Cordia subcordata (Boraginaceae), a distylous species on oceanic coral islands, is self-compatible and pollinated by a passerine bird

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Background and aims – Distyly is usually rare on oceanic islands, which is probably due to the difficulty for distylous plants to colonize those islands. However, *Cordia subcordata* was observed to be distylous with short- and long-styled morphs on the Xisha Islands in the South China Sea. To characterize the reproduction system of *Cordia subcordata* and to understand how this distylous species maintains itself on these islands, we studied its reproductive and pollination biology.

Methods – Seed set and pollen tube growth under manipulated intermorph, intramorph, and self-pollination were examined to investigate self-incompatibility in the species. The number of pollen grains deposited on the stigmas after a single pollinator visit were counted to investigate the pollination efficiency of different visitors.

Key results – Our study indicated that *Cordia subcordata* shows reciprocal herkogamy as is typical in distylous species. Pollen tubes could reach the base of the style and move into the ovules under all the manipulated pollination treatments in both morphs. Seed set resulting from four hand-pollination experiments did not show any differences between both morphs, suggesting that *Cordia subcordata* lacks heterostylous self-incompatibility. The most frequent flower visitors, *Zosterops japonicus* and *Apis cerana*, were observed foraging on the large volumes of nectar and pollen grains, respectively, with *Zosterops japonicus* being the most effective pollinator, depositing large number of pollen grains on the stigmas during their visits.

Conclusions – Our findings show that *Cordia subcordata* established itself and persists in the archipelago by producing fruits through a combination of self-compatibility and pollination by the most common passerine bird on the oceanic islands.

Keywords – *Cordia subcordata*; distyly; heterostylous self-incompatibility; oceanic island; passerine pollination.

INTRODUCTION

On oceanic islands, breeding systems can influence the colonization, establishment, and maintenance of plants (Crawford et al. 2011). Oceanic islands have a relative paucity of insects, which limits the number of potential pollinators available to resident angiosperms (McMullen 1993; Hervías-Parejo & Traveset 2018). As a result of this scarcity of pollinators, the majority of angiosperms are reported to be self-compatible on islands (McMullen 1987; Philipp et al. 2004), since plants with a self-incompatible breeding system are more dependent on appropriate pollinators to ensure their reproduction. Hence, self-compatible plants would be preferred for establishment after a long-distance dispersal event because they only need one self-fertilizer for reproduction, whereas self-incompatible plants require at least two individuals (Stebbins 1957). In fact, heteromorphic and homomorphic incompatible plants are rarer on oceanic islands than

on continents (Barrett 1996; Crawford et al. 2011). A recent study indeed found significantly more self-compatible species on islands than on the mainland across a sample of more than 1500 species (Grossenbacher et al. 2017).

It is generally acknowledged that heterostyly is rare or absent on remote oceanic islands (Pailler et al. 1998; Kondo et al. 2007; Watanabe & Sugawara 2015). This is probably due to the fact that most heterostylous plants are characterized by a heteromorphic incompatibility system (Barrett & Cruzan 1994), and simultaneous colonization of both morphs is difficult and pollinators are scarce on islands (Watanabe & Sugawara 2015). The Xisha Islands (Paracel Islands) is a remote group of small coral islets in the South China Sea. Recently, the pollination biology and sexual system of *Guettarda speciosa* L. (Rubiaceae) has been studied, and it showed that the species is distylous with heteromorphic self-incompatibility (Xu et al. 2018). However, heterostyly may be more common on oceanic islands than indicated by previous studies. For example, heterostylous plants have been reported on the Indian Ocean Islands (Lewis 1975), La Réunion (Pailler & Thompson 1997; Pailler et al. 1998; Meeus et al. 2011), the Canary Islands (Olesen et al. 2003), the Galápagos Islands (McMullen 2012; Bramow et al. 2013), and the Bonin Islands (Kondo et al. 2007; Sugawara et al. 2014; Watanabe et al. 2014). Self-compatible plants more often occur on an island than self-incompatible species (Grossenbacher et al. 2017) and as a consequence, the breeding system might be an important factor for the maintenance of heterostylous plants. Studies on mechanisms of reproduction and pollination can reveal how distylous breeding systems of these plant species have been maintained on oceanic islands.

Boraginaceae is one of the families containing the largest number of heterostylous plant species, and heterostyly is present in at least nine genera in this family (Ganders 1979; Naiki 2012). In *Cordia* L., heterostyly was first recorded by Darwin (1877), and distyly can be considered the most prominent feature of the reproductive system in this genus (Opler et al. 1975; Gibbs & Taroda 1983; Taisma & Varela 2005). Flower dimorphism in *Cordia* is found in at least 12 of the 250 or more species (Opler et al. 1975; Gibbs & Taroda 1983; Machado & Loiola 2000; McMullen 2012; Naiki 2012; Canché-Collí & Canto 2014; Martínez-Adriano et al. 2016). Studies on *Cordia* species revealed a wide range of reproductive systems, ranging from heterostyly to homostyly and dioecy, including those adapted to pollination by a broad spectrum of insect assemblages (Opler et al. 1975; de Stapf & dos Santos Silva 2013). Although heterostyly on remote islands can provide excellent opportunities to deepen our understanding of plant reproductive strategies, and *Cordia* provides an ideal research system, the study of heterostylous species is still rare in tropical Asia, especially on oceanic islands.

In this study, we investigated the reproductive and pollination biology of *Cordia subcordata* Lam. (Boraginaceae), a typical inhabitant and dominant tree of coral reef islands in tropical Asia. Friday & Okano (2006) described its biological characteristics but failed to recognize the distyly of the species. Therefore, the study of *C. subcordata* on the Xisha Islands may provide the opportunity to understand the colonization and establishment of heterostylous species on is-

lands in general, and to accumulate knowledge necessary for a better conservation of this species in particular. Our aims are to characterize the reproduction systems of *C. subcordata* and to understand how distyly is maintained on these islands.

