

1 **Tritrophic interactions involving a dioecious fig tree, its**
2 **fig pollinating wasp and fig nematodes**

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23 **Abstract**

24

25 Many species of fig trees (*Ficus* spp., Moraceae) have nematodes that develop
26 inside their inflorescences (figs). Nematodes are carried into young figs by females of the
27 trees' host-specific pollinating fig wasps (Agaonidae) that enter the figs to lay their eggs.
28 The majority of Asian fig trees are functionally dioecious. Pollinators that enter figs on
29 female trees cannot reproduce and offspring of any nematodes they carry will also be
30 trapped inside. The biology of the nematodes is diverse, but poorly understood. We
31 contrasted the development of nematodes carried by the pollinating fig wasp *Ceratosolen*
32 *solmsi marchali* into figs on male and female trees of *Ficus hispida* in Sumatra, Indonesia.
33 Figs were sampled from both male and female trees over a six-month period, with the
34 nematodes extracted to record their development of their populations inside the figs.
35 Populations of three species of nematodes developed routinely inside figs of both sexes:
36 *Caenorhabditis* sp. (Rhabditidae), *Ficophagus* cf. *centerae* and *Martininema baculum*
37 (both Aphelenchoididae). This is the first record of a *Caenorhabditis* sp. associated with
38 *F. hispida*. Mean numbers of nematodes reached around 120-140 in both male and
39 female figs. These peak population sizes coincided with the emergence of the new
40 generation of adult fig wasps in male fig trees. We conclude that figs on female trees can

41 support development and reproduction of some nematode species, but the absence of
42 vectors means that their populations cannot persist beyond the lifetime of a single fig.
43 Just like their fig wasp vectors, the nematodes cannot avoid this routine source of
44 mortality.

45

46 **Keywords** - Agaonidae, *Caenorhabditis*, *Ficus r*, phoresy, vector

47 **Introduction**

48

49 Fig trees (*Ficus* spp., Moraceae) often produce large crops of figs all year around.

50 This has resulted in many species of vertebrates