant (Pearson correlation, *r*=0.06, *F*=0.05, *P*>0.83) (Figure 5). Overall, gall size did not influence the size of the fig wasps within the same variety (*P*>0.05), but there was a strong relationship between the overall body size and head width of male fig wasps across the three varieties (linear regression, R^2=0.77, *F*=47.39, *df*=44, *P*<0.05).

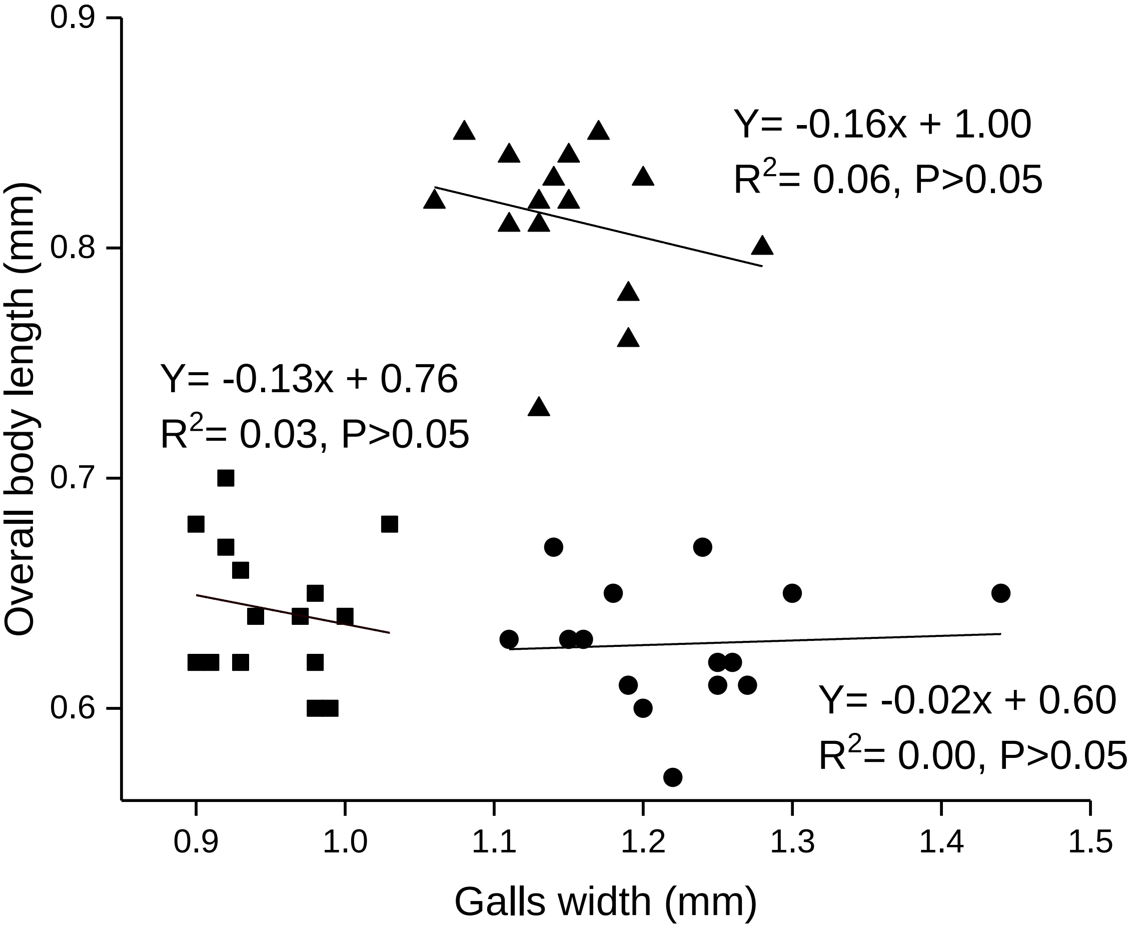

Figure 5. Relationship between overall body length of male fig wasps and gall width in three *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*