MATERIAL AND METHODS

Study sites and species

The Xisha Islands (Paracel Islands), with an elevation of 2.6–15.9 m, are located in the South China Sea (15°46′–17°08′N, 110°11′–112°54′E) and were formed about 7000 years ago as a result of coral growth and crust uplift (Taylor & Hayes 1980). Yongxing Island (16°50.1′N, 112°19.8′E), which is located 320 km from Hainan Island, has a total area of 2.6 km² and is the largest islet of the archipelago. The second largest island in the archipelago, Dong Island (16°40′N, 112°44′E), with 1.7 km², is located 337 km from Hainan Island (Tong et al. 2013). A map of the geographical location of Dong Island and Yongxing Island is shown in supplementary file 1. Our field studies were undertaken from the beginning of May until mid-June 2017, and again in November 2017 on both islands.

Cordia subcordata (Boraginaceae), an evergreen tree with a broad, dense, wide crown, is native to the Xisha Islands (Tong et al. 2013). *Cordia subcordata* bears cymes of about ten bright orange flowers at the terminal ends of its branches and in leaf axils, opening one to five flowers per cyme every day (Friday & Okano 2006). The large, funnel-shaped flowers have five to seven slightly wrinkled lobes. *Cordia subcordata* involves two discrete floral morphs differing in style length: flowers of the longistylous morph (L-morph) have the stigma positioned above the anthers and slightly exserted out of corolla, while flowers of the brevistylous morph (S-morph) have the stigma positioned below the anthers (fig. 1). *Cordia subcordata* flowers and sets fruit year-round. Its bright orange flowers provide one of the archipelago's few showy floral displays. However, there are no studies about its reproductive and pollination biology.

Measurements of floral and pollen traits

Floral longevity was assessed by tagging flowers in the late bud stage and observing them from anthesis to flower wilting at Yongxing Island. For floral biometry, we randomly selected 15 trees of each morph, and measured one to three flowers from different inflorescences for each plant in May 2017. In total, we collected 34 flowers of the L-morph and 40 flowers of the S-morph in anthesis and measured six morphological traits (fig. 1) immediately with digital calipers with an accuracy of 0.01 mm.

Nectar volume was determined in flowers from ten individuals of each morph from the Yongxing Island population. Before anthesis, flowers were covered with mesh bags to exclude flower visitors. Nectar volume (μl) was estimated using 100-μl glass micropipette from 20 bagged flowers of the L-morph and S-morph, respectively. Nectar sugar concentration (g solute per 100 g solution) was measured with a hand-held sucrose refractometer (Atago Co., Ltd., Tokyo, Ja-

pan). A total of 40 flowers (L-morph, 20; S-morph, 20) were sampled for these measurements.

Flower buds for determining the pollen-ovule ratio and pollen size of two morphs were collected and stored in FAA solution (formalin: glacial acetic acid: 70% ethanol = 5: 5: 90) at Yongxing Island. Anthers chosen for study were mature but not dehisced. Ovule counts were conducted using a dissecting scope (Leica EZ4W). Pollen counts were made using a light microscope. Pollen-ovule ratios were determined for five L-morph individuals and five S-morph individuals, sampling one flower from each. Pollen morphology of the two morphs was compared with a scanning electron microscope (JSM-6360LV, Japan). Equatorial and polar diameters of 34 and 40 (L-morph and S-morph) pollen grains were measured using a light microscope by taking digital images in Image-Pro Plus v.6.0 of 15 plants for each morph. We excluded pollen grains that were extremely small or had irregular shapes.

Flower morph ratios

Cordia subcordata populations on Yongxing Island and Dong Island were investigated to determine the relative abundance of the two morphs. We sampled individuals on the two islands by walking through the whole habitat from east to west and from south to north in May 2017.

Pollination experiments

Natural fruit set of the two morphs was investigated for the Yongxing Island and Dong Island populations. We randomly selected ten trees of each morph, and counted the fruit number of three to five inflorescences. Fruit set was calculated based on the traces left by flowers. To examine the heteromorphic incompatibility system, hand-pollination was performed on ten individuals of each morph at Yongxing Island. Isolation of flowers was accomplished by enclosure in bags made of cotton mesh netting. We performed five treatments: (1) emasculated, hand inter-morph pollination using pollen from the opposite flower morph; (2) emasculated, hand intra-morph pollination using pollen from the same flower morph; (3) hand self-pollination using pollen from the same flower; (4) bagged without hand-pollination; and (5) marked flowers without treatments as control. To allow fruits to mature naturally, the mesh bags were removed after corolla wilting. Three months after pollination, fruits were collected and seed set was recorded. Pollen-tube growth was examined *in vivo*. Newly open virgin flowers were hand-pollinated by fresh pollen with the above (1), (2), and (3) treatments. Five styles with ovary were collected at each time interval (2 h, 6 h, 12 h, and 24 h) after pollination and fixed in FAA solution. In the lab, the fixed styles were softened in 4 mol/L NaOH for 1 h at 100°C, rinsed with water, and then stained with 0.005% aniline blue in Na_2HPO_4 (pH 11) for 30 min at 100°C (Kearns
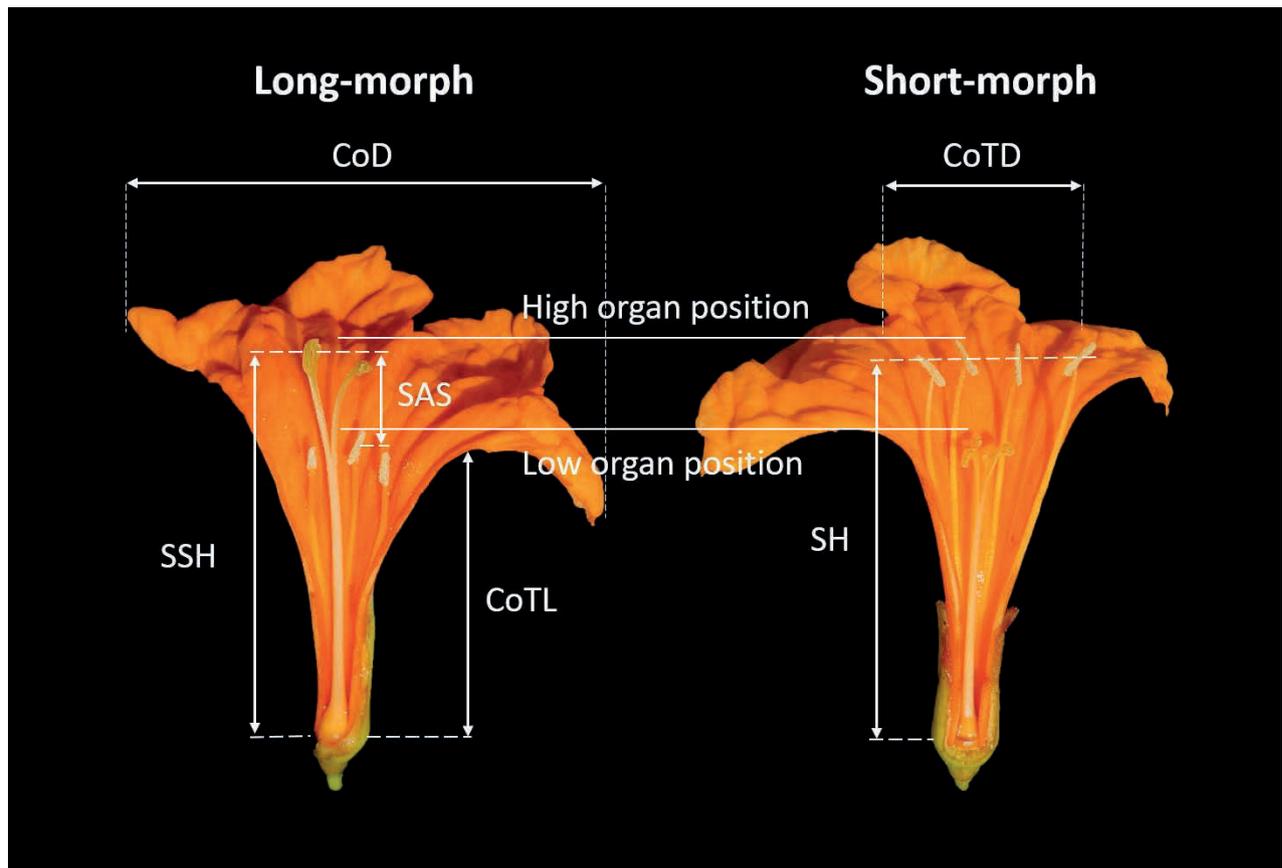

