Maintenance of specificity in sympatric host-specific fig/wasp pollination mutualisms

Hua Xie [1,†], Pei Yang [2,†,*], Yan Xia [1], Finn Kjellberg [3], Clive T. Darwell [4] and Zong-Bo Li [1,*]

[1] Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Southwest Forestry University, Kunming 650224, China

[2] Yunnan University of Chinese Medicine, Kunming 650500, China

[3] CEFE, CNRS, Université De Montpellier, EPHE, IRD, Montpellier, France

[4] Biodiversity and Biocomplexity Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa 904-0412, Japan

[†] Equal first author

Corresponding Author:

Pei Yang and Zong-Bo Li

Email address: 521yangpei@163.com; lizb@swfu.edu.cn

Abstract

Background: Fig/wasp pollination mutualisms are extreme examples of species-specific plant-insect symbioses, but incomplete specificity occurs, with potentially important evolutionary consequences. Why pollinators enter alternative hosts, and the fates of pollinators and the figs they enter, are unknown.

Methods: We studied the pollinating fig wasp, *Ceratosolen emarginatus*, which concurrently interacts with its typical host *Ficus auriculata* and the locally sympatric alternative host *F.*

23 *hainanensis*, recording frequencies of the wasp in figs of the alternative hosts. We measured

24 ovipositor lengths of pollinators and style lengths in female and male figs in the two host species.

25 Volatile organic compounds (VOCs) emitted by receptive figs of each species were identified

26 using GC-MS. We tested the attraction of wasps to floral scents in choice experiments, and

27 detected electrophysiologically active compounds by GC-EAD. We introduced *C. emarginatus*

28 foundresses into figs of both species to reveal the consequences of entry into the alternative host.

29 **Results:** *C. emarginatus* entered a low proportion of figs of the alternative host, and produced

30 offspring in a small proportion of them. Despite differences in the VOC profiles of the two fig

31 species, they included shared semiochemicals. Although *C. emarginatus* females prefer receptive

32 figs of *F. auriculata*, they are also attracted to those of *F. hainanensis*. *C. emarginatus* that

33 entered male figs of *F. hainanensis* produced offspring, as their ovipositors were long enough to

34 reach the bottom of the style, but broods were larger and offspring smaller than in the typical

35 host. Female figs of *F. hainanensis* failed to produce seeds when visited by *C. emarginatus*.

36 These findings advance our current understanding of how these species-specific mutualisms

37 usually remain stable and the conditions that allow their diversification.

Introduction

39 Obligate species-specific pollination mutualisms are important and unique parts of natural

40 ecosystems that facilitate efficient reproductive isolation between plant species (Schiestl &

41 Schlüter 2009). However, species-specificity is not absolute and numerous examples of alternative

42 host-use by pollinators have been recorded (Kawakita 2010; Rasplus 1996; Starr et al. 2013; Zhang

43 et al. 2012). The ongoing occurrence of such events without partner fidelity mechanisms that

44 regulate species-specificity have the potential to undermine extant biodiversity patterns by either

45 creating hybrid swarms among closely related species or by instigating hybrid-induced speciation

46   events (Coyne & Orr 2004). The proximate mechanisms that facilitate alternative host-use and

47   those that help maintain species-specificity, alongside their potential evolutionary consequences,

48   are largely unknown.

49   Partners in species-specific mutualisms have evolved sensory-mechanical filters including

50   production of, and response to, particular mixes of volatile organic compounds (VOCs) that

51   mediate host/pollinator encounter, and matching morphological traits that enforce specificity of

52   the interaction (Cornille et al. 2012; Okamoto et al. 2007; Raguso 2008). Such relationships have

53   evolved between several plant and insect lineages, including *Ficus* (Moraceae) and their fig wasp

54   pollinators (Cook & Rasplus 2003), Yucca (Asparagaceae) and yucca moth pollinators (Pellmyr

55   et al. 1996), and Phyllantheae and leafflower moth pollinators (Kawakita 2010). In these systems,

56   pollinators rear offspring exclusively within the reproductive structures of their host plants and

57   they are the plants' sole pollinators (Dufaÿ & Anstett 2003).

58   Strict specificity is predicted to lead to co-diversification over evolutionary timescales (Silvieus

59   2006). However, numerous exceptions to this have been reported. They may involve multiple

60   pollinators breeding on a single host, or, less frequently, a pollinator species locally interacting

61   with different hosts (Kawakita 2010; Rasplus 1996; Starr et al. 2013; Su et al. 2022; Zhang et al.

62   2012). Among fig wasps, cases where a pollinator uses two hosts may result in interspecific

63   hybridization among both hosts and wasps (Molbo et al. 2003; Parrish et al. 2003; Wei et al. 2014).

64   While interspecific introgression may be a genetic dead-end if selection counters hybridization, it

65   can also promote speciation (Abbott et al. 2013; Su et al. 2022; Wang et al. 2016). Cases of

66   incomplete specificity in species-specific mutualisms have received considerable attention (de

67   Vienne et al. 2013; Su et al. 2022; Whittall & Hodges 2007), but several important questions have

68   not been addressed. For example, why would an exclusive pollinator associating with its own

69   obligate host species interact with an alternative host plant that presumably provides sub-optimal

70   breeding conditions? What are the consequences of this behavior for the reproductive success of

71   pollinators? And most importantly, why does such behavior not result in the breakdown of species-

72   specific mutualisms?

73   Among plants, barriers promoting reproductive isolation are typically classified as either pre- or

74   post-pollination (Baack et al. 2015). In species-specific pollination systems, most studies have

75   focused pre-pollination barriers with plants emitting distinctive volatile organic compound (VOC)

76   mixes that attract specific pollinators (Okamoto et al. 2007; Raguso 2008; Scopece et al. 2013;

77   Whitehead & Peakall 2014). However, post-pollination barriers (including pre- and post-zygotic

78   mechanisms) do occur in some cases and are typically mediated by pollen-stigma incompatibility,

79   pollen competition, embryo abortion and hybrid sterility (Scopece et al. 2013).

80   *Ficus* species feature a unique globular inflorescence called a syconium or fig (Janzen 1979). They

81   are one of the largest genera of terrestrial plants and are keystone species in many tropical biomes

82   (Nason et al. 1998). These figs usually undergo 5 developmental phases, including pre-female

83   phase (A phase), female phase (B or receptive phase), interfloral phase (C phase), male-floral

84   phase (D phase), and postfloral phase (E phase), over several weeks for a full developmental

85   course. Only receptive figs allow pollinating wasps to enter the fig through the apical bracts

86   forming the ostiole, which constitutes a mechanical filter to the pollinators. In the absence of

87   pollinator visits, these receptive figs will cease development and abort. The pollinating wasps are

88   mostly species-specific (Cook & Rasplus 2003) with larvae developing within the fig's ovules

89   (Borges & Kjellberg 2014; Jansen-González et al. 2012). In dioecious species, figs of male trees

90   produce wasps and pollen, whereas figs of female trees produce only seeds. When figs become

91   receptive, they emit floral scents comprising a mix of VOCs, which constitute the main signal used

92   by a typically exclusive pollinator to locate its host plant. Floral scents exhibit quantitative and

93   qualitative variation among host plant species in their VOC composition (Souto-Vilarós et al.

94   2018). Pollinating insects can therefore primarily rely on these specific signals as semiochemicals

95   to locate their typical hosts, thus promoting species-specific interactions (Cornille et al. 2012;

96   Raguso 2008). A key determinant of oviposition success is relative ovipositor to style length. The

97   egg is laid in the flower ovule between the inner integument and the nucellus. In male figs, female

98   flowers have short styles, which allow easy access to the oviposition site by the ovipositor. On the

99   contrary, on female figs these flowers have long styles and the oviposition site are, therefore,

100   inaccessible (Shi et al. 2006).

101   Incomplete specificity of fig-wasp mutualisms has been documented in around 30-40% of cases

102   (Cook & Segar 2010; Machado et al. 2005; Rasplus 1996; Segar et al. 2014; Yang et al. 2015). In

103   most of these, multiple pollinators are associated with a widely distributed *Ficus* species in

104   different parts of its range (Bain et al. 2016; Cornille et al. 2012; Darwell et al. 2014; Rasplus

105   1996; Rodriguez et al. 2017; Su et al. 2022; Yu et al. 2019). However, in some cases, figs are

106   entered not only by the typical pollinator but also by a pollinator from a sympatrically occurring

107   *Ficus* species (Machado et al. 2005; McLeish & van Noort 2012; Moe et al. 2011; Ramírez 1970;

108   Souto-Vilarós et al. 2018; Yu et al. 2022). Fig wasps may enter an alternative host because it

109   produces floral scents similar to those of its typical host. However, little is known about how

110   pollinating fig wasps perceive the odours of receptive figs and may be induced to visit them.

111   Indeed, studies have typically focused on differences in VOC composition of floral odours among

112   host plant species (Ackerman 1983; Starr et al. 2013; Sutton et al. 2017; Wang et al. 2016; Zhang

113   et al. 2012) rather than on their similarities.

114   The evolutionary consequences of alternative pairings for figs and for wasps is also unknown.

115   Limited recent gene exchange among *Ficus* species has been observed in cases where pollinators

116   of one species regularly visit (normally at low frequencies) an alternative host (Machado et al.

