



## Genetic data revealed co-diversification and host switching in the *Wiebesia pumilae* species complex, pollinators of *Ficus pumila*

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**ABSTRACT:** Figs and their pollinating fig wasps constitute a classic example of co-evolution. Recent molecular studies revealed that multiple cryptic fig wasp species may interact with single or different fig hosts; these species include pollinators associated with *Ficus pumila*. However, previous studies focused on different geographical ranges and fig varieties. The full evolutionary picture of *F. pumila* and its pollinating wasp, *Wiebesia pumilae*, has not been unraveled. Here, we collected mitochondrial COI sequences from the literature and studied the evolutionary history and phylogeography of the pollinating fig wasps of *F. pumila* L. var. *pumila* (creeping fig) and *F. pumila* var. *awkeotsang* (Makino) Corner (jelly fig). The *W. pumilae* contains three distinct lineages, of which Sp. 3 is mostly associated with *F. pumila* var. *awkeotsang* and Sp. 1 and Sp. 2 exclusively with *F. pumila* var. *pumila*. *Wiebesia* sp. 3 showed no genetic differentiation between sample collected from China and Taiwan. Besides, Sp. 3 samples from China exhibits only approximately 50% of genetic diversity compared to samples from Taiwan, suggesting Sp. 3 in China was recently introduced from Taiwan. Our study clarified the co-evolutionary relationships among *F. pumila* varieties and their pollinators, while showing how human activities have influenced their distribution. We also provide support for the endemism of both *F. pumila* var. *awkeotsang* and Sp. 3 in Taiwan.

**KEY WORDS:** Co-evolution, *Ficus pumila* var. *awkeotsang*, fig wasp, host switching, introduced species, phylogeography.