Figure 1 – Floral morphology of *C. subcordata*, showing the position of the anther and stigma, as well as the measurements of the floral characters. CoD: corolla diameter; CoTD: corolla tube diameter; CoTL: corolla tube length; SSH: style plus stigma height; SH: stamen height; SAS: stigma-anther separation.

& Inouye 1993; Wu et al. 2010). The styles were mounted on a slide in glycerol beneath a cover glass and gently pressed to spread the tissue. Pollen grains on the stigmas and pollen tube in the styles were observed using a fluorescence light microscope (BX41, Olympus; excited with UV light) and imaged with Image-Pro Plus (Kho & Baer 1968).

Visitation observations

To quantify visitation rates (visits/flower/hour) of different visitors to *C. subcordata*, we recorded visitors on different patches at Yongxing Island from 10 to 19 Nov. 2017, typically at a distance of not less than five meters, using binoculars. Patches containing one or two trees with about 50 open flowers were randomly selected. Sessions for pollination observation lasted 30 min each and were performed between 8:00 and 18:00 on sunny days. We observed seven days in total at Yongxing Island. The numbers of flowers visited by any visitor in one session were recorded. At the end of an observation period, the open flowers in the patch were counted. Two visitor species were observed visiting the flowers, and
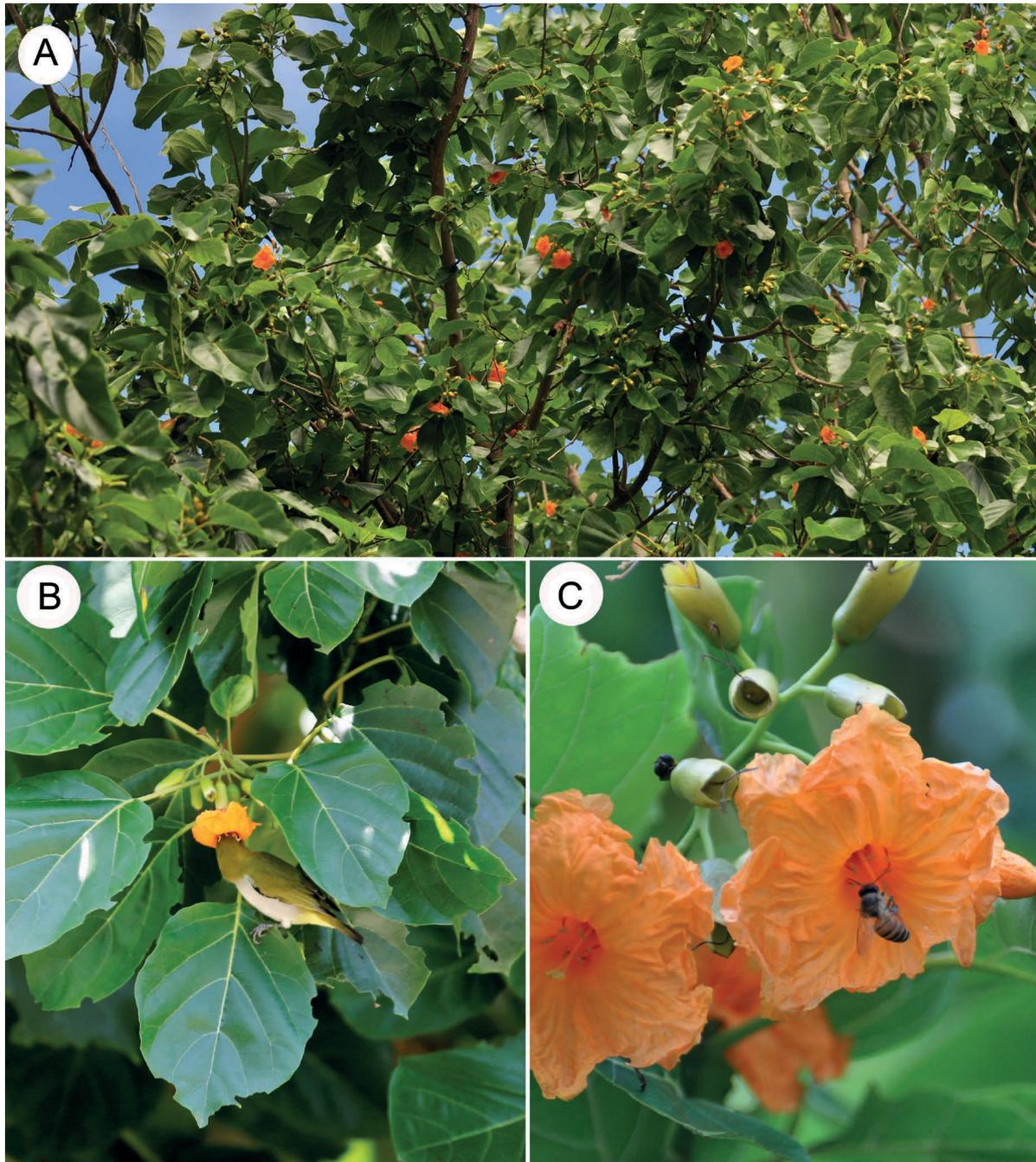