117   2005; Su et al. 2022; Wang et al. 2016; Wei et al. 2014; Yang et al. 2015). This finding is consistent

118   with data from studies of controlled pollinator introductions into alternative hosts. Introduction of

119   four fig wasp species from other *Ficus* species into *F. turbinata* in Venezuela showed that while

120   these wasps produced offspring, no viable seeds were produced (Ramírez 1970). In dioecious *F.*

121   *montana*, its pollinator, *Kradibia tentacularis*, produced no progeny when introduced into male

122   figs of *F. asperifolia*, but female figs of *F. asperifolia* produced viable seeds after introduction of

123   *K. tentacularis* bearing *F. montana* pollen (Ghana et al. 2015). Few experimental studies of

124   pollinating wasps entering alternative hosts have examined the fates of resulting pollinator

125   offspring and fig seeds. Where wasp offspring are produced in alternative hosts, no information

126   exists on resultant morphological traits and whether fitnesses are affected. It is also unknown

127   whether pollinators and plants might also hybridize.

128   The fig pollinating wasp association between *F. auriculata* and *Ceratosolen emarginatus*,

129   provides an ideal study system in which the pollinators occasionally visit figs of the alternative

130   host, *F. hainannensis* (Fig. 1; Yang et al. 2012; further see study system below). Here we address

131   the following questions: i) What are the frequencies of pollinator visitation on alternative hosts in

132   natural populations? ii) What are the differences and the similarities in profiles of the VOCs

133   produced by receptive figs of the two host species? iii) Do receptive figs of both hosts attract *C.*

134   *emarginatus*? iv) Do the two fig species share semiochemicals that are electrophysiologically

135   active in *C. emarginatus*? v) Does relative ovipositor length of *C. emarginatus* among both

136   typical and alternative hosts vary? vi) What are the potential evolutionary consequences of entry

137   by *C. emarginatus* into an alternative host, for both wasps and figs?

138   **Materials & Methods**

139   ***Ficus* choice in study sites**

140   This study was conducted in Xishuangbanna, Yunnan, southwestern China. *Ficus auriculata* is

141   distributed in moist valleys in rain forests, whereas *F. hainanensis* is distributed in limestone

142   areas along rivers. We chose the natural habitats of the two species as study sites, the former in

143   the rain forest in XTBG (Xishuangbanna Tropical Botanical Garden) in Menglun town, and the

144   latter along the Mengxing River in Mengxing town. All study trees are non-cultivated and their

145   identity was previously validated by genetic data presented in Wei et al. (2014). We use the same

146   nomenclature as in that study. The two study sites are 10km apart.

147   **General biology and pollinator resource of the fig-wasp pairing study system**

148   The two study *Ficus* species, *F. auriculata* and *F. hainanensis*, are dioecious species (subsection

149   *Neomorphe*, section *Sycomorus*, subgenus *Sycomorus*). *F. auriculata* exhibits a heart-shaped to

150   round leaves (15-55 × 10-27 cm), and its figs are produced on cauliforous branches on the main

151   trunk and main branches. they are pear-shaped, 3-6 cm in diameter, and green-reddish when

152   unmature (A-D phase). *F. hainanensis* is distinguishes from *F. auriculata* by its narrower

153   obovate to elliptic leaves (12-25 × 6-23 cm). The figs are borne on long stoloniflorous branches

154   expanding from the base of the trunk. The pear-shaped, 1-2 cm diameter figs are sparsely

155   speckled with globose tuberculate over the reddish surface (A-E phase). They usually bear figs in

156   December every year at our study site. Their fruiting period is synchronous within species. *F.*

157   *auriculata* is typically pollinated by the species traditionally identified as *Ceratosolen*

158   *emarginatus* Mayr (Clade 1 in Wang et al., 2016), and occasionally by what could be another

159 *Ceratosolen* species involved in two study species and *F. oligodon* (Clade 3 in Wang et al.,

160 2016), and produced offspring in *F. hainanensis* (Fig 1; Yang et al. 2012). However, *F.*

161 *hainanensis* is almost exclusively pollinated by a closely related species, *Ceratosolen* sp. (Clade

162 2 in Wang et al., 2016), which also enter figs of *F. auriculata* successfully, but leads to a high

163 fig abortion rate (88.3%, Yang et al. 2012). This is the reason we selected *F. auriculata - C.*

164 *emarginatus* as a study system. To distinguish *C. emarginatus* from *Ceratosolen* sp., we

165 examined their external morphology (Fig. 2): among other traits, most obviously, the appendages

166 of the mandibulae are notably closer to the maxilla in *C. emarginatus* than in *Ceratosolen* sp.. To

167 further control which wasp species were used in our experiments (e.g., bioassay, GC-EAD, and

168 introduction), we introduced a single female wasp of *C. emarginatus* into each fig so that we

169 could use the resulting offspring for experimentation without need for species identification.

170 These offspring were temporary stored into a 120-mesh nylon bag (to prevent escape) at room

171 temperature after emergence from the male-phase figs.

172 **Investigation of pollinator host use in natural populations**

173 We investigated the numbers of *C. emarginatus* and *Ceratosolen* sp. that had entered and

174 produced offspring into the figs of *F. auriculata* and *F. hainanensis* by collecting post receptive

175 figs, 5 days after the end of receptivity and male-floral phase, respectively. For the former, we

176 collected at least 20 post-receptive figs from each of four male and four female trees. For the

177 latter, we collected 20 male-phase figs per male tree at the same tree about two months later.

178 Each post-receptive fig was cut open and effective foundresses were collected from the cavity

179 and preserved in 75% alcohol for morphological species identification and counting. Every male-

180 phase fig was put into a nylon bag (120 mesh) shortly before wasp emergence. Once wasps had

181 emerged into the bag, 10 wasps per bag were randomly selected and preserved in 75% alcohol

182   for subsequent species identification. Combing with the aforementioned morphological criteria

183   (Fig. 2), we finally identified these wasp species using a key table to the species of fig wasps

184   associated with the *Ficus* subgenus *Sycomorus* in Xishuangbanna, then counted and classified

185   them into each host species.

186   **Behavioral bioassays**

187   To test whether *C. emarginatus* was preferentially attracted to its typical over alternative figs,

188   behavioral choice experiments were performed using a Y-tube olfactometer (ID:1.5 cm, length of

189   each arm 9 cm, stem 8 cm; for further information see Chen et al., 2009). Two arms of the Y-

190   tube were each connected to a polyethylene terephthalate bag (Toppits® GmbH, Germany)

191   containing a source of odour. Airflow was pumped by a mini-vacuum pump (Xinweicheng®

192   Xinweicheng Machinery & Electric Co., Ltd, Chengdu, China) through the bags into the arms of

193   the Y-tube, after being purified by passing through an activated charcoal filter. The flow rate

194   through each arm was maintained at 100 ml/min. A wasp was deposited in the third arm of the

195   olfactometer for behavioral observation. Preliminary experiments showed that *C. emarginatus*

196   was equally attracted by male and female figs of *F. auriculata* (male Vs female =18 Vs 16;

197   χ^2=0.118, P=0.732), so we used only male figs as a source of odours.

198   To prepare the odour sources, pre-receptive stage figs of *F. auriculata* and *F. hainanensis* were

199   enclosed in nylon bags to protect them from oviposition by wasps until the figs became

200   receptive. Male figs were collected just before pollinator emergence to obtain freshly emerged

201   fig wasps, and only one wasp was selected from each fig. To test the response of *C. emarginatus*

202   to receptive figs of typical and alternative host, three treatments were carried out: receptive figs

203   of *F. auriculata* versus air, receptive figs of *F. hainanensis* versus air, and receptive figs of *F.*

204   *auriculata* versus receptive figs of *F. hainanensis*. Because of differences in fig size between

205 species, equal weights of figs of the two hosts were used (*F. auriculata*: 5-6 figs, diameter range:

206 36.55 - 59.41 mm; *F. hainanensis*: 22-28 figs, diameter range: 12.35 - 21.70 mm). All bioassays

207 were performed in a darkened room, between 10:00-12:00 hours, within three hours after

208 collection of fresh figs from trees. Each of the female fig wasps tested was positioned at the

209 entrance to the stem of the olfactometer, and the arm it selected was recorded as well as the time

210 to decision. If the wasp did not make a decision within 5 minutes, it was excluded from the total

211 number counted and from statistical analysis. After testing five successive wasps, Y-tube arms

212 were reversed to cancel out any orientation-bias effect between the two arms. Each wasp

213 individual was tested only once, and, after 10 successive wasps, the Y-tube was replaced with a

214 new one to avoid any influence of residual materials remaining in the apparatus. Three

215 treatments were repeated with total 31, 38 and 41 fig wasps that made a decision.