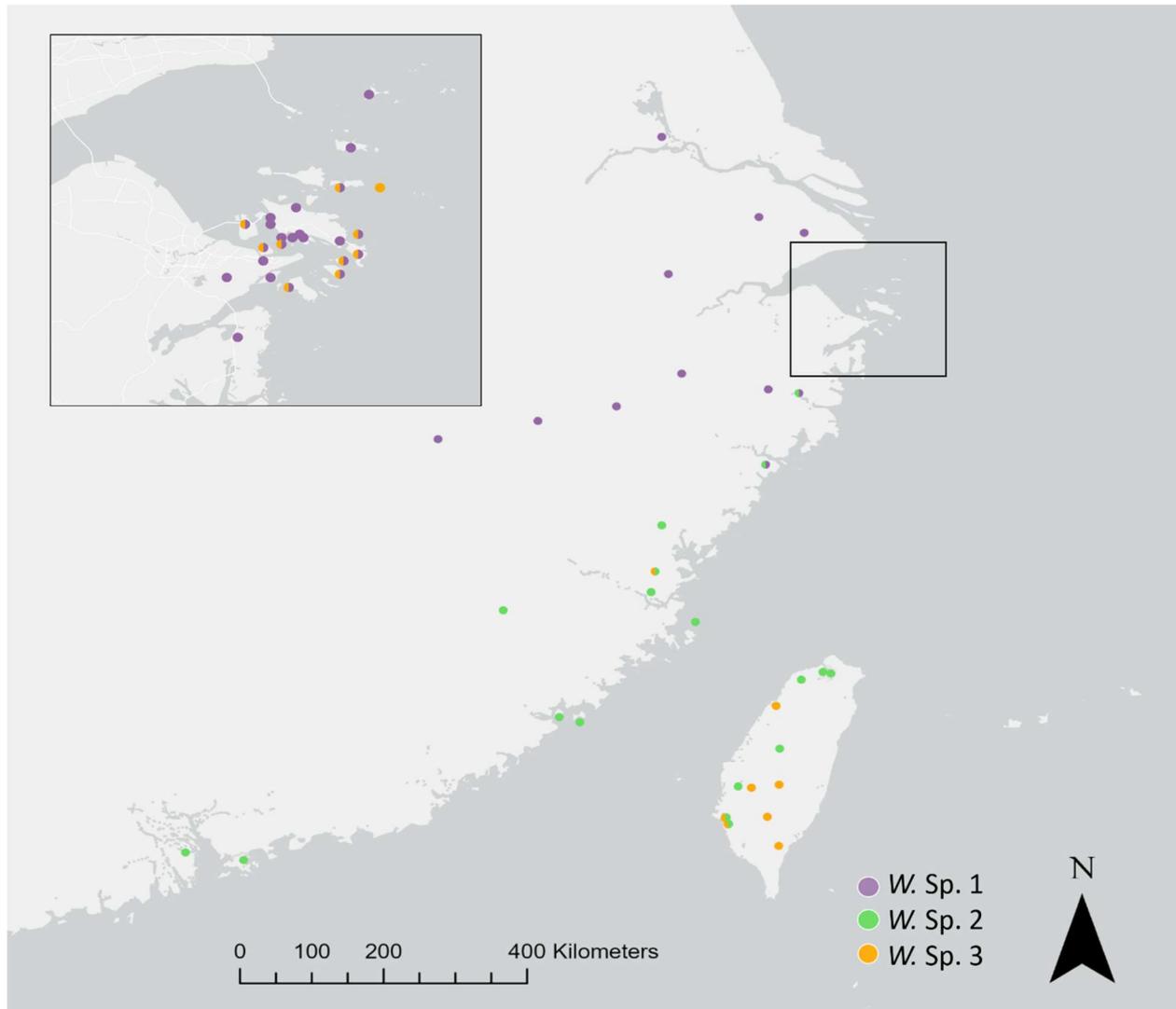
### INTRODUCTION

The obligate mutualism between figs (Moraceae: *Ficus*) and fig wasps is a classic co-evolutionary system (Cruaud *et al.*, 2012; Machado *et al.*, 2005; Wiebes, 1979). Figs and pollinating fig wasps were once thought to have evolved under strict co-speciation. However, molecular evidence of species delimitation acquired over the last two decades has undermined the universality of strict co-speciation (Cook and Segar, 2010; Jackson *et al.*, 2008; Machado *et al.*, 2005; Yang *et al.*, 2015). Many of the fig trees that deviates from strict co-speciation were widely distributed and has multiple geographically isolated subspecies/varieties (Rasplus, 1996; Rodriguez *et al.*, 2017), such as *Ficus pumila* L.

*Ficus pumila* is a functional dioecious fig. Male syconia (singular = syconium, an enlarged, fleshy, hollow receptacle which contains many highly simplified flowers or florets on its inner surface), which nourish pollinating fig wasps and produce pollen, and female syconia, which produce seeds, grow on separate plants. *Ficus pumila* occurs widely across East Asia, including Vietnam, China, Taiwan, and Japan (Hsieh *et al.*, 1993). Two varieties have been described: the nominate variety, *F. pumila* L. var. *pumila*, commonly known as the creeping fig; and *F. pumila* L. var. *awkeotsang* (Makino) Corner, commonly known as the

jelly fig. The former is widespread and spans most of the species' range, while the latter is likely endemic to Taiwan, although some argue that it also sporadically occurs in coastal regions of south China (Wu *et al.*, 2003; but see Chen *et al.*, 2012). The two varieties differ not only in their distribution but also in several morphological and physiological traits, including leaf shape, syconia size, and types of pectin-methylesterase isoforms inside drupes (Hsieh *et al.*, 1993; Lin *et al.*, 1990). They also exhibit different habitat preference: *F. pumila* var. *pumila* favors lowlands, while *F. pumila* var. *awkeotsang* favors altitudes between 1200 and 1900 meters (Hsieh *et al.*, 1993). As a result, the two varieties are largely allopatric in Taiwan.

Several molecular studies have examined the infrastructure of the pollinators associated with *F. pumila*, *Wiebesia pumilae*. The first large-scale exploration on the phylogeography of pollinators of *F. pumila* in China and Taiwan found three pollinator species (Chen *et al.*, 2012). These species are *Wiebesia* sp. 1, which is found north of the Wuyi mountains, located in northern Fujian province near the border with Jiangxi province, China; *Wiebesia* sp. 2, which is found south of the Wuyi mountains; and *Wiebesia* sp. 3, which is unexpectedly and discontinuously scattered on offshore islands of Zhejiang and Fujian, China. In another study that focused on the co-evolution and co-



**Fig. 1.** Geographical distribution of the three *Wiebesia* species.

adaptation of the two *F. pumila* varieties and their pollinators in Taiwan (Wang *et al.*, 2013), researchers discovered that creeping and jelly figs are pollinated by genetically distinct pollinators, namely creeping-fig and jelly-fig wasps, respectively, with evidence of adaptation to local climate in each of the two pollinator species.