Figure 2 – Flowers and visitors of *Cordia subcordata*. **A**. Orange flowers in a tree on Yongxing Island (visitor observation area). **B**. *Zosterops japonicus* visiting flowers for nectar. **C**. *Apis cerana* visiting flowers for pollen. A photographed by Xiangping Wang; B & C photographed by Meihong Wen.

Table 1 – A comparison of floral traits and pollen traits between L-morph and S-morph flowers in *Cordia subcordata* (mean ± SE) by Mann-Whitney U test (0.05 level).
Number of flowers per morph (long-styled and short-styled) is indicated between brackets. All data were obtained from the Yongxing Island population.

Trait	L-morph (30)	S-morph (30)	Z value	P value
corolla diameter (mm)	42.17 ± 1.01	45.16 ± 0.76	-1.656	0.098
corolla tube diameter (mm)	16.94 ± 0.46	18.40 ± 0.27	-2.528	**0.011**
corolla tube length (mm)	28.42 ± 0.48	30.52 ± 0.42	-3.068	**0.002**
stigma height (mm)	33.18 ± 0.39	27.78 ± 0.30	-6.328	**< 0.001**
anther height (mm)	26.42 ± 0.28	32.72 ± 0.32	-6.587	**< 0.001**
stigma-anther separation (mm)	6.76 ± 0.30	4.94 ± 0.35	-3.704	**< 0.001**
P/O ratio (n = 5)	4714.8 ± 469.7	4802.0 ± 502.6	-0.104	0.917
equatorial axis (μm)	45.74 ± 0.41	46.93 ± 0.34	-1.989	**0.047**
polar axis (μm)	36.17 ± 0.35	39.44 ± 0.39	-4.879	**< 0.001**
E/P ratio	1.27 ± 0.02	1.19 ± 0.01	-3.330	**0.001**

we compared the difference of visit frequency between these two species: *Zosterops japonicus* Temminck & Schlegel, 1845 and *Apis cerana* Fabricius, 1793 (fig. 2). We also found ants within the corolla tubes of *C. subcordata* but because of their limited movement, these ants seemed to be ineffective pollinators. We also observed visitors on *C. subcordata* at Dong Island in November 2017 for two days.

Pollen deposition on stigma after a single visit

To compare the pollination efficiency of the two most frequently visiting species (*Z. japonicus* and *A. cerana*), we estimated pollen receipt per flower per visit. Virgin (previously unvisited) inflorescences were bagged with cotton mesh until the flowers opened the next morning. These flowers were uncovered and then one visit by *Z. japonicus* or *A. cerana* was allowed. Stigmas of the visited flowers were collected and pollen counting was performed in the lab. For the L-morph flowers, eleven stigmas were collected after visits by *Z. japonicus*, and five stigmas were collected after *A. cerana* visits. For the S-morph flowers, three stigmas were collected after *Z. japonicus* visits. As the stigma was large enough, pollen grains on the stigma were taken off with gelatine cubes (Kearns & Inouye 1993), and pollen from each stigma was then transferred to a clean slide. The slides were warmed gently to melt the gelatine and pollen grains were counted under a light microscope. We counted the number of conspecific pollen and heterospecific pollen using an electron microscope.

Data analyses

We used the Reciprocity Index, calculated with Recipro-V2 (Sanchez et al. 2008), to represent the stigma-stigma reciprocal degree between two floral morphs. Morphological differences between the two floral morphs was compared using a Mann-Whitney U test as most data were not normally distributed. A one-way analysis of variance (ANOVA) was used to detect the difference of natural fruit set among the L-morphs and S-morphs on Yongxing Island and the S-morphs on Dong Island, and seed set among the different hand-pol-

lination treatments. Tukey honest significant (HSD) multiple comparisons were conducted if significant differences were revealed among different morphs and hand-pollination treatments. To compare the difference of visitation rate from two pollinator species, we used a Mann-Whitney U test of the mean data. All statistical analyses were carried out in SPSS v.19.0 and at a 0.05 significance level.

RESULTS

Floral and pollen traits

Individual flower longevity of *C. subcordata* was one day with flowers opening around 6:00, followed by anther dehiscence around 8:30, and finally corolla wilting around 18:00. All examined flowers had a single style, four ovules per ovary, and six anthers. The Reciprocity Indices v.2.0 recorded were: for the whole level R = 0.954, the higher level R = 0.978, and the lower level R = 0.990 (fig. 3). *Cordia subcordata* nectar was secreted at the bottom of corolla tube. Mean nectar standing crop volumes were 124.74 ± 4.90 μl
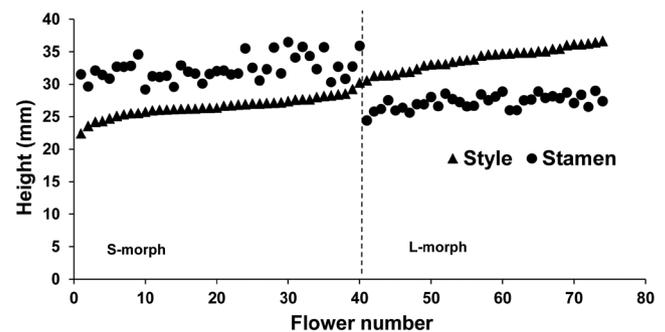