216 **Comparison of VOCs emitted by two host species**

217 VOCs were collected using the dynamic headspace technique (Chen et al. 2009). Small pre-

218 receptive figs for each sex of *F. auriculata* and *F. hainanensis* were enclosed in nylon bags on

219 trees to prevent wasps from entering and ovipositing. When the figs had reached receptive phase

220 (*F. auriculata*: 4-8 figs, *F. hainanensis*: 20-35 figs), they were enclosed in polyethylene

221 terephthalate bags for the collection of VOCs. Airflow, purified by passing through a filter of

222 activated charcoal (20-40 mesh, Supelco® Sigma-Aldrich, USA), was maintained through the

223 bag by a mini-vacuum pump connected to the entrance by flow-meters with a flow rate of 300

224 ml/min, while a VOC trap containing 80 mg Porapak® Q adsorbent (80-100 mesh, Supelco®

225 Sigma-Aldrich, USA) was connected to the exit of the bag at a flow rate of 300 ml/min. To

226 check for possible contaminant compounds sampled during collection, empty bags were used as

227 blanks for extraction by means of the same dynamic headspace technique and equipment. VOC

228   collection was performed for four hours from 10:00 to 14:00, the period of the day when fig

229   wasps are most active. Three repeats were performed for each sex of each tree species. After

230   VOC collection, the adsorbents were eluted three times with a total of 500 μl of dichloromethane

231   and concentrated down to 100 μl with 99.99% N_2. Then, two internal standards (octane and

232   dodecane, at 200 ng/μl) were added to every sample prior to gas chromatography.

233   All VOC samples were analysed using gas chromatography-mass spectrometry (GC-MS, Agilent

234   Technologies 7890A-5975C, USA) with an HP-5MS column (30 m, ID: 250 μm, film thickness

235   0.25 μm). Helium was used as carrier gas at a flow rate of 1ml/min. The injector split vent was

236   set at a ratio of 1:4 and the injector temperature was 250 °C. Oven temperature was set at 40 °C,

237   and then programmed to rise to 150 °C at a rate of 3 °C/min, then at 10 °C/min to 260 °C, and

238   finally temperature was maintained at 260 °C for 5 minutes. Compound identification was based

239   on comparison of retention times (RT), matching of the mass spectra with the NIST 08 MS

240   library, and Kovats retention indices (RI) taken from both the NIST Chemistry Web Book

241   (http://webbook.nist.gov) and the RI database (Adams 2007). Where available, we used synthetic

242   compounds as a more precise reference (see Supplementary material Table S1).

243   **Electrophysiological responses of *C. emarginatus* to VOCs of the two host species**

244   To identify which VOCs of the receptive fig odours were detected by the wasps and thus

245   constituted candidate semiochemicals, we performed electrophysiological tests. The responses of

246   *C. emarginatus* antennae to odours from receptive figs of *F. auriculata* and *F. hainanensis* were

247   recorded using gas chromatography-electroantennography (GC-EAD, Agilent, USA, Syntech,

248   Netherlands). VOCs were collected for injection in the GC-EAD as for the VOC analysis

249   procedures except that we extended the collection duration to six hours in order to extract larger

250   quantities of VOCs. The GC program was the same as that used for the analysis of VOCs

251   presented above. A head with an antenna was placed on a micro-operating platform (MP-15,

252   Syntech, Netherlands), and two glass electrodes filled with saline solution (NaCl, 4 g; Na_2HPO_4

253   0.57 g; KH_2PO_4, 0.1 g; KCl, 0.1 g in 500 ml distilled water; pH 7.4) were connected to the distal

254   tip of the antenna and to the antennal scape. Antenna depolarization was recorded using the

255   Electroantennography version 2.5 software package (Syntech, Netherlands). Three antennae

256   from *C. emarginatus* were tested for *F. auriculata* VOCs and three for *F. hainanensis* VOCs.

257   **Relative ovipositor to style length**

258   As pollinating wasps oviposit by inserting their ovipositors into the female styles of pistillate

259   flowers, we investigated matching relationship between style length in both host *Ficus* species

260   and ovipositor length of the pollinator *C. emarginatus*. We enclosed the pre-receptive figs of *F.*

261   *auriculata* and *F. hainanensis* in nylon bags (120 mesh) to prevent wasp entrance. When the figs

262   became receptive to the pollinators, we collected 32 figs from four *F. auriculata* male trees and

263   30 figs from three female trees; we collected 30 figs from three *F. hainanensis* male trees and 31

264   figs from three female trees. Then 20 pistillate flowers per fig were randomly selected to

265   measure style length. Ninety male figs of *F. auriculata* were collected and placed in separate

266   bags before female wasp emergence. After emergence we selected one *C. emarginatus* from

267   every fig and measured the length of the ovipositor, which was excised from the wasp abdomen

268   and removed the ovipositor sheath. All measurements of style length of figs and ovipositor

269   length of pollinators were conducted using a micrometer in a dissection microscope (Olympus

270   SZX12-3141, Tokyo, Japan).

271   *C. emarginatus* **introduction experiments**

272   Pre-receptive figs of *F. auriculata* and *F. hainanensis* were enclosed in large nylon bags (120

273   mesh) to prevent wasp oviposition. When these figs reached receptive phase, one *C. emarginatus*

274   emerging from *F. auriculata* was introduced into each fig. Wasps were introduced into at least

275   20 figs for each tree. We chose three trees of each sex for each host species. After introduction,

276   figs were re-enclosed in large nylon bags until just before wasps emerged from the figs. The figs

277   were then removed from the tree and enclosed in individual nylon bags. All emerging wasps

278   were preserved in 75% alcohol for subsequent counting and measurements. According to

279   morphological convergence produced by the selective pressures from the ostiole (Liu et al. 2011;

280   van Noort & Compton 1996), three traits representing fig wasp size, including head width,

281   thorax width, and ovipositor length, were measured on the offspring for comparison with the

282   foundresses. At least 5 wasps from each fig for each tree were measured. All measurements were

283   carried out under a stereomicroscope.

284   **Data analysis**

285   Data analyses were mostly performed in R version 4.0.5 (R Development Core Team; URL

286   http://www.R-project.org). For VOC analyses, non-metric multi-dimensional scaling (NMDS)

287   methods were conducted using the Vegan package and the Bray-Curtis distance was used to find

288   the best two-dimensional representation of the distance matrix. A Permutational Multivariate

289   Analysis of Variance (PERMANOVA) was used to compare the VOC composition between *F.*

290   *auriculata* and *F. hainanensis*. Chi-square tests were used to determine whether pollinators

291   showed preferences for their typical or alternative host. Mann-Whitney U tests were used to test

292   the time that pollinators took to make a choice in the behavior-choice experiment. ANOVAs or

293   Kruskal-Wallis test with unequal variances were used to examine differences in style length

294   among figs and trees as well as head width, thorax width and ovipositor length between

295   foundresses and offspring in the two treatments. Style length, ovipositor length and number of

296   offspring in the two treatments were also compared using Mann-Whitney U tests.

297 **Field Study Permissions**

298 The following information was supplied relating to field study approvals (i.e., approving

299 body and any reference numbers): the agonid wasps, *C. emarginatus* and *Ceratosolen* sp., are

300 common insects and collection permitted by the leader of Department of Horticulture and

301 Landscape, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science.

302 ## Results

303 **Frequency of *C. emarginatus* entering the alternative host and offspring production**

304 We collected a total of 2251 wasps (1036 wasps in 86 male figs and 1215 in 88 female figs) that

305 had entered 175 receptive figs of *F. auriculata* (Table 1). Among these, each fig contained at

306 least one *C. emarginatus* (one exception), but the total number of foundresses entering into a fig

307 showed a significant variation (male fig:1-38 wasps, female fig: 1-25 wasps). There was no

308 difference in average of foundresses numbers between male (11.4 ± 7.8) and female fig (11.6 ±

309 6.5). Totally, the proportions of *C. emarginatus* entering figs of *F. auriculata* were 94.31% in

310 male figs and 98.02% in female figs, respectively. We collected a total of 931 wasps (757 in 60

311 male figs and 174 in 60 female figs) that had entered 120 receptive *F. hainanensis* figs. There

312 was a small number of *C. emarginatus* (11.89% in male figs and 6.32% in female figs). Among

313 these, 24 out of 60 male figs and 11 out of 60 female figs contained *C. emarginatus*. The total

314 number of *C. emarginatus* in a male fig was larger than in a female fig (male fig: 1-14 wasps,

315 female fig: 1 wasp). Results for offspring production were qualitatively similar to those for fig

316 visitation, but the differences between typical and alternative hosts were more extreme (Table 1).

317 In *F. auriculata*, 793 *C. emarginatus* offspring from 80 male figs (occurring rate of 100%) were

318 found while from 9 figs from 60 male *F. hainanensis* figs (occurring rate of 15%), there were of

319 total 30 *C. emarginatus* individuals.

320 **Pollinator bioassays**

321 Female *C. emarginatus* individuals were strongly attracted by receptive figs of their typical host,

322 *F. auriculata*, when confronted with a choice between it and air or the alternative host *F.*

323 *hainanensis*. The wasps preferred *F. hainanensis* receptive figs over air (Fig. 3). When the wasps

324 were given a choice between *F. auriculata* and *F. hainanensis*, there was a reduction in the

325 proportion of wasps choosing *F auriculata* figs when compared to the *F. auriculata* versus air

326 choice (χ^2=8.656, P=0.003). When given a choice between *F. auriculata* odour and air, *C.*

327 *emarginatus* spent the shortest mean recorded time before entering a branch of the olfactometer

328 (39.91 ± 22.35 s, P=0.036). *C. emarginatus* preferred *F. auriculata* odour when given a choice

329 between it and *F. hainanensis*, with the time taken independent of final arm choice (*F.*

330 *auriculata*: 54.78 ± 36.41 s, *F. hainanensis*: 56.75 ± 46.60 s, Z=-0.078, P=0.938). In the *F.*

331 *hainanensis* versus air treatment, *C. emarginatus* took longer before entering an arm (63.99 ±

332 38.29 s) in comparison to the *F. auriculata* versus air treatment (Z=-2.641, P=0.008).