They also found segregated differences between *F. pumila* var. *pumila* and *F. pumila* var. *awkeotsang* in chloroplast DNA (cpDNA), although the nuclear and microsatellite markers are undifferentiated, suggesting there is admixture between the two varieties or the loci are still under incomplete lineage sorting. So far, the relationship between all the reported pollinating fig wasp species associated with the two *F. pumila* varieties remains unclear. Further complicating the situation, in the last few decades, *F. pumila* var. *awkeotsang* has been cultivated in southern China after being imported from Taiwan due to its agricultural importance (Chen *et al.*, 2008). A study that includes data from both Taiwan and

China, and that covers pollinators of both *F. pumila* varieties, is needed to understand the biogeography and evolutionary history of *F. pumila* and its pollinating fig wasp species.

Here, using mitochondrial cytochrome oxidase I (COI) sequences from the literature, we provide a phylogenetic and population genetic study on pollinating fig wasps of *F. pumila*. Our results reconstruct the co-evolutionary relationships between the three *Wiebesia* species and the two *F. pumila* varieties. In addition, we reveal that co-diverging pollinators of *F. pumila* var. *awkeotsang* had shifted hosts onto *F. pumila* var. *pumila* after being introduced by humans to China, where the original host is probably not there. Finally, by revisiting the past literature of *F. pumila* associated with different pollinators, we argue that male fig flowering time is plastic and can be adjusted according to the symbiotic wasp species.



## MATERIALS AND METHODS

Mitochondrial cytochrome oxidase subunit I (COI) sequences belonging to pollinators of *F. pumila* were downloaded from the National Center for Biotechnology Information (NCBI) nucleotide database (Benson *et al.*, 2017). Keywords used for searching were “*Wiebesia*” and “cytochrome oxidase subunit I.” Articles containing searched results were examined; only sequences from studies that featured pollinators of *F. pumila* and had geographical information of collecting site were retained.

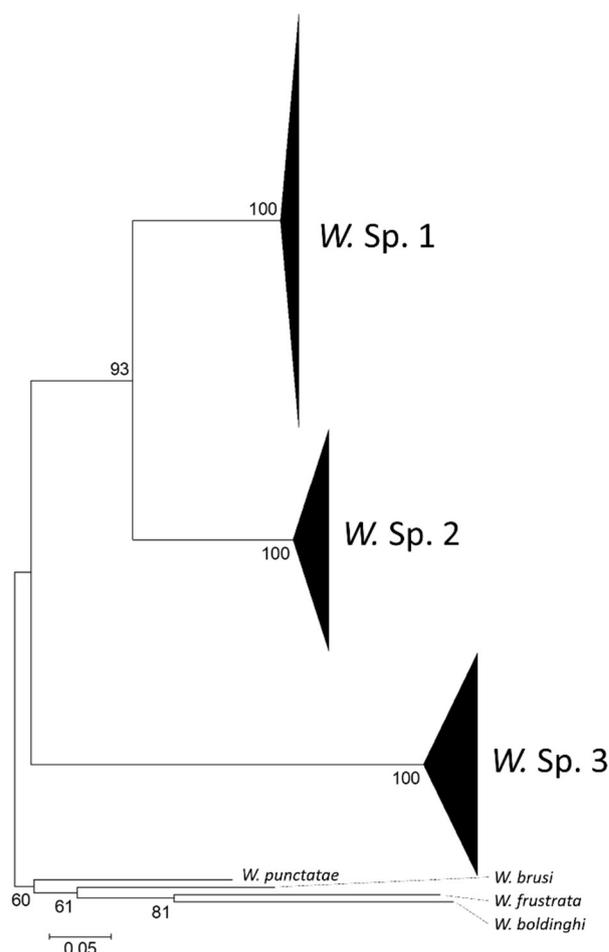
The sequences were aligned by codon using ClustalW (Larkin *et al.*, 2007) implemented in MEGA7 (Kumar *et al.*, 2016). A neighbor-joining phylogenetic tree (Nei and Gojobori, 1986; Saitou and Nei, 1987) with 1000 bootstrap replicates and pairwise genetic distance matrices were calculated using MEGA7. Four additional sequences from *W. brusi*, *W. frustrate*, *W. punctatae* (GenBank accession: AF200412-AF200414; Weiblen 2001), and *W. boldinghi* (JN103288; Cruaud *et al.*, 2012) were used as outgroups in phylogenetic analysis. Three distinct species were defined by phylogenetic analysis. The haplotype diversities and nucleotide diversities ( $\pi$  and  $\theta$ ) were calculated using DNAsp v6 (Rozas *et al.*, 2017). To explore intra-species relationships, the TCS network (Clement *et al.*, 2000) was calculated and visualized with PopART 1.7 (Leigh and Bryant, 2015), and colored according to the source population. Because two of the three wasp species were distributed both in Taiwan and China, to investigate the genetic architecture across the Taiwan Strait, analysis of molecular variation (AMOVA) (Excoffier *et al.*, 1992) was performed on these two species to determine the composition of genetic variation using Arlequin 3.5 (Excoffier and Lischer, 2010).