Figure 3 – Patterns of variation in style and stamen height in S-morph and L-morph flowers of *C. subcordata*. Styles are indicated by solid triangles and stamens are indicated by solid circles. The dotted line in the middle separates the two floral morphs: the S-morph flowers are on the left, while the L-morph flowers are on the right. The x-axis shows the flowers investigated numbered from 1 to 80.
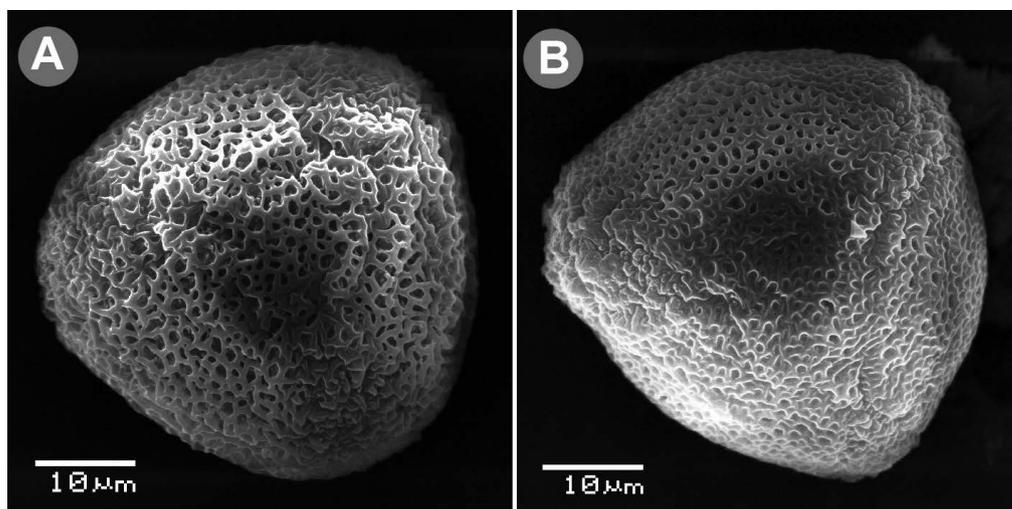
Figure 4 – SEM micrographs of pollen grains of *C. subcordata*. **A**. Pollen of L-morph flowers. **B**. Pollen of S-morph flowers.
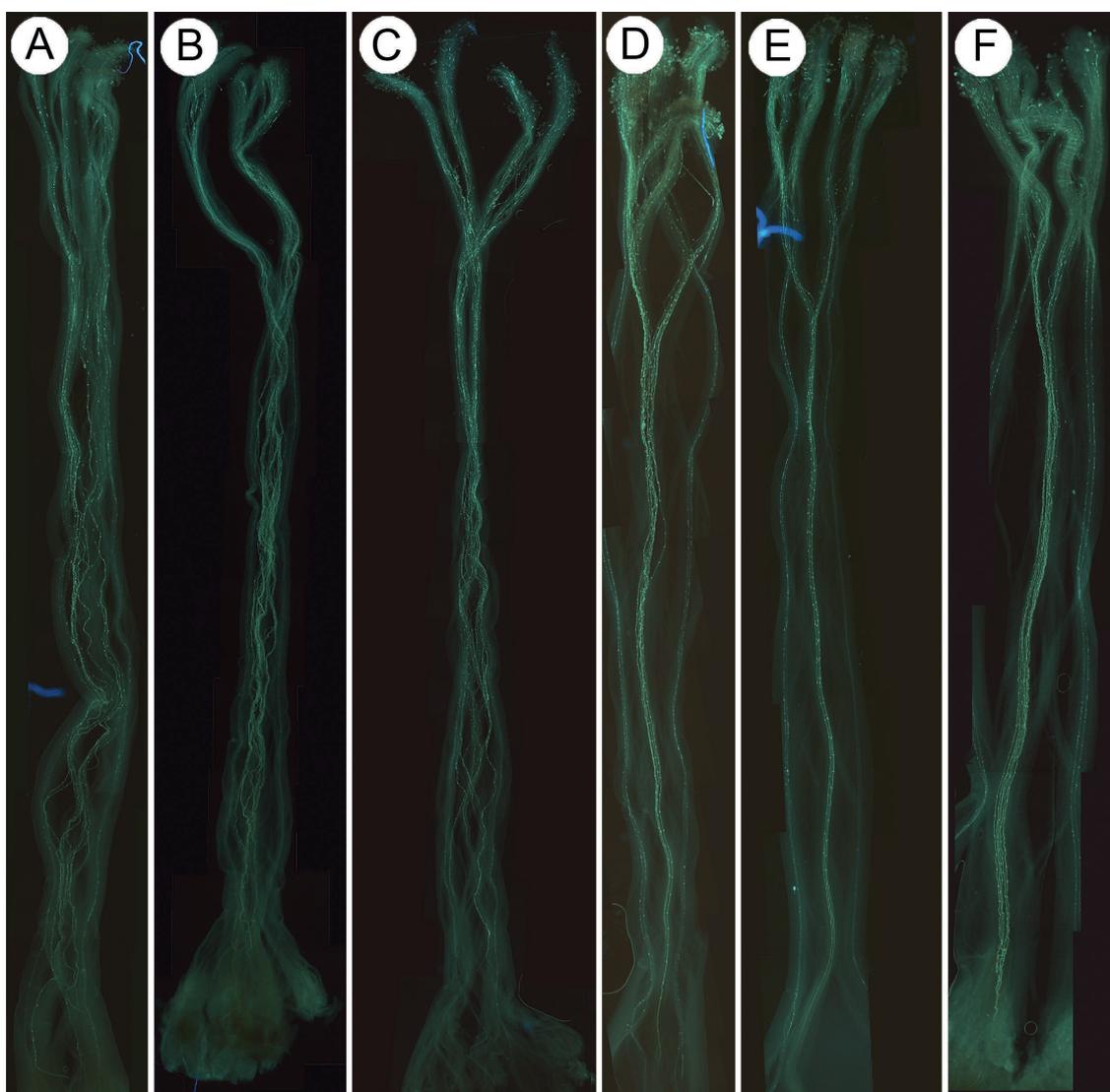

Figure 5 – Pollen tube growth *in vivo* of *C. subcordata* after hand-pollination. **A**. L-morph style 24 h after intermorph cross pollination. **B**. L-morph style 24 h after intramorph cross pollination. **C**. L-morph style 24 h after self-pollination. **D**. S-morph style 24 h after intermorph cross pollination. **E**. S-morph style 24 h after intramorph cross pollination. **F**. S-morph style 24 h after self-pollination.