333 **Comparison of the VOCs emitted by the two *Ficus* species**

334 We identified a total of 78 VOCs in scents emitted by receptive figs of *F. auriculata* and *F.*

335 *hainanensis*. VOCs emitted by receptive figs did not differ significantly between sexes within

336 species (Fig. 4, PERMANOVA; for *F. auriculata*, F=2.68, P=0.062, for *F. hainanensis*, F=3.95,

337 P=0.100; Supplementary material Table S1). Thirty-four VOCs were shared between the odours

338 produced by the two fig species, and among these, ten VOCs were abundant (> 5%) in one or

339 both species. In particular, the relative ratios of β-funebrene were high in both species (*F.*

340 *auriculata*: 12.22 ± 5.50%, *F. hainanensis*: 26.83 ± 5.48%). Nevertheless, the complement of

341 VOCs produced by *F. auriculata* and *F. hainanensis* were distinguishable (PERMANOVA,

342 F=11.297, P=0.002).

343   **Electrophysiological responses of *C. emarginatus* to VOCs of the two *Ficus* species**

344   *C. emarginatus* presented electroantennographic responses to nine compounds in the odours

345   from *F. auriculata* and to six in the odours from *F. hainanensis* (Fig. 5). Among these

346   compounds, α-copaene and β-funebrene were produced by both host trees. These two

347   compounds represented 38% and 31% of the scents emitted by male and female *F. hainanensis*

348   figs respectively.

349   **Matching of style length and ovipositor length**

350   Style lengths were bimodally distributed in both *F. auriculata* and *F. hainanensis*, while they

351   significantly differed among figs and trees (Table 2; Fig. 6). Style lengths of figs of female trees

352   (*F. auriculata*: 2.03 ± 0.75 mm, *F. hainanensis*: 1.54 ± 0.18 mm) were much longer than in figs

353   of male trees in both *Ficus* species (*F. auriculata*: Z=-25.534, P<0.001; *F. hainanensis*: Z=-

354   30.292, P<0.001). Style length in figs of male trees of *F. auriculata* (0.97 ± 0.11 mm) was

355   around 0.05 mm longer than that for figs of male trees of *F. hainanensis* (0.92 ± 0.05 mm)

356   (Z=9.295, P<0.001). Ovipositor of *C. emarginatus* was 1.19 ± 0.09 mm (n = 90) in length, which

357   were very similar to the styles of male figs from the two *Ficus* hosts (Fig. 6), but shorter than the

358   styles in female figs (*F. auriculata*: Z=11.10, P<0.001; *F. hainanensis*: Z=-8.93, P<0.001).

359   **Consequences of introduction of *C. emarginatus***

360   Single *C. emarginatus* females produced significantly fewer offspring in *F. auriculata* figs than

361   in *F. hainanensis* figs (Z=-4.490, P<0.001; Table 3). When *C. emarginatus* was introduced into

362   female figs of *F. hainanensis*, all treated figs aborted. There was no significant fig and tree effect

363   on wasp-size of female *C. emarginatus* reared in *F. auriculata* figs (head width: P=0.097 and

364   P=0.076, thorax width: P=0.709 and P=0.512; ovipositor length: P=0.073 and P=0.510) and *F.*

365   *hainanensis* (head width: P=0.204 and P=0.070, thorax width: P=0.649 and P=0.695; ovipositor

366   length: P=0.853 and P=0.159) among figs and trees, respectively. All components of these same

367   data types were combined into one for subsequent analysis. Further, female *C. emarginatus*

368   reared from *F. hainanensis* were significantly smaller than those reared from their typical host *F.*

369   *auriculata* (head width: P=0.018; thorax width: P= 0.0002), but similar to those *Ceratosolen* sp.

370   associated with *F. hainanensis* (head width: P=0.925; thorax width: P=0.999). their ovipositor

371   was shorter than those of both *C. emarginatus* and of *Ceratosolen* sp. (P=0.034) raised on their

372   respective typical hosts (P<0.001; Table 4).

373   ## Discussion

374   Imperfect host-symbiont interactions, particularly in the case of highly species-specific

375   pollination mutualisms, maintaining low rates of the wrong symbiont associating with the

376   alternative host likely involve a fitness cost for both partners (e.g. Janzen 1979, Ghana et al.

377   2015). Moreover, given that these phenomena naturally occur, it is unknown what mechanisms

378   impede either the formation of hybrid swarms among closely-related species or the evolution of

379   hybridization-induced speciation events – both of which may cause breakdown of species-

380   specificity with resultant fluxes in extant biodiversity patterns. Here we present a novel study

381   that incorporates comprehensive sampling alongside detailed ecological data to investigate both

382   the mechanisms and potential evolutionary outcomes of alternative host-use events. We show

383   that while similarity in semiochemicals of two *Ficus* species may facilitate alternative host-use,

384   partner fidelity mechanisms may regularly operate that select against sustained introgression and

385   help maintain species-specificity patterns.

386   **Pollinator visits to alternative hosts**

387   Frequent use of several host species by a pollinator species has been reported in a few cases for

388   American and African monoecious *Ficus* lineages (Cornille et al. 2012; Machado et al. 2005;

389 McLeish & van Noort 2012) and in some Asian dioecious *Ficus* lineages (Su et al. 2022; Wang

390 et al. 2016; Yu et al. 2022). In some of these cases olfactive messaging has converged showing

391 that pollinator sharing is a plant adaptation (Cornille et al. 2012; Wang et al. 2016). In our study,

392 we investigated a case in which a pollinator species, *C. emarginatus*, has a main host and

393 alternative host. The two host species' populations, were located several kilometers apart in their

394 natural habitat. We identified to species 3182 wasps that had entered 295 receptive phase figs

395 and 1400 offspring wasps emerging from 140 figs. Our results reveal strong specialization with a

396 low frequency of individuals colonizing the alternative host species, as seen in some other

397 dioecious *Ficus* species (Moe et al. 2011; Silvieus 2006; Weiblen et al. 2001). Further, the

398 typical host *F. auriculata* was predominantly visited by its own associated pollinators, *C.*

399 *emarginatus*.

400 We document a reduction in the proportion of alternative pollinator offspring relative to the

401 initial proportion of foundresses in natural populations. Moreover, female *C. emarginatus*

402 experimentally introduced into figs of *F. hainanensis* produced larger broods than on its typical

403 host and more than is produced by *F. hainanensis*'s typical pollinator, *Ceratosolen* sp. (Yang et

404 al. 2012). However, our results suggest that *C. emarginatus* individuals visiting figs of *F.*

405 *hainanensis* have reduced fitness due to trait mismatch resulting from reduced offspring

406 ovipositor length (Table 2) that is likely to compromise oviposition ability (Liu et al. 2011; Yang

407 et al. 2012) and due to the reduced fecundity associated with reduced size (Moore & Greeff

408 2003). It is possible that individuals of *C. emarginatus* visiting figs of *F. hainanensis* are wasps

409 that have failed to locate receptive figs of *F. auriculata* (Liu et al. 2015), and have become less

410 choosy towards the end of their life spans. In some cases, wasps entering alternative hosts failed

411 to reproduce. Additionally, our results show that 41.67% of receptive male *F. hainanensis* figs

412 harbored pollinating wasp *C. emarginatus*. This is reminiscent of the situation for the pollinators

413 of *F. hirta* and *F. triloba* in Guangdong province, China, though in that case fitness

414 consequences were not investigated (Yu et al. 2022). Co-occurrence of the pollinator species

415 across two fig species indicates potential for hybridization, although we did not observe any

416 wasps possessing morphological characters suggestive of hybridization. In *F. rubiginosa*, three

417 cryptic species of *Pleistodontes imperialis* coexist in the same localities while retaining

418 reproductive isolation (Sutton et al. 2017), with *Wolbachia* identified as the most likely

419 candidate of post-zygotic reproductive isolation (Haine & Cook 2005). Similarly, no

420 hybridization was detected between pollinating-wasps of genus *Pegoscapus* in Panama (Satler et

421 al. 2022).

422 **Host volatile semiochemicals attracting pollinators**

423 It has been suggested that closely related sympatric figs species may emit similar floral scents to

424 attract pollinators and this could result in pollinators confusing typical and alternative host

425 species (Moe et al. 2011; Wang et al. 2013). The VOC composition of the receptive fig odours of

426 *F. auriculata* and *F. hainanensis* were clearly differentiated, consistent with the different VOC

427 profiles between sister species of section *Papuacyse* and between subspecies of *F. trichocerasa*

428 in Papua New Guinea (Souto-Vilarós et al. 2018), and between *F. hirta* and *F. triloba* (Yu et al.