The overall length of the female fig wasps from var. *angustifolia* (Pearson correlation, *r*=−0.03, *F*=0.01, *P*>0.05) and var. *deltoidea* (Pearson correlation, *r*=−0.20, *F*=0.54, *P*>0.05) were negatively correlated with gall width, while a positive correlation was found between the overall body length of female fig wasps and gall size in var. *trengganuensis* (Pearson correlation, *r*=0.63, *F*=8.51, *P*<0.05) (Figure 6). We also observed the relationships across and within varieties. A strong relationship between overall body size and gall width in the female figs across varieties (linear regression, R^2=0.89, *F*=164.34, *df*=44, *P*<0.05) was only significant within var. *trengganuensis* (linear regression, R^2=0.06, *F*=0.86, *df*=14, *P*>0.05).

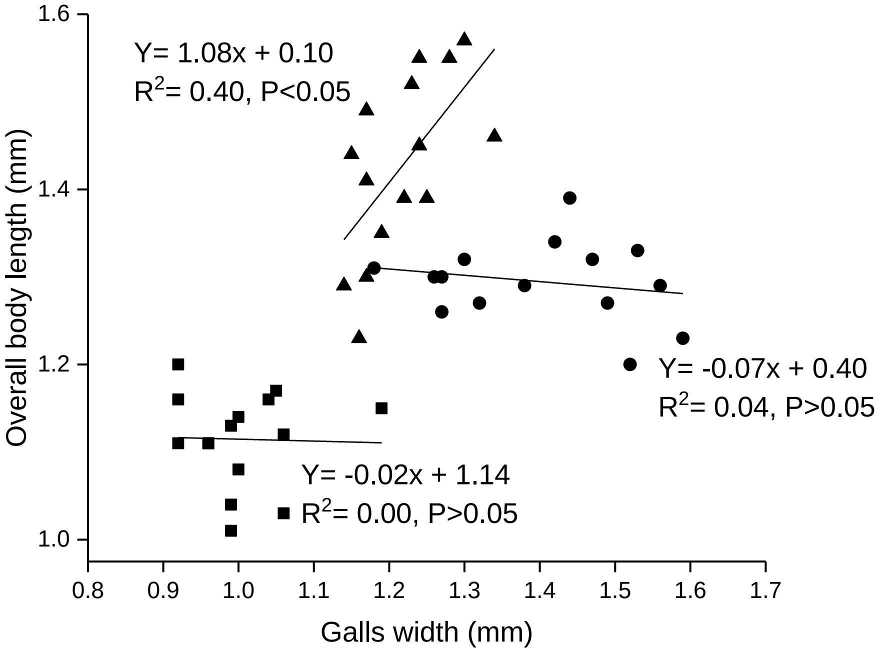

Figure 6. Relationship between overall body length of female fig wasps and gall width in three *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*

A positive correlation was found between fig wall thickness and mandible size of male fig wasps in var. *angustifolia* (Pearson correlation, r=0.10, F=0.14, P>0.05) and var. *deltoidea* (Pearson correlation, r=0.22, F=0.13, P>0.05), and they were negatively correlated in var. *trengganuensis* (Pearson correlation, r =–0.22, F=0.67, P>0.05) (Figure 7). A strong relationship between mandible size and fig wall thickness was observed in the male wasps when the varieties were compared (linear regression, R^2=0.43, F=15.92, df=45, P<0.05), but not in the female wasps (linear regression, R^2=0.09, F=1.62, df=45, P>0.05). Within varieties, there was no relationship between mandible size and fig wall thickness of the female (linear regression, R^2=0.04, F=0.86, df=45, P=0.43) and male pollinators (linear regression, R^2=0.01, F=0.12, df=45, P=0.88).