Table 2 – Seed set under five pollination treatments of *Cordia subcordata* in the Yongxing Island population.
Numbers between brackets indicate the number of flowers.

Treatment	Seed set	
	L-morph	S-morph
intermorph crosses	0.79 ± 0.06 (13)	0.70 ± 0.06 (14)
intramorph crosses	0.66 ± 0.07 (14)	0.67 ± 0.07 (12)
self-pollination	0.75 ± 0.07 (15)	0.71 ± 0.07 (12)
control	0.58 ± 0.03 (75)	0.60 ± 0.03 (71)
bagged	0 (15)	0 (15)

(L-morph) and 161.70 ± 7.57 μl (S-morph), and mean sugar concentrations were 13.79 ± 0.36% (L-morph) and 13.16 ± 0.20% (S-morph). Furthermore, ancillary polymorphism occurred in other parts of the flowers. S-morph flowers possessed larger corolla, longer corolla tube, larger pollen grains but lower equatorial axis/polar axis ratio than L-morph flowers (table 1). However, the exine sculpture of pollen was similar for the two flower morphs (fig. 4).

Morph ratios

Floral morph ratio (L-morph: S-morph) in *C. subcordata* was about 1:3 (34 L-morph: 105 S-morph) on Yongxing Island. The Dong Island population only had 27 S-morph individuals (0 L-morph: 27 S-morph). The ratios of L-morph and S-morph individuals therefore deviated from the 1:1 equilibrium.

Pollination experiments

Results of pollen tube growth under three hand-pollination treatments suggested that pollen tubes entered the ovary 24 h after pollination of intermorph, intramorph, and selfing (fig. 5). Natural fruit set of *C. subcordata* (S-morph) on Dong Island (0.22 ± 0.03, n = 102) was significantly lower than the natural fruit set on Yongxing Island (S-morph, 0.49 ± 0.03, n = 58; L-morph, 0.48 ± 0.03, n = 54; df = 2, F = 40.163, P < 0.001). In the flower bagging experiments, bagged flowers (n = 15) had zero fruit set and did not set any seeds. One-way ANOVA revealed that seed set in the L-morph differed among four hand-pollination treatments (df = 3, F = 4.152, P = 0.008). Multiple comparisons indicated that intermorph pollinated seed set was higher than for the control group (fig. 6). Seed set under the four hand-pollination treatments did not show any difference in the S-morph individuals (df = 3, F = 1.174, P = 0.323; table 2, fig. 6). These results indicate that *C. subcordata* lacks heteromorphic incompatibility on the Xisha Islands.

Pollinators and pollination observation

Our observation of pollinators covered around 50 flowers each time period within our observation area (fig. 2A). Two visitor species were observed during seven days of observations. One species, *Zosterops japonicus* (Passeriformes), a passerine bird, was observed visiting to suck nectar, while the other species, *Apis cerana* (Apidae), was observed collecting pollen grains during visits (fig. 2). The visitation fre-

quency of *A. cerana* was significantly higher (2.39 ± 0.42 visits per flower per hour) than *Z. japonicus* (1.04 ± 0.20) (Z = -2.326, P = 0.020). *Apis cerana* visited flowers almost throughout the whole day, while *Z. japonicus* was mainly active during the mornings (fig. 7). On Dong Island, only *Z. japonicus* was observed visiting flowers, and its visitation rate was very low (0.11 ± 0.06).

Pollen deposition after a single visit

In L-morph flowers of *C. subcordata*, *Z. japonicus* deposited significantly more conspecific pollen grains on stigmas after one visit than *A. cerana* (Z = -2.662, P = 0.008); while *A. cerana* deposited more heterospecific pollen grains after one visit than *Z. japonicus* (Z = -2.736, P = 0.006; table 3).

DISCUSSION

We have demonstrated that *Cordia subcordata* (Boraginaceae) shows typical distyly with S- and L- morphs with highly reciprocal positioning of the sexual organs, and that *C. subcordata* lacks heterostylous self-incompatibility, as manipulated inter-, intra-, and self-pollination experiments yielded similar seed set, and with similar patterns of pollen tube growth. The variation of floral traits relating to distyly was consistent with that reported for other *Cordia* species (Ganders 1979; Canché-Collí & Canto 2014). In *C. subcordata*, S-morph flowers had larger corollas, longer corolla tubes, and larger pollen grains than L-morph flowers. Increased floral size in S-morph flowers has been suggested to be mainly derived from their long corolla tubes, and this phenomenon has been found in many other distylous species, as well as in two other *Cordia* species (Ganders 1979; Richards & Koptur 1993; Pailler & Thompson 1997; de Castro & Araujo 2004; Ferrero et al. 2011; Valois-Cuesta et al. 2011; Faria et al. 2012; Canché-Collí & Canto 2014; Xu et al. 2018). In *C. subcordata*, the filaments which coherent stamens are epipetalous and adnate to the corolla in the lower portion. This structure implies that stamen height increases as the co-
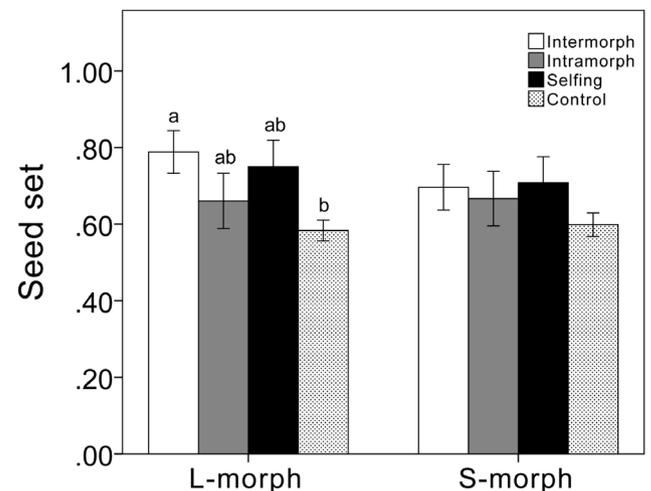

Figure 6 – Differences of seed set (mean ± SE) of four hand-pollination treatments in L-morph and S-morph flowers. Different letters indicate significant differences between the means under Tukey HSD multiple comparisons (P < 0.05).