429 2022). They shared 34 VOCs including 10 quantitatively important compounds (i.e. >5%), but

430 only two that were shown to elicit wasp antennal response as semiochemicals. The shared

431 semiochemicals eliciting antennal response represented 19% of receptive male fig odours and

432 13% of receptive female fig odours in *F. auriculata* and 38% and 31% for male and female *F.*

433 *hainanensis* figs respectively, indicating a large degree of chemical attractant overlap. Wasp

434 attraction to receptive figs may be from long and short distance alongside contact attractants. In

435 *F. curtipes* it has been shown that one VOC is mainly responsible for long distance attraction

436 while another is more important for fig entry (Gu & Yang 2013). When *C. emarginatus* were

437 given a choice between *F. auriculata* and *F. hainanensis* receptive figs, 30% chose *F.*

438 *hainanensis* figs, a figure much higher than the observed frequency of alternative pollinators on

439 our wild figs and suggesting either that initial contact with typical host odour in the stem of the

440 Y-tube may result in reduction in choosiness.

441 **Offspring of pollinating fig wasps in alternative host species**

442 *C. emarginatus* produced more but smaller offspring in *F. hainanensis* than in its typical host

443 (Table 3). *F. auriculata* and *F. hainanensis* fit the general pattern by which style lengths in male

444 figs are shorter than the ovipositor of pollinators (Fig. 6), allowing wasps to oviposit into

445 pistillate flowers of male figs (Ganeshaiah et al. 1995; Shi et al. 2006; Weiblen 2002). *C.*

446 *emarginatus* ovipositors are longer than the styles in male figs of *F. hainanensis* which have

447 shorter styles than *F. auriculata*. This may explain why *C. emarginatus* produced more offspring

448 in *F. hainanensis* than in *F. auriculata*, as oviposition can be more rapid in flowers with shorter

449 styles. However, offspring body size was reduced in comparison with *C. emarginatus* developing

450 in *F. auriculata* (Table 4) and body size is known to correlate with fecundity in fig pollinating

451 wasps (Moore & Greeff 2003). This probably results from the developing larva consuming the

452 endosperm of the smaller plant ovules of *F. hainanensis* (0.61 ± 0.01 mm), compared with those

453 of *F. auriculata* (0.88 ± 0.01 mm), which may cause a concomitant reduction in fitness due to

454 reduced body size featuring shorter ovipositors which should impinge subsequent oviposition

455 attempts (Moore et al. 2004; Stone et al. 2002). Nevertheless, *C. emarginatus* offspring will

456 leave male *F. hainanensis* figs carrying pollen. These wasps are likely to search for *F. auriculata*

457 figs and may subsequently initiate host hybridization (Machado et al. 2005). However, small

458   wasps will probably have reduced capacity to reach receptive figs and will therefore be poor

459   pollen vectors. In comparison, smaller sized *C. emarginatus* developing in the alternative host *F.*

460   *hainanensis* may be fully capable of entering and ovipositing in *F. hainanensis* figs. If their

461   parents had entered the atypical host because of a mutation in its olfactory system, or if host

462   choice ability is formed through host environmental effect, then the evolution of a new host

463   preference is possible. Such variant *C. emarginatus* are likely to be outcompeted by *Ceratosolen*

464   sp. Only in locations where *Ceratosolen* sp. is absent would these genetic variants thrive and

465   progressively adapt to the new host. This may happen, as populations of *F. hainanensis* are

466   generally small and localized. The case of *C. emarginatus* also visiting figs of *F. oligodon* is

467   somewhat different as the two host species produce identical receptive fig odours suggesting

468   selection on the two host species to mimic each other and attract a same population of pollinating

469   wasps (Wang et al. 2016).

470   In experimental introductions into receptive figs of *F. hispidioides* of wasps originating from

471   four other *Ficus* species, no wasps developed, although oviposition attempts led to ovule

472   development in male figs (Moe et al. 2011). Beyond the *F. auriculata* species complex which

473   includes *F. auriculata*, *F. oligodon* and *F. hainanensis*, successful emergence of offspring wasps

474   of a same species from two or several locally co-occurring alternative host figs has been reported

475   for *Elisabethiella stuckenbergi* visiting *F. burkei* and *F. natalensis*, subgenus *Urostigma* in South

476   Africa (Cornille et al. 2012) and for *Blastophaga silvestriana* visiting a number of species of

477   subgenus *Ficus*, section *Ficus* in China (Su et al. 2022). In this later case some genetic data

478   suggests the presence of hots races (Wachi et al. 2016), while other data suggests that a same

479   population of wasp visits several host species (Su et al. 2022). Nevertheless, none of these

480   studies includes the experimental confirmation we provide here on successful offspring

481   development in alternative host. Hence, despite reports of fig pollinating wasps visiting several

482   host species the outcome of these visits should be ascertained before drawing conclusions on

483   their evolutionary significance (Moe et al. 2011). Mechanisms can therefore exist that allow

484   alternative interactions while simultaneously preventing introgression and species-specificity

485   break-down. However, it can be further envisaged that this system could be easily reconfigured

486   to favour hybridization events if partner fidelity mechanisms fail or are selectively removed,

487   potentially due to changes in abiotic conditions and local extinctions of the population of

488   pollinators of a potential alternative host.

489   **Hybridization implied by pollinator's presence in an alternative host**

490   With respect to potential plant hybridization, our data show that all female *F. hainanensis* figs

491   visited by *C. emarginatus* undergo selective abortion (Table 3). High abortion levels (88.3% and

492   100% in male and female figs, respectively) have also been documented for *F. auriculata* figs

493   visited by wasps (most probably *Ceratosolen* sp.) emerging from *F. hainanensis* (Yang et al.

494   2012). As viable hybrids have been observed between phylogenetically more distant *Ficus*

495   species (e.g. Condit 1950), we suggest that the lack of seed production results from variation

496   between wasp species in pollination behavior and trait matching. Indeed, artificial pollination

497   leads to the production of viable offspring (Wei et al. 2014).

498   Four genetic studies based on RFLPs (Parrish et al. 2003) and on microsatellite data (Moe et al.

499   2011; Wang et al. 2016; Wei et al. 2014) have suggested the presence of hybrids between closely

500   related *Ficus* species. In two of those studies, genetic results confirmed that morphologically

501   intermediate individuals were interspecific hybrids (Parrish et al. 2003; Wei et al. 2014). We cut

502   open all aborted female figs to inspect the ovules in every cavity containing a *C. emarginatus*

503 foundresses. We observed no swelling of the ovules. This indicates that strong pre-zygotic

504 isolation mechanisms prevent the hybridization of *F. auriculata* and *F. hainanensis*.

505 Interestingly, although our experiments suggest that *F. auriculata* and *F. hainanensis* do not

506 typically hybridize, some hybrids have been documented between them (Wang et al. 2016). *F.*

507 *oligodon* which is sympatric with both species hybridized with *F. auriculata* and produced a

508 normal number of seeds (Yang et al. 2012), but hybridization events with *F. hainanensis*

509 produced few seeds and a high fig abortion ratio (Table 3). This suggests that *F. oligodon* may

510 act as a bridge species facilitating introgression between *F. auriculata* and *F. hainanensis*.

511 Therefore, in addition to sharing pollinators, such a bridge species may play a pivotal role in

512 speciation events and co-diversification with some wasp exchanges between closely related

513 *Ficus* species (Cornille et al. 2012).

Conclusions

515 Our findings show that a low frequency of *C. emarginatus* associated with the typical host *F.*

516 *auriculata* enter the alternative host *F. hainanensis* in natural populations, and that a small

517 proportion of wasps' offspring complete their development in the alternative host. *C.*

518 *emarginatus* appear to enter receptive figs of the alternative host because emitted signal scents

519 share some of the main semiochemicals, although their overall VOC compositions are different.

520 However, mismatches between the length of ovipositors of fig wasps and of styles in male figs

521 appear to limit successful reproduction of pollinators in the alternative host, *F. hainanensis*, and

522 further reduces the body size of progeny that are produced. Our findings also show that no seeds

523 were produced when *C. emarginatus* with *F. auriculata* pollen were introduced into female figs

524 of *F. hainanensis*, owing either to mismatches between wasp behavior and plant anatomy, or to

525 interspecific pre or post zygotic incompatibility, or both. Thus, we show that despite a potential

526 for multiple host use and genetic introgression, specificity in sympatric fig-wasp pollination

527 mutualisms can be maintained. Moreover, our data indicate that suites of mechanisms either

528 promoting or hindering hybridization may co-exist, with eventual outcomes contingent on

529 dominant partner fidelity mechanisms. However, over evolutionary timescales, occasional use of

530 alternative hosts may provide opportunities for host shifts, hybridization or other events leading

531 to diversification of both figs and wasps if partner fidelity mechanisms fail or are selectively

532 removed.

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551   **Competing Interests**

552   The authors declare there are no competing interests.

553   **Author Contributions**

554   Xie Hua performed field experiments and laboratory work, analyzed the data, prepared figures

555   and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

556   Pei Yang conceived and designed the experiments, analyzed the data, prepared figures and/or

557   tables, authored or reviewed drafts of the paper, and approved the final draft.

558   Yan Xia performed field experiments, authored or reviewed drafts of the paper, and approved the

559   final draft.

560   Finn Kjellberg analyzed the data, authored or reviewed drafts of the paper, and approved the

561   final draft.

562   Clive T. Darwell analyzed the data, authored or reviewed drafts of the paper, and approved the

563   final draft.

564   Zong-Bo Li conceived and designed the experiments, analyzed the data, prepared figures and/or

565   tables, authored or reviewed drafts of the paper, and approved the final draft.