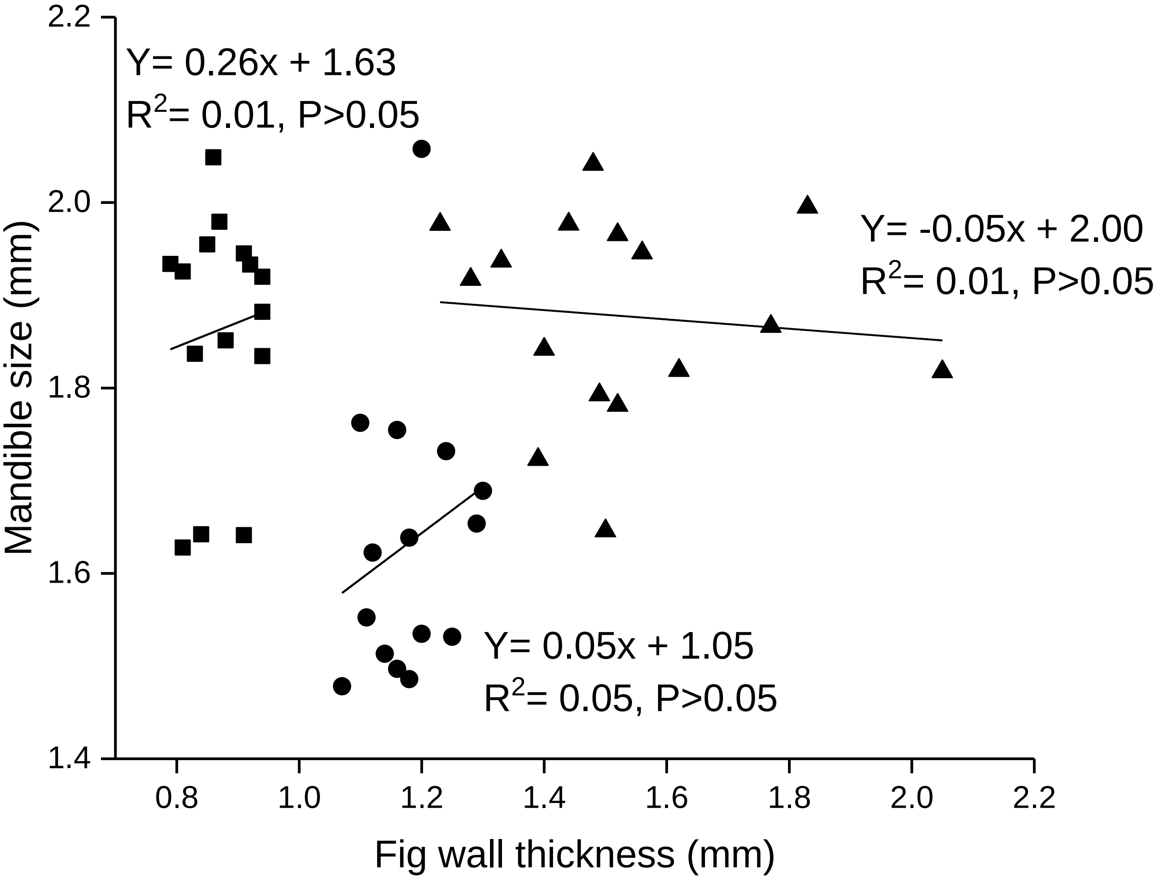

Figure 7. Relationship between mandible size of male fig wasp and rind thickness of figs from three *F. deltoidea* varieties. (■) = var. *angustifolia*, (●) = var. *deltoidea*, (▲) = var. *trengganuensis*