Table 3 – **The number of pollen grains (conspecific pollen and heterospecific pollen) deposited on the stigmas of *C. subcordata* after a single visit from *Z. japonicus* or *A. cerana* on Yongxing Island.**
Numbers between brackets indicate the number of collected stigmas.

Pollinator	Pollen grains number (L-morph)		Pollen grains number (S-morph)	
	Conspecific	Heterospecific	Conspecific	Heterospecific
Zosterops japonicus	153.7 ± 51.7 (11)	0.8 ± 0.6 (11)	87.3 ± 35.4 (3)	0.0 (3)
Apis cerana	13.0 ± 3.2 (5)	19.6 ± 6.5 (5)	/	/

rolla tube elongates, causing a position correlation between stigma-anther separation distance and corolla tube length in S-morph flowers, as reported in *Primula vulgaris* Huds. and *P. veris* L. (Primulaceae) (Kálmán et al. 2007). Larger size of pollen grains in S-morph flowers than in L-morph flowers has been documented for other heterostylous species as well (Ganders 1979; Dulberger 1992; Pailler et al. 1998). Larger pollen in S-morph flowers is thought to be related to the need for longer pollen tubes to reach the ovules through the L-morph styles (Darwin 1877). However, several studies have found that pollen grain size (S/L) had no correlation with style length (L/S) in some distylous species (Ganders 1979; Cruden 2009). Recently, Wang et al. (2016) suggested that pollen size was positively correlated with stigma depth rather than style length among species in the genus *Pedicularis* L. (Orobanchaceae). Thus, in distylous plants, the functional basis and the evolutionary significance of pollen grain size dimorphism remains to be explained.

The results of the hand-pollination experiments indicated that heteromorphic self-incompatibility is not functional in *C. subcordata*. Bagged flowers did not set seed, which suggested that *C. subcordata* cannot autonomously self-pollinate and requires pollinators for fruit and seed set. There

was a trend toward higher seed set for flowers experiencing intermorph cross-pollination, intramorph cross-pollination, and open pollination in both morphs. This fact suggests that pollen limitation occurs in the Yongxing Island population. Self- and intramorph- incompatibility is commonly observed in distylous plants (Klein et al. 2009). In addition, most continental species of *Cordia* have been reported to maintain heteromorphic self-incompatibility (Opler et al. 1975; Canché-Collí & Canto 2014). However, *C. lutea* Lam. and *C. revoluta* Hook.f. are reported to be partly self-compatible (heteromorphic incompatibility system is incomplete) on the Galápagos Islands (Philipp et al. 2006; McMullen 2012). In this study, *C. subcordata* was revealed to be completely self- and intra-morph compatible. Mating systems that strictly enforce out-crossing are characteristically underrepresented on oceanic islands (Baker 1967), and heterostyly is notably absent in such locations (McMullen 1987; Pailler et al. 1998). During colonization, pollinator availability and low density of conspecifics may select for changes in mating patterns in distylous species leading to the evolution of self-compatibility (Barrett 2002). On the Xisha Islands, only *Guettarda speciosa* and *C. subcordata* are found to be distylous so far (Xu et al. 2018; this study). *Guettarda speciosa* is distylous
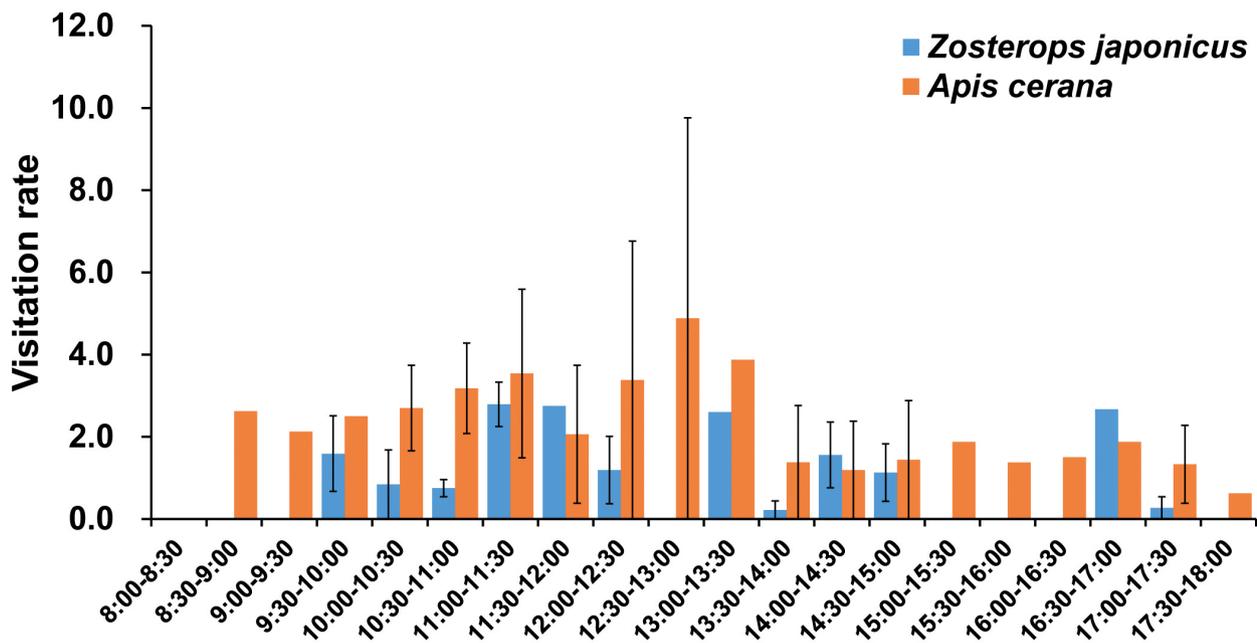

Figure 7 – Visitation rate (visits/flower/hour) of *Zosterops japonicus* and *Apis cerana* at different time periods within a day. Error bars depict the Standard Error.

with strict self- and intra-morph incompatibility, which can persist due to acclimatized long-distance dispersal of *Agrius convolvuli* (Linnaeus, 1758) (the convolvulus hawk-moth) that provides pollination for *G. speciosa* on a wide scale (Xu et al. 2018). Different from *G. speciosa*, self-compatible distyly in *C. subcordata* was favoured by island floras as would be expected according to Baker's law (Pannell 2015). Most likely, this self-compatibility has been selected because of reproductive assurance during the colonization process to the remote Xisha Islands. Thus, the distyly of *C. subcordata* populations on the Xisha Islands became established and has persisted in the archipelago by producing fruits through a combination of self- and cross-pollination, facilitated by the paucity of pollinators on the remote coral islets.

Flowers of *C. subcordata* exhibit the ornithophilous syndrome by having large, tubular corollas, open-shaped, orange flowers with large volumes of nectar. Our results suggest that *Z. japonicus* serves as an effective pollinator for *C. subcordata*, and *A. cerana* also frequently visits flowers on Yongxing Island. The large volume of nectar in *C. subcordata* is characteristic of ornithophilous species (Nicolson 2002; Cronk & Ojeda 2008), and nectar sugar concentration was similar to that of typical bird-pollinated flowers (10–15%; Nicolson 2002). *Zosterops japonicus* probed the majority of freshly opened flowers on plants when foraging, and it was mainly active during the morning, in agreement with previous reports of bird-pollinated plants (Micheneau et al. 2006). As *C. subcordata* is self-compatible, self-pollination could be facilitated by the behaviour of *Z. japonicus* on the inflorescences. *Zosterops japonicus* deposited enough conspecific pollen grains on stigmas after one visit to potentially promote self-pollination. The introduced honeybee, *A. cerana*, was a more frequent visitor to flowers in order to collect pollen at Yongxing Island than *Z. japonicus*. However, because *A. cerana* has a short tongue that cannot reach the nectar secreted from the base of the corolla tube, they only collected pollen grains located near the corolla tube entrance. Thus, *A. cerana* deposited significantly less conspecific pollen grains on stigmas after one visit than *Z. japonicus* in L-morph flowers. We predicted that similar results would occur in S-morph flowers, although data of pollen deposition on stigmas are lacking. As *A. cerana* is not able to touch the stigma hidden within the corolla tube of the S-morph flowers, they may carry pollen grains from the S- to the L-morphs unidirectionally. This case was similar with *Psychotria homalosperma* A.Gray (Rubiaceae), a distylous tree on the Bonin Islands (Watanabe et al. 2018). *A. cerana* deposited more heterospecific pollen grains on stigmas than *Z. japonicus*. One explanation is that *A. cerana* is a more general pollinator, while *Z. japonicus* only visits *C. subcordata* in the Yongxing Island community (Wang et al. unpublished data). Opportunistic nectarivory by generalist passerines is considered casual in mainland ecosystems, but it is a relatively frequent phenomenon on oceanic islands (Valido et al. 2004), such as the Galápagos Islands (Safford & Jones 1998), Mauritius (Hansen et al. 2002), the Hawaiian Islands (Lammers et al. 1987), Trinidad (Feinsinger et al. 1982), the Canary Islands, and Madeira (Valido et al. 2002; Olesen & Valido 2003). However, pollinators such as nectar-feeding birds have been poorly studied in East Asia (Gu & Zhang 2009; Ren et al. 2018; Funamoto 2019), and

previous studies suggest that passerines mainly visit flowers during winter time when other food resources are lacking (Gu & Zhang 2009; Gu et al. 2010). Zosteropidae have colonized more oceanic islands than any other passerine family (Gill 1971), suggesting that Zosteropidae may play an important role in the pollination system on oceanic islands. However, evidences about passerine pollination on the Xisha Islands is very scant so far. This study can provide new evidence for the passerine pollination on oceanic coral islands.

The floral morph-ratio in *C. subcordata* deviated significantly from 1:1 and was not typical for distylous plant species (Hodgins & Barrett 2008). Furthermore, smaller populations with only S-morph individuals occurred on Dong Island. Distyly is usually accompanied by self-incompatibility, genetically promoting symmetrical intermorph mating, resulting in equal morph ratios within populations (Hodgins & Barrett 2008). The floral morph ratio in *C. dodecandra* A.DC. is almost 1:2, while in *C. sebestena* L. it approached 1:1, and both species have a self-incompatibility system (Canché-Collí & Canto 2014). There may be some influences of self- and intramorphic compatibility in the morph ratio (Baker et al. 2000). The uneven morph ratio of *C. subcordata* may be the result of self-compatibility, and S-morph individuals may have higher reproductive success than L-morph. Since our investigation is restricted to only two populations of *C. subcordata* on the Xisha Islands, further studies including more populations are needed to illustrate the ecological factors that caused the biased morph ratio in this species. Natural fruit set of S-morph flowers on Dong Island were much lower than those of the S- and L-morph flowers on Yongxing Island. This disproportion of fruit set of *C. subcordata* between the two islands is probably due to the fact that Dong Island is smaller in size, with a smaller and less active population of the *Z. japonicus* (based on our observations), and the greater distance from the mainland than Yongxing Island. Further studies may reveal other factors involved in this phenomenon.

In summary, our study showed that *C. subcordata* is a distylous species. On the Xisha Islands, distyly is only found in *Guettarda speciosa* (Xu et al. 2018) and *C. subcordata* (this study) so far. As distyly is rare on oceanic islands, it is important to illuminate how these species reproduce and maintain themselves on isolated oceanic islands. *Cordia subcordata* lacks heterostylous self-incompatibility and requires pollinators to develop fruit and set seed. All elements of the floral syndrome of *C. subcordata*, including shape, size, and colour of the corolla, nectar volume and nectar sugar concentration, indicate an adaptation for passerine pollination on the Xisha Islands. These findings show that *C. subcordata* established itself and persists in the archipelago by producing fruits through a combination of self-compatibility and pollination by the most common passerine bird on the oceanic islands in the South China Sea. Further studies are needed to elucidate the reproductive systems of this species in other regions, especially on the continent, and how distyly is maintained on the Xisha Islands after the breakdown of heteromorphic incompatibility.

SUPPLEMENTARY FILE

One supplementary file is associated with this paper:

Supplementary file 1: Map (generated with ArcGIS software) showing the geographical location of the two *C. subcordata* populations on Dong Island and Yongxing Island (black triangles).

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