566   **Field Study Permissions**

567   The following information was supplied relating to field study approvals (i.e., approving

568   body and any reference numbers):

569   The agaonid wasps, *C. emarginatus* and *Ceratosolen* sp., are common insects and collection

570   permitted by the leader of Xishuangbanna Tropical Botanical Garden, Chinese Academy of

571   Science.

572 **Data Availability Statement**

573 The following information was supplied regarding data availability:

574 The raw data is provided in the Supplemental Files.

575 **Supplemental Information**

576 Table S1: Occurrence (Occ) and relative amounts of tentatively identified volatile organic

577 compounds emitted by receptive figs of *Ficus auriculata* and *F. hainanensis*. a compound with

578 their identity confirmed by mass spectrometry analysis; b compounds with their identity

579 confirmed by comparison of Kovats retention index with the NIST chemistry Web Book

580 (http://webbook.nist.gov) and literature; c compounds with their identity confirmed by

581 comparison of MS and retention time with laboratory standards (Sigma-Aldrich, USA).

582

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Figure 1

Illustration of sharing the pollinator between two distinct host *Ficus* species.

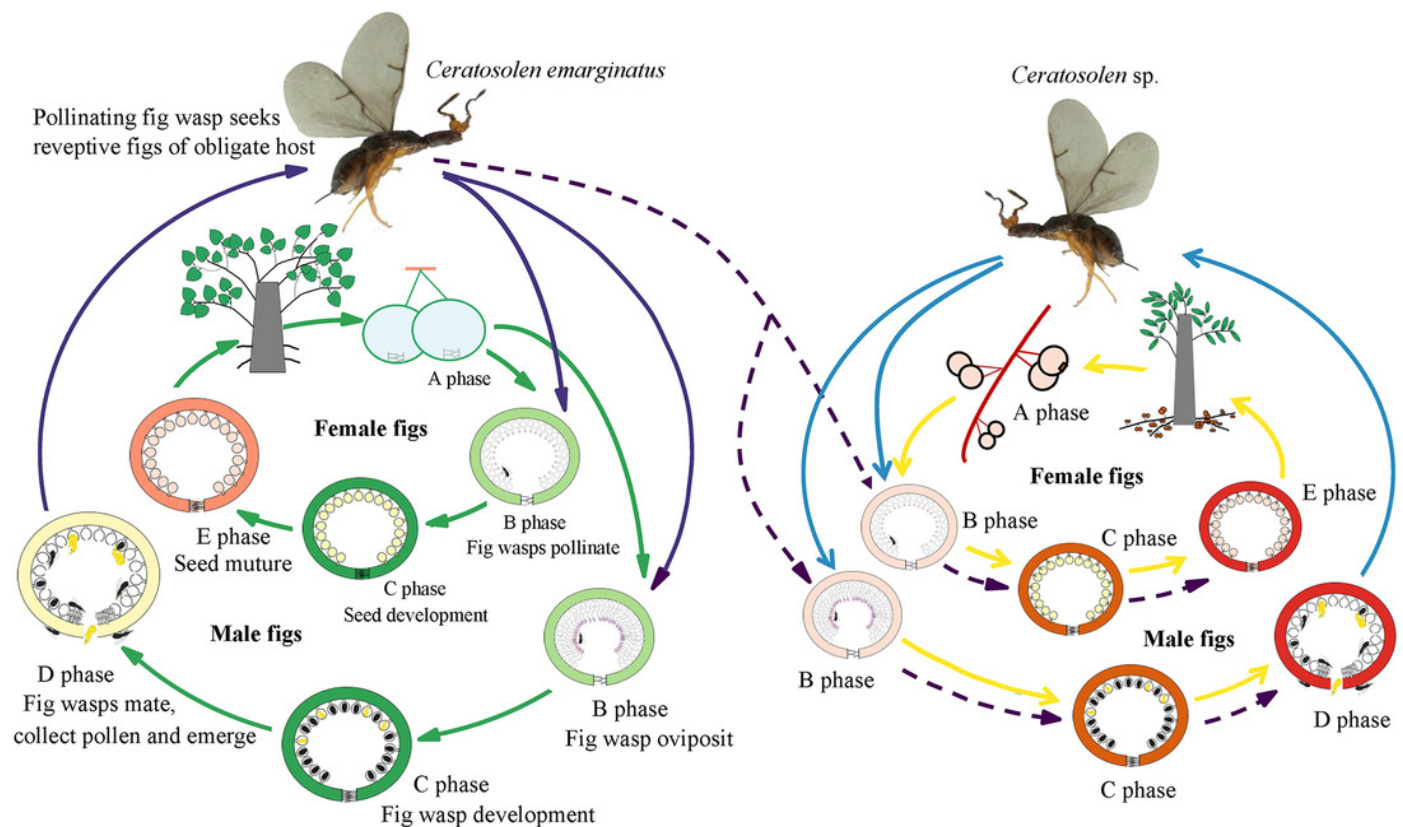



Figure 2

Characteristics of *Ceratosolen emarginatus* and *Ceratosolen* sp.

(A) dorsal side of head of *C. emarginatus*; (B) ventral side of head of *C. emarginatus*; (C) dorsal side of head of *Ceratosolen* sp.; (D) ventral side of head of *Ceratosolen* sp.; (E) dorsal side of hind leg of *C. emarginatus*; (F) ventral side of hind leg of *C. emarginatus*; (G) dorsal side of hind leg of *Ceratosolen* sp.; *(H)* ventral side of hind leg of *Ceratosolen* sp.. Scale: 200 µm. Note that, when the mandibulae are closed, the appendages of the female mandibulae are close to the maxilla in *C. emarginatus* while the mandibular appendages are more divergent from each other and hence more separated from the maxilla in *Ceratosolen* sp.. Furthermore, the hind legs of *C. emarginatus* have a large ventral tooth in the coxa and a sharp tooth in the femur, thus differing from those of *Ceratosolen* sp..

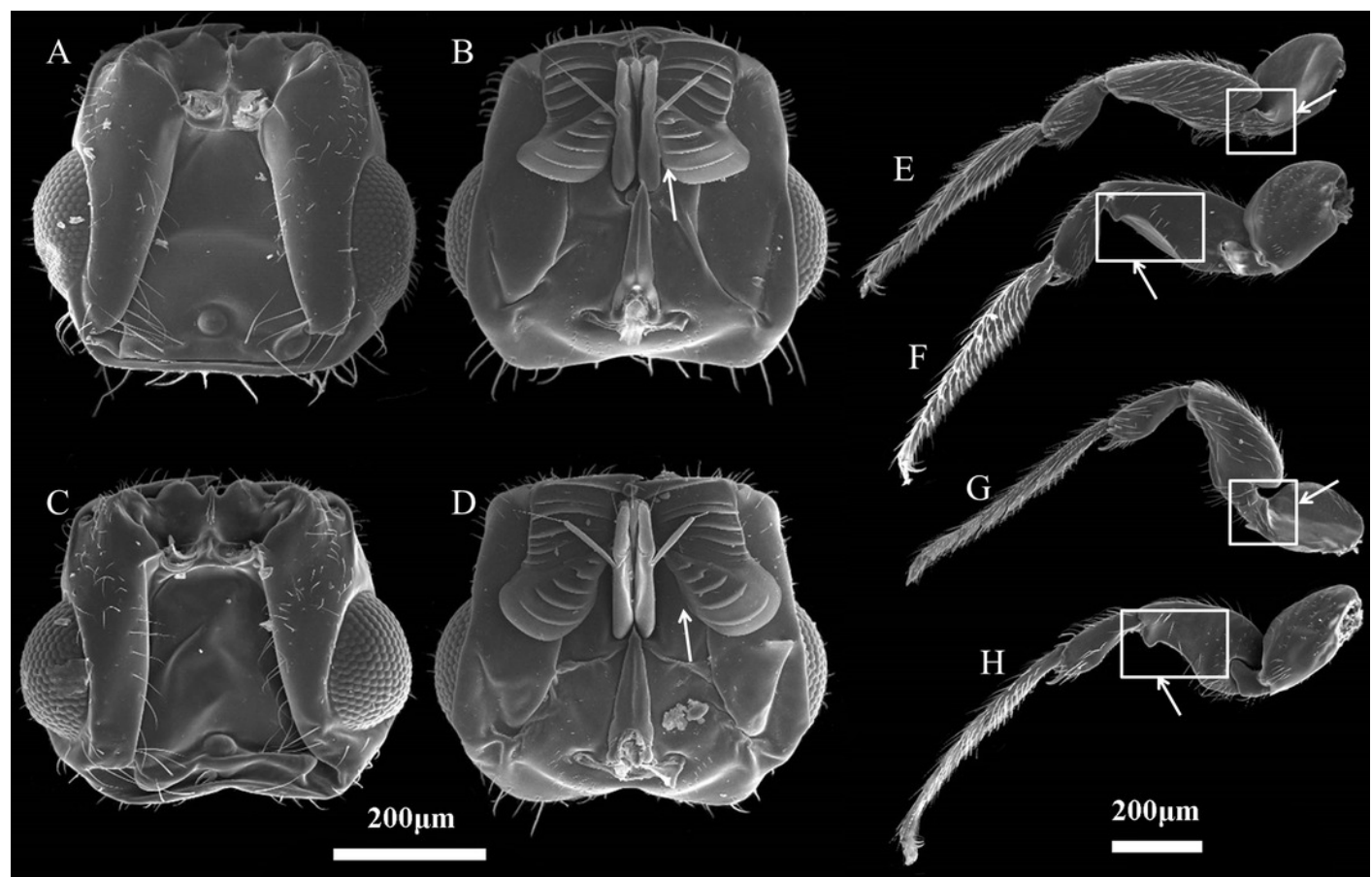



Figure 3

Bioassays of female Ceratosolen emarginatus responses to receptive figs of Ficus auriculata and F. hainanensis performed using Y-tube olfactometer tests