DISCUSSIONS

Currently, *F. deltoidea* is regarded as one species with different varieties, but some of these occur in sympatry, which suggests that they are distinct biological species. There is a significant difference in the morphometrics of the fig and fig wasps from different varieties (Mohd Hatta 2019) as well as the reproductive outputs of this mutualism. Floral number, seed number, and offspring numbers are different for each variety (Noor Nasuha et al. 2017). In contrast to their host plants, differences between female and male pollinators were significant, although nonsignificant differences were present in tibia length and mandible size in female pollinators and mandible width in male pollinators. *F. deltoidea* var. *trengganuensis* wasps recorded the largest wing length followed by var. *deltoidea* and var. *angustifolia* wasps. Insects need to generate loads equal to their weight to fly (Cheng & Sun 2016). This indirectly supports the results of our study; the overall size of the fig wasps from var. *trengganuensis* is the largest, causing them to have large wings to accommodate their weight compared to fig wasps from other varieties. Male fig wasps are wingless to aid them in moving around in the fig cavity (Zavodna et al. 2005). Male fig wasps also differ from female fig wasps because they have reduced eyes, reduced middle legs, and shortened antennae (Kjellberg et al. 2005).

The shortest mean ovipositor length was recorded in wasps from var. *angustifolia*, which has small figs, and the longest ovipositors were from wasps of var. *deltoidea*, which also has small figs. The ovipositor lengths of the pollinator of var. *trengganuensis,* which has big figs, were in between. The shortest ovipositor length (in pollinators from var. *angustifolia*) was still sufficient to penetrate the longest style lengths in male figs in var. *trengganuensis* (Mohd Hatta 2019), making the morphological compatibility for gene flow to occur across varieties. Among plants in general, the specificity of the relationship is maintained before and after pollination. Pre-pollination barriers include the production and detection of cues produced by the host plant (Hossaert-McKey et al. 2010), morphological compatibilities, and pollinator behaviours (Sedeek et al. 2014). Meanwhile, post-pollination filters potentially include pollen competition, gametic mismatches, negative fitness, and hybrid sterility (Coyne & Orr 2004). In nurseries, the specificity of pollination mutualisms between plants and pollinators are often very high, with one plant species being regularly pollinated by one or a few insect species (Rodriguez et al. 2017). The obligate mutualism between fig and fig wasps can be used as a model of evolution and speciation (Wei et al. 2014). Entering atypical hosts can lead to hybrids (Ghana et al. 2017). This hybridisation may contribute to the speciation and diversification that have occurred in the long history of the association between fig trees and fig wasps (Kusumi et al. 2012).

Ficus deltoidea and its fig wasp are involved in a strict one-to-one relationship (Mohd Hatta 2019) at the variety level, and this usually arises from a high degree of behavioural and morphological adaptation (Liu et al. 2013). Fig wasps that come from the same fig tree species but different varieties are usually indistinguishable morphologically, but in fact, they are biologically distinct sibling species (Schneider 1997). In this study, the fig wasps' morphological characteristics showed compatibility for the cross between the varieties, but the gene flow is absent even though in some plantations, two varieties live sympatrically and they remain distinct varieties (Mohd Hatta 2019). Different varieties may emit different attractant volatiles, and pollinator sharing by different varieties is likely to be rare, even when they are living sympatrically (Moe et al. 2011).

The results of our study show that there are morphological differences between the fig wasps of three different fig varieties, including head morphometrics. The head length and width of *Blastophaga* from var. *angustifolia* and var. *trengganuensis* are wider compared to those from var. *deltoidea*. The head shape of the female fig wasp is usually influenced by the morphology of the fig's ostiole (Ramirez-Benavides 1974). A significant relationship between the head width of the female fig wasp and the ostiole size between different varieties was also observed. The morphology of female fig wasps, especially their heads, is correlated with the morphology of the ostiole length and fig wall thickness, further ensuring that pollination is species specific (van Noort & Compton 1996). These morphological traits act as a filter and help maintain the specificity of the relationship between the fig wasp and its host species (Souto-Vilaros et al. 2018), even if they are from the same host species.

Our study shows that the head width of the male fig wasp has no significant relationship with the ostiole diameter. These results are parallel with the findings of van Noort and Compton (1996), which showed that the head size of the male fig wasp has no significant correlation with the fig as well as the female fig wasp. Their morphology is well adapted to conditions in the fig lumens, where they may spend their entire life (Compton & McLaren 1989).