## RESULTS

The 187 potentially relevant results found from the NCBI database are a mixture of both unique haplotypes within populations and haplotype of single individuals. After reviewing the source literature, 594 COI sequences belonging to pollinators of *F. pumila* with geographical information were included in this study. Of all the sequences, 10 are unique haplotypes within populations that cannot be assigned to particular individuals (HQ398108-HQ398117; Liu *et al.* 2014), and all 10 were collected in China; these sequences were only used in phylogenetic and haplotype network analysis. Furthermore, of all the sequences, 584 represent haplotypes of individual wasps (JN183988-JN184049; Chen *et al.*, 2012, KC579186-KC579356; Wang *et al.*, 2013, KC593629-KC593631, and MZ695804); 89 were pollinators of *F. pumila* var. *awkeotsang* collected in Taiwan, and 4 were collected in China (all from Ningde, Fujian province); 57 and 439 individuals were

pollinators of *F. pumila* var. *pumila* in Taiwan and China, respectively (Table 1 & Figure 1).

A 908-base pair region of COI gene was aligned and used for further analysis. A neighbor-joining phylogenetic tree showed three distinct clades of pollinator wasps, which is consistent with Chen *et al.*, 2012 (Figure 2). Sp. 1 (*Wiebesia* sp. 1 in Chen *et al.*, 2012) was found almost exclusively north of the Wuyi mountains, except one sequence, which was found in Wenzhou (Zhejiang), China; Sp. 2 (*Wiebesia* sp. 2 in Chen *et al.*, 2012, and creeping fig wasp in Wang *et al.*, 2013) was found south of the Wuyi mountains; Sp. 3 was found on Zhoushan Islands (Zhejiang) and Ningde (Fujian), China (*Wiebesia* sp. 3 in Chen *et al.*, 2012), and in Taiwan (jelly fig wasp in Wang *et al.*, 2013). Regarding host plants, both Sp. 1 and Sp. 2 originated exclusively from *F. pumila* var. *pumila*, while Sp. 3 emerged from both *F. pumila* var. *pumila* (Chen *et al.*, 2012; Wang *et al.*, 2013) and *F. pumila* var. *awkeotsang* (Wang *et al.*, 2013) (Table 1).