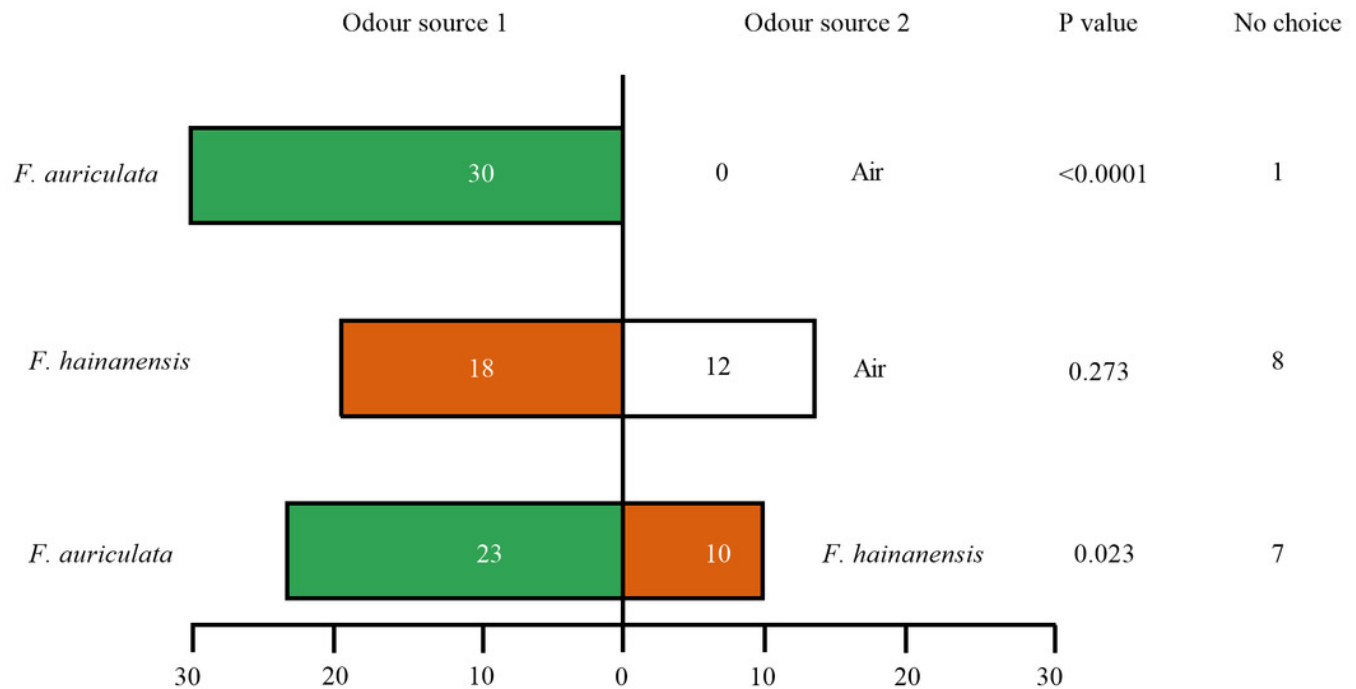



Figure 4

Non-metric multi-dimensional scaling of the relative proportions of VOCs emitted by receptive figs of Ficus auriculata and F. hainanensis based on Bray-Curtis dissimilarity index (stress=0.062).

The tendency for a slight difference in receptive fig odour between sexes is non-significant whereas the difference between species is significant.

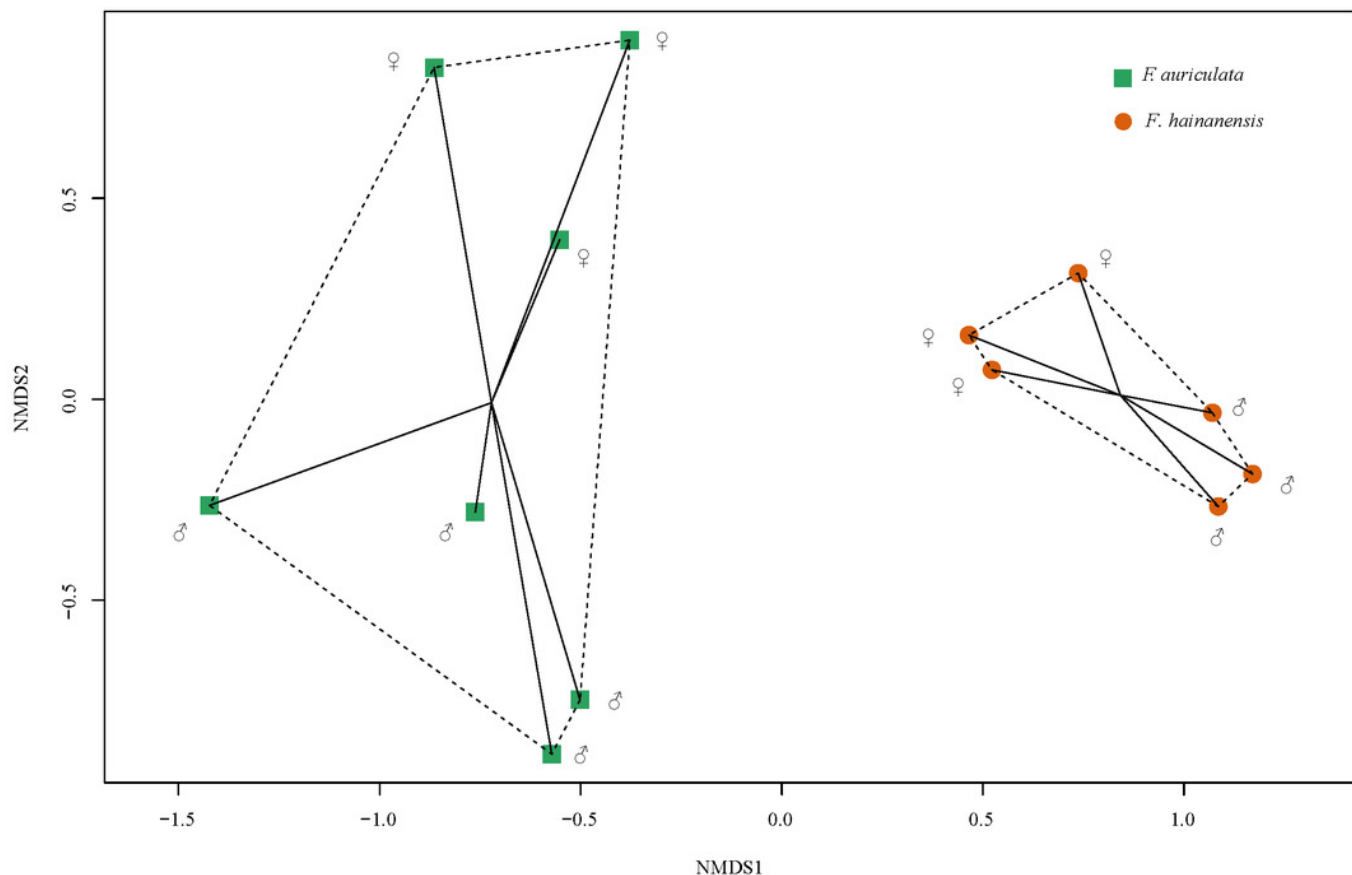



Figure 5

Electroantennographic responses of Ceratosolen emarginatus to receptive fig scent extracts of (A) Ficus auriculata and (B) F. hainanensis.

GC-FID (black line), and GC-EAD responses of C. emarginatus antennae (inverted blue line).

VOC identification: 1: 2-Heptanone; 2: Ylangene; 3 and 1': α-Copaene; 4: α-Funebrene; 5: α-Gurgujene; 6 and 3': β-Funebrene; 7: trans-β-Farnesene; 8: α-Patchoulene; 9: β-Cadinene; 2': α-Cedrene; 4': β-Cedrene; 5': α-Guaiaene; 6': Aromadendrene).