The body size of the fig wasps is an example of selection pressure in ecology. The selection pressure that works on body size of the fig wasp requires more evidence and studies. An adult fig wasp does not eat; thus, the size of the body and its fecundity are determined before it comes out of the gall, which are usually influenced by the quality of nutrients provided by the fig tree (Liu et al. 2013). The body size of adult fig wasps is almost similar from one species to another as food competition and cannibalism do not occur, because each of them is raised independently in female flowers, which then develop into galls (Ramírez-Benavides et al. 2009). Our study shows that the overall body length of fig wasp offspring has a significant relationship with gall width. Small figs in var. *angustifolia* and var. *deltoidea* produced small galls and small overall body length of fig wasps, and vice versa in var. *trengganuensis*. The fig wasp size reflects the size of the gall it occupies (Compton et al. 2017). The gall location in a fig also influences gall size, where female fig wasp tends to be bigger in the central cavity (Liu et al. 2013). However, Peng et al. (2014) did not find any relationship between gall size and location in an individual fig, but the frequency mating did influence the galls size and the size of the female pollinators. Some foundresses mate only once, but others can do multiple mating up to four times.

The tibia length of the hind leg indicates insect size (Darwell & Cook 2017). The longest tibia was recorded in wasps from var. *trengganuensis* that showed the largest overall body length compared to the other varieties. This parameter has been widely used in determining the key features of other chalcids and wasps. The spiny tibia holds a great function in facilitating the foundress through the ostiole after the head has successfully penetrated the ostiole (Berg & Wiebes 1992). The body size of the fig wasp can also be determined via measurement of head length, head area, and mandible length of fig wasp (Liu et al. 2013). The mandible length in male fig wasp showed a clear relationship with the fig wall thickness. The male fig wasp has well-developed mandibles and a telescopic gaster (Yang et al. 2002). In a similar trend, var. *trengganuensis* wasps again recorded the highest value for mandible length for both males and females. The big figs of var. *trengganuensis* might have influenced the male fig wasp to have larger mandibles.

The correlation and regression analyses show that the mandible size (length/width) of males has a significant relationship with fig wall thickness when different varieties were compared. The male fig wasp has well-developed mandibles (Yang et al. 2002) as they need to bite holes into the female galls and insert their genitalia in order to mate (Cook & Segar 2010). In some species, the male will also bite holes from the fig wall to help the newly mated foundress get out from the fig (Cook & Rasplus 2003) but in *F. deltoidea*, the foundress gets out from the fig trough the ostiole (personal observation). The mandibles are also important for the fig wasp to compete with another male when mating with female fig wasps (Moore et al. 2009). In contrast to the male fig wasps, no relationship was found between the mandible (length/size) and fig wall thickness for the female fig wasps. The mandible functions are different in female fig wasps, where it comes with rows of back-pointing teeth or ridges to aid in passing through the ostiole and preventing them from slipping backwards while passing through the ostiole bract (Al-Khalaf et al. 2018).

CONCLUSION

The fig from family Moraceae and fig wasp from family Agonidae show a notable species-specific as well as mutualistic relationship, as specific figs would exclusively interact with only specific pollinator fig wasps in allowing pollination to occur and development to take place in the cavity, even under the same species. Morphological differences were present between the fig wasps of different varieties in almost all parameters, especially the overall body length. We conclude that the specificity between fig trees and fig wasps in the many different *F. deltoidea* varieties is likely to be high due to the differences in their morphological parameters. Previously, all varieties under *F. deltoidea* were defined based on morphological characteristics only. However, according to the biological species concept, they are said to be a same species if they interbreed when living together. The major implication from this study is that taxonomists may have underestimated the number of *Ficus* species. *F. deltoidea* may represent a complex of closely related but biologically distinct species.

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