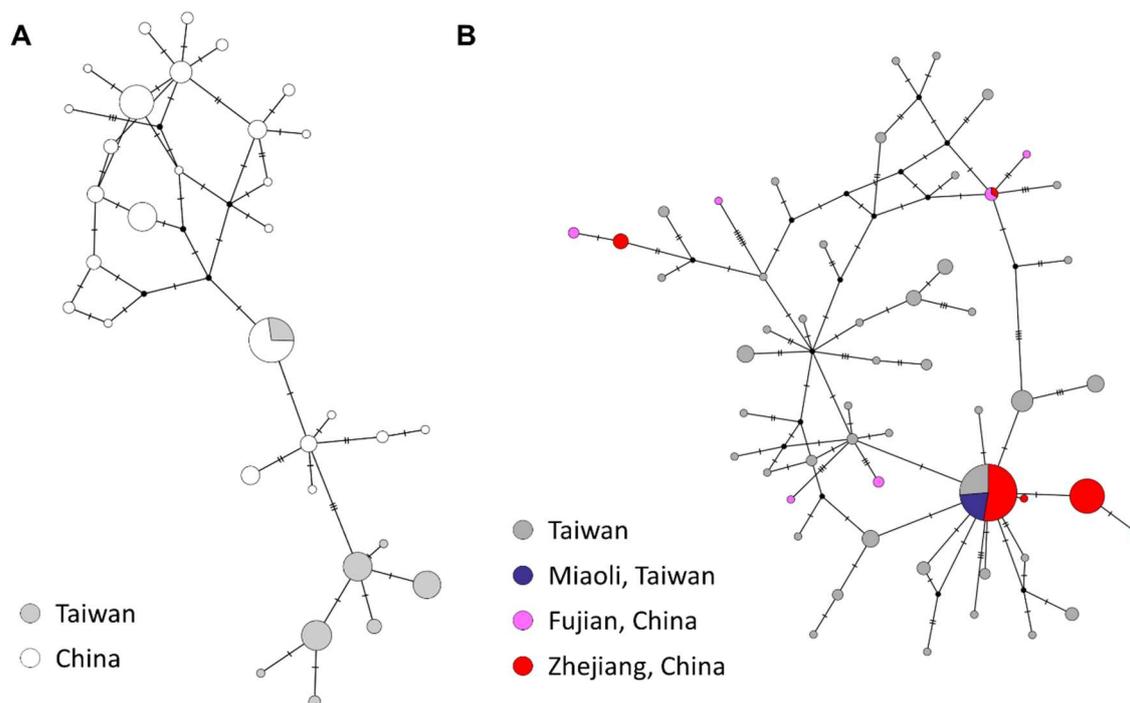
**Fig. 2.** Neighbor-joining phylogenetic tree of *Wiebesia* wasps associated with *Ficus pumila* based on mitochondrial COI sequences. Bootstrap values are indicated at each branching point.

**Table 1.** Sampled *Wiebesia* populations and their corresponding *Ficus pumila* variety.

Location*	Sample size	Species	Host**	Accessions
Yangzhou, ZJ	10	Sp. 1	Creeping fig	JN183994
Suzhou, ZJ	10	Sp. 1	Creeping fig	JN183994
Shanghai	9	Sp. 1	Creeping fig	JN184045, JN184046, JN183994
Shengsi island, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183989
Tianmushan, ZJ	10	Sp. 1	Creeping fig	JN183994
Qushan island, ZJ	8	Sp. 1	Creeping fig	JN183988, JN183997
Dachangtu island, ZJ	10	Sp. 1 & 3	Creeping fig	JN183988, JN183990, JN183991, JN184022
Mao, ZJ	10	Sp. 1	Creeping fig	JN183988
Fuchi island, ZJ	8	Sp. 1	Creeping fig	JN183988, JN183992, JN183998, JN184003
Cengang, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183990, JN183991
Jintang island, ZJ	10	Sp. 1 & 3	Creeping fig	JN183988, JN184004, JN184020, JN184021
Dinghai, ZJ	10	Sp. 1	Creeping fig	JN183988, JN184001
Putuoshan island, ZJ	10	Sp. 1 & 3	Creeping fig	JN183988, JN183992, JN184020
Aoshan island, ZJ	9	Sp. 1	Creeping fig	JN183988, JN183989
Panzhi island, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183990, JN183991
Xixiezhi island, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183990, JN183991, JN183995
Qionglongshan, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183990
Damao island, ZJ	9	Sp. 1 & 3	Creeping fig	JN183988, JN184020, JN184021
Daxie island, ZJ	8	Sp. 1 & 3	Creeping fig	JN183988, JN183989, JN183990, JN184021, JN184025
Zhujiajian island, ZJ	10	Sp. 1 & 3	Creeping fig	JN183988, JN184020, JN184021, JN184025
Baifeng, ZJ	9	Sp. 1	Creeping fig	JN183988, JN183989, JN183995, JN184010, JN184011
Dengbu island, ZJ	10	Sp. 1 & 3	Creeping fig	JN183990, JN183993, JN183994, JN184020, JN184021, JN184025, JN184027
Taohua island, ZJ	10	Sp. 1 & 3	Creeping fig	JN183988
Meishan island, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183989, JN183990
Tiantong, ZJ	8	Sp. 1	Creeping fig	JN183988, JN183990, JN183991
Liuheng island, ZJ	10	Sp. 1 & 3	Creeping fig	JN183988, JN183990, JN184002, JN184005, JN184020, JN184021
Xianshan, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183990
Jinhua, ZJ	10	Sp. 1	Creeping fig	JN183988, JN183993, JN183994, JN184006
Xianju, ZJ	9	Sp. 1	Creeping fig	JN183988, JN183999, JN184007, JN184009
Linhai, ZJ	10	Sp. 1 & 2	Creeping fig	JN183988, JN183996, JN184008, JN184009, JN184012, JN184014
Quzhou, ZJ	10	Sp. 1	Creeping fig	JN183993, JN183994, JN184006
Shangrao, ZJ	7	Sp. 1	Creeping fig	JN183993, JN183994, JN184029
Dongxiang, ZJ	10	Sp. 1	Creeping fig	JN183993, JN183994, JN184028
Dongji island, ZJ	10	Sp. 3	Creeping fig	JN184020, JN184021
Fodu island, ZJ	10	Sp. 3	Creeping fig	JN184020, JN184021
Wenzhou, ZJ	10	Sp. 1 & 2	Creeping fig	JN183993, JN184012, JN184040, JN184041, JN184043, JN184044
Fuan, FJ	10	Sp. 2	Creeping fig	JN184012, JN184013, JN184015, JN184019
Ningde, FJ	14	Sp. 2 & 3	Creeping fig, Jelly fig	JN184012, JN184013, JN184016, JN184017, JN184018, JN184023, JN184024, JN184026, JN184049, KC593629–KC593631
Fuzhou, FJ	9	Sp. 2	Creeping fig	JN184012, JN184013, JN184033, JN184039
Yongan, FJ	10	Sp. 2	Creeping fig	JN184030, JN184036, JN184037, JN184038
Kinmen, FJ	14	Sp. 2	Creeping fig	KC579290–KC579303
Matsu, FJ	20	Sp. 2	Creeping fig	KC579304–KC579323
Xiamen, FJ	9	Sp. 2	Creeping fig	JN184031, JN184032, JN184034, JN184039
Hong Kong	14	Sp. 2	Creeping fig	JN184033, JN184035, JN184039, JN184042, KC579285–KC579289
Chiayi-1, TW	10	Sp. 2	Creeping fig	KC579275–KC579284
Nantou-1, TW	6	Sp. 2	Creeping fig	KC579324–KC579329
Tainan, TW	12	Sp. 2 & 3	Creeping fig	KC579330–KC579340, MZ695804
Taoyuan, TW	3	Sp. 2	Creeping fig	KC579346–KC579348
Kaohsiung-1, TW	12	Sp. 2 & 3	Creeping fig	KC579349–KC579356, MZ695804
Taipei, TW	13	Sp. 2	Creeping fig	JN184047, JN184048, KC579341–KC579345
Chiayi-2, TW	19	Sp. 3	Jelly fig	KC579186–KC579204
Kaohsiung-2, TW	20	Sp. 3	Jelly fig	KC579205–KC579224
Nantou-2, TW	23	Sp. 3	Jelly fig	KC579225–KC579247
Taitong, TW	15	Sp. 3	Jelly fig	KC579248–KC579262
Miaoli, TW	12	Sp. 3	Jelly fig	KC579263–KC579274

\* ZJ: Zhejiang; FJ: Fujian; TW: Taiwan

\*\* Creeping fig: *F. pumila* var. *pumila*; Jelly fig: *F. pumila* var. *awkeotsang*



**Fig. 3.** TCS haplotype networks of *Wiebesia* species that were found in both Taiwan and China. **A.** the network of Sp. 2. **B.** the network of Sp. 3. Population Miaoli from Taiwan is colored separately to demonstrate its close relatedness to multiple haplotypes from Zhejiang, China.

**Table 2.** Genetic diversity of COI in the three *Wiebesia* species.

	Sp. 1	Sp. 2			Sp. 3*		
		All	China	Taiwan	All	China	Taiwan*
No. of wasps	284	154	103	51	149	67	82
No. of haplotypes	28	33	26	10	53	11	44
Haplotype diversity	0.754	0.926	0.909	0.819	0.92	0.703	0.975
$\pi$	0.0033	0.0049	0.0040	0.0024	0.0058	0.0037	0.0069
$\theta$	0.0057	0.0055	0.0047	0.0025	0.0136	0.007	0.0126

\*Population Miaoli was excluded from the analysis because it was an inbred strain.

$\pi$  and  $\theta$ , respectively, are Nei and Li (1979) and Watterson's (1975) estimators of nucleotide diversity.

Among the three clades, Sp. 3 exhibited the highest genetic diversity ( $\theta = 1.36\%$ ) followed by Sp. 1 (0.57%) and Sp. 2 (0.55%), respectively (Table 2). Samples from two sides of Taiwan Strait were analyzed separately: Sp. 3 from Taiwan showed approximately two-fold more genetic diversity (1.26%) than that from China (0.70%); Sp. 2 showed the opposite trend (0.25% and 0.47% for samples from Taiwan and China, respectively). In general, ancestral populations are expected to exhibit more genetic diversity than descendant populations. Therefore, it is reasonable to assume that Sp. 2 initially originated from China, whereas Sp. 3 from Taiwan. They achieved their current distribution by cross-strait migration.

To further address the above issue, intra-species relationships of Sp. 2 and Sp. 3 were visualized by TCS haplotype network (Figure 3A & B). Samples of Sp. 2 from Taiwan were distinct from those from China, indicating that the Taiwan Strait is a barrier to gene flow between the two regions. A different pattern was observed in Sp. 3: samples from China were mixed with samples from Taiwan. In particular, a majority of individuals from Zhejiang have identical or closely related haplotypes compared to samples collected from Miaoli, Taiwan, indicating that Miaoli is likely the direct source population. Samples from Miaoli came from pollinators of cultivated *F. pumila* var. *awkeotsang* grown at an agricultural research center (Miaoli District Agricultural Research and Extension Station). These contain no genetic variation (Table 1 of Wang *et al.*, 2013), likely because of an initial bottleneck followed by generations of inbreeding in captivity.

The lack of genetic differentiation between populations of Sp. 3 from two sides of the Taiwan Strait was also seen through the AMOVA results. Percentage of genetic variation of Sp. 3 across the Taiwan Strait is 5.69% (Table 3), indicating that the differentiation is essentially zero (Meirmans, 2006). Majority of genetic variation (78.23%) are within the populations of Sp. 3. In contrast, in Sp. 2, around 43% of the overall genetic variation can be explained by differences between Taiwan and China.

**Table 3.** Analysis of molecular variance (AMOVA) results of *Wiebesia* sp. 2 and sp. 3

	Percentage of variation	
	Sp. 2	Sp. 3
Among groups (across Taiwan Strait)	42.99%	0(-5.69)%
Among populations within groups	21.96%	27.46%
Within populations	35.04%	78.23%

## DISCUSSION

Our analysis, which integrated the currently available data, supports the finding of Chen *et al.*, 2012 that *Wiebesia pumilae*, the pollinator of *F. pumila*, actually contains three species. As the model specimen of *W. pumilae* was collected in Hong Kong, where only Sp. 2 can be found, Sp. 2 should retain the species name *W. pumilae*. Our results also clarify the evolutionary relationships of *F. pumila* and its pollinator species seen in the literature. The speciation of Sp. 3 from the common ancestors of Sp. 2 and Sp. 1 corresponds to the divergence of *F. pumila* var. *awkeotsang* and *F. pumila* var. *pumila*. Due to different altitudinal preferences (see Introduction), the largely non-overlapping distributions of the two *F. pumila* varieties and their associated fig wasp species suggest that the figs and wasps most likely diversified allopatrically in separate glacial refugia at a sequential time point. Post-glacial population expansion then lead to secondary contact of the figs and wasps in their current territories (Chen *et al.*, 2012). Altitudinal adaptations can lead to genetic incompatibility between wasp species (Wang *et al.*, 2013). Together with different habitat preference and partial pollinator specificity, these mechanisms impose pre- and post-zygotic gene flow barriers to the secondary contacted figs and fig wasps in Taiwan.

The speciation of Sp. 1 and Sp. 2 did not seem to be associated with the split of their fig hosts (Chen *et al.*, 2012). The divergence of the two pollinators may have preceded that of their fig hosts, a phenomenon that has often been noted in fig-fig wasp co-evolution (Herre, Jandér, and Machado, 2008; Lin *et al.*, 2011; Rodriguez *et al.*, 2017). Alternatively, the divergence between hosts of Sp. 1 and Sp. 2 may be subtle. For example, in Taiwan, *F. pumila* var. *awkeotsang* and *F. pumila* var. *pumila* only segregated at two nucleotides and a 1-bp indel among 1102 bp long intergenic region of chloroplast trnT-trnL (Wang *et al.*, 2013). They share identical sequences of nuclear ribosomal inter-genic spacer-1 (651 bp) and the third intron of the nitrate reductase gene (1159 bp). Consequently, the pollinating fig wasps may have diverged substantially, while their hosts remained partially interconnected. Sp. 1 and Sp. 2 are separated by the Wuyi mountains, which may also have facilitated the differentiation of their hosts. Further study focused on genetic differences between the hosts is necessary.

Sp. 3 is not as host-specific. Multiple independent

host switching from *F. pumila* var. *awkeotsang* to *F. pumila* var. *pumila* were seen in populations of China and Taiwan, which is consistent with behavioral experiments (Kong, 2011; Wang *et al.*, 2013; Yang *et al.*, 2017). A phenological study of *F. pumila* var. *pumila* in Zhejiang discovered that even within the same host variety, the emergence times of Sp. 1 and Sp. 3 are reliably distinct (Liu *et al.*, 2014), with the emergence of Sp. 1 followed by that of Sp. 3. There is a similar pattern in the emergence time of pollinators of *F. pumila* var. *pumila* (presumably Sp. 2) and that of pollinators of *F. pumila* var. *awkeotsang* (Sp. 3) in Fujian, China (Chen *et al.*, 2002). The late emergence of Sp. 3, compared to that of Sp. 1/Sp. 2, regardless of which fig variety it inhabits, indicates that the flowering time of the male fig may be influenced by its symbiotic fig wasp.

We also demonstrate that the Sp. 3 wasps found in China today were recently introduced by humans from Taiwan and then switched from their original host, *F. pumila* var. *awkeotsang*, to *F. pumila* var. *pumila*. Reduced genetic diversity (Table 2) accompanied by low genetic differentiation (Table 3) and shared haplotypes (Figure 3B) across the Taiwan Strait indicates that Sp. 3 found in China are recently introduced from Taiwan. If Sp. 3 had originated in Taiwan and subsequently colonized to China sometime in the ancient past (e.g. several thousand years ago), we would expect some samples from China to form distinct clades, as shown in the case of Sp. 2 (Figure 3A), despite concurrent gene flow across the Taiwan Strait. In contrast, all samples from China had their closest related haplotype in Taiwan, demonstrating they were independently introduced from Taiwan in the recent past (perhaps from 20 to 30 years ago). Most of the haplotypes can be traced back to a strain from the Miaoli District Agricultural Research and Extension Station, which is responsible for providing pollinator strains to farmers in Taiwan. Those wasps were probably then shipped across the sea in attempts to cultivate *F. pumila* var. *awkeotsang*. Although there is no large-scale genetic study of *F. pumila* covering Taiwan and China to date, field surveys in China failed to find wild *F. pumila* var. *awkeotsang* (Chen *et al.*, 2012). More importantly, as shown in (Kong, 2011), while the Sp. 3 is able to develop within male syconium of *F. pumila* var. *pumila*, the development of Sp. 2 within male syconium of *F. pumila* var. *awkeotsang* is unsuccessful. With no evidence of Sp. 3 existence in China before recent past (~ 20 years ago), the nature occurrence of *F. pumila* var. *awkeotsang* in China is strongly in doubt. In conclusion, our results support previous notions that *F. pumila* var. *awkeotsang* and its pollinators are endemic to Taiwan (Hsieh *et al.*, 1993).

## Data availability

The sequences discussed in this publication are all



accessible through NCBI GenBank. MZ695804 was published for this article, but it was collected with other samples in Wang *et al.*, 2013.

## ACKNOWLEDGMENTS

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