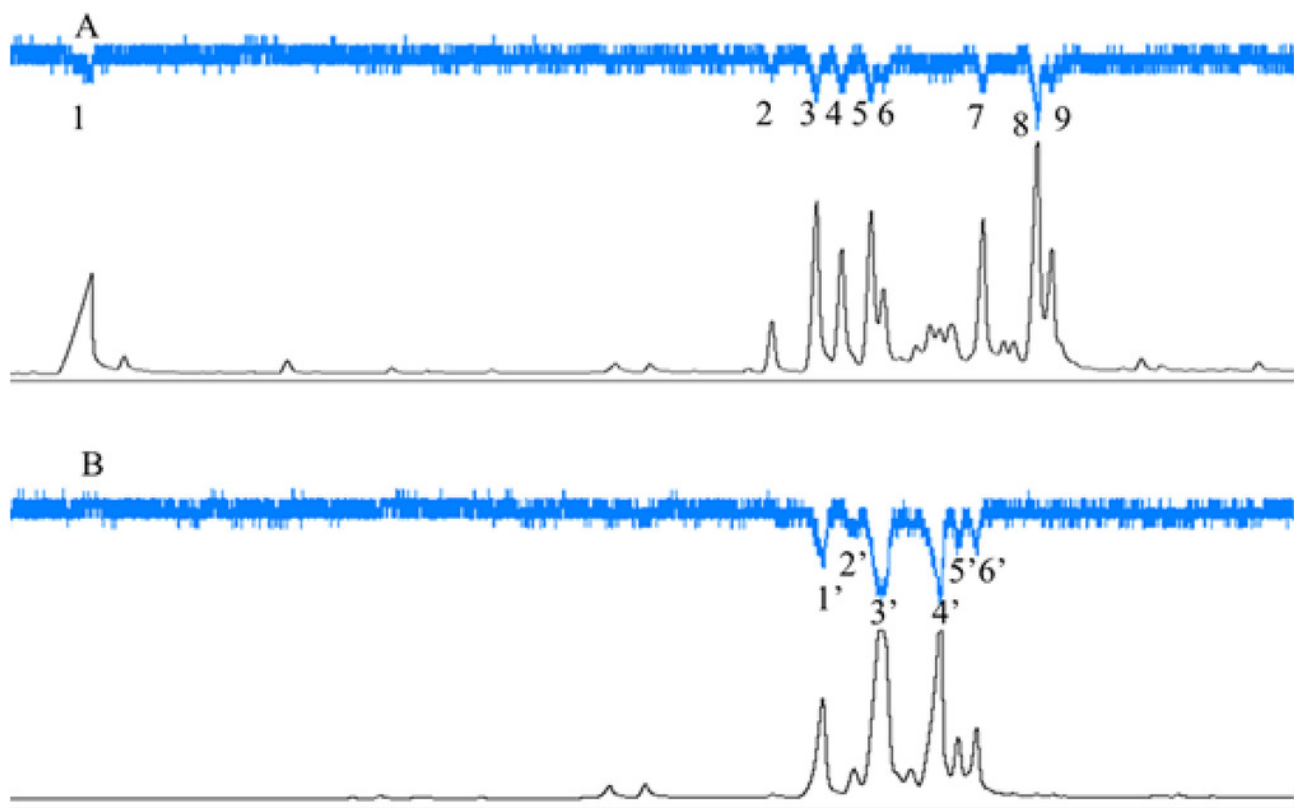
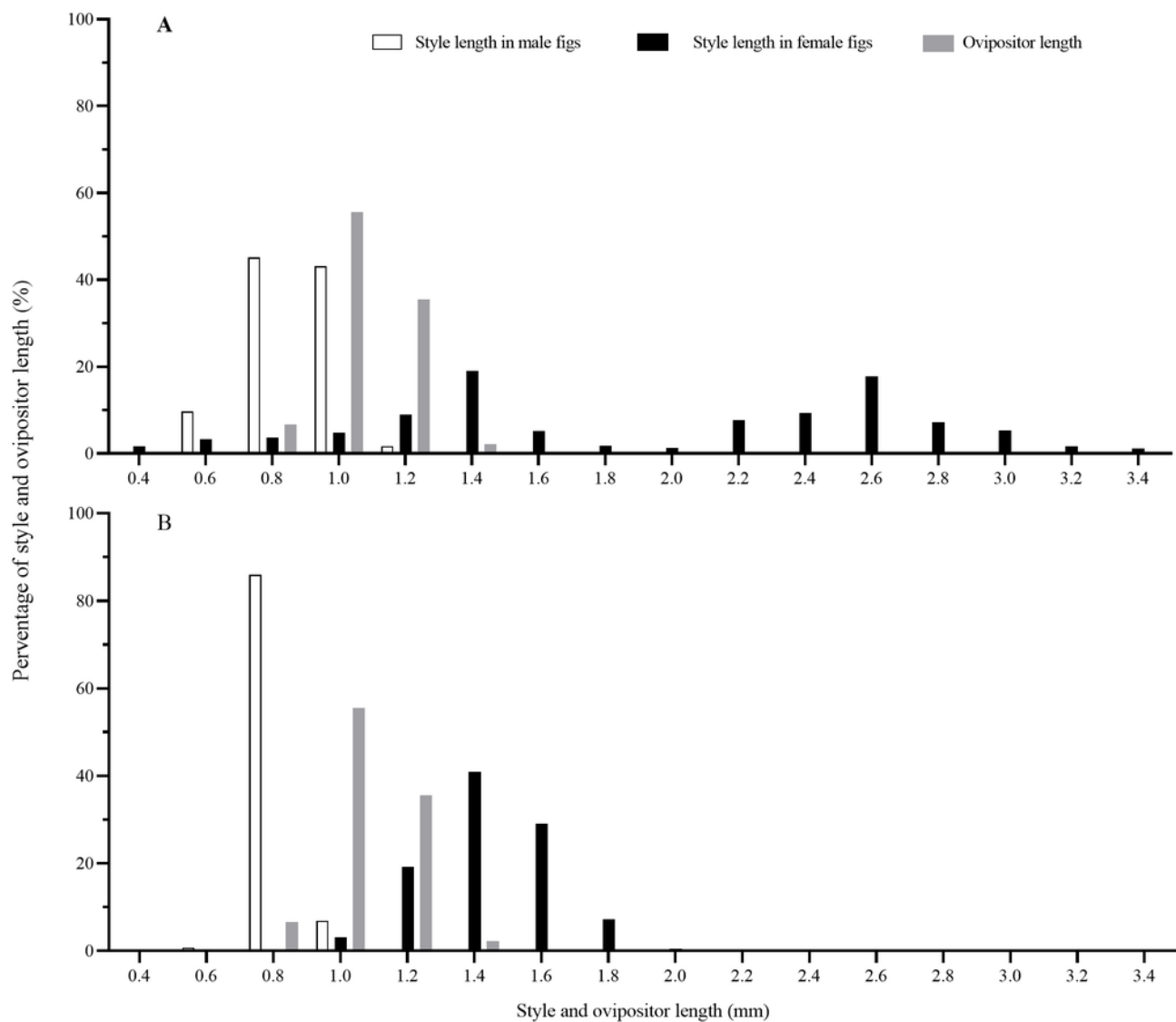



Figure 6

The distribution of fig style length and ovipositor length of Ceratosolen emarginatus.

(A): Style length of receptive figs from Ficus auriculata; (B): Style length of receptive figs from F. hainanensis.

Number of foundresses entering receptive figs of two host *Ficus* species and offspring production at the male-floral phase.

1 Table 1

Ficus species	Number of foundresses entering receptive figs					Offspring produced by figs at the male-floral phase		
	Sample	Occurrence rate	Range	Mean	Total	Sample	Occurrence rate	Total
F. auriculata								
Male figs	86	100%	1 - 38	11.4	1036	80	100%	793
Female figs	88	100%	1 - 25	11.6	1215	-	-	
F. hainanensis								
Male figs	60	41.7%	1 - 14	1.5	24	80	15%	30
Female figs	60	18.3%	1	0.2	11	-	-	

Comparisons on the style length among figs and trees for *F. auriculata* and *F. hainanensis*.

1   Table 2

Ficus species	Figs	Trees	Among figs		Among trees	
			χ^2	p	χ^2	p
F. auriculata						
Male figs	32	4	635.99	<0.001	584.53	<0.001
Female figs	30	3	597.48	<0.001	531.46	<0.001
F. hainanensis						
Male figs	30	3	68.81	<0.001	16.09	<0.001
Female figs	31	3	597.18	<0.001	549.64	<0.001

Consequences of offspring and seeds produced by single foundress of *Ceratosolen emarginatus* in *Ficus auriculata* and *F. hainanensis*.

Abortion rate: the aborted figs to all sampling figs.

Ce: Ceratosolen emarginatus, Fa: Ficus auriculata, Fh: F. hainanensis

1 Table 3.

Treatments	Sample	Abortion rates (%)	No. of offspring			No. of seeds		
			Mean	SD	CV	Mean	SD	CV
Ce–Fa, male	60	7.69	472.85	144.67	0.306			
Ce–Fh, male	60	41.67	618.49	211.55	0.342			
Ce–Fa, female	60	11.76				974.21	642.48	0.659
Ce–Fh, female	60	100						

Body size and ovipositor length of *Ceratosolen emarginatus* emerging from figs of the typical host *F. auriculata*, and of *Ceratosolen* sp. and *C.emarginatus* emerging from figs of alternative host *F. hainanensis*.

Ce: *Ceratosolen emarginatus*, Csp: Ceratosolen sp., Fa: *Ficus auriculata*, Fh: *F. hainanensis*

1 Table 4.

Species	Sample	Head width (mm) (mean ± SD)	Thorax width (mm) (mean ± SD)	Ovipositor length (mm) (mean ± SD)
Ce	77	0.48±0.05	0.59±0.05	1.15±0.11
Csp	69	0.46±0.04	0.55±0.04	1.07±0.11
Ce from Fh figs	114	0.46±0.04	0.55±0.07	1.03±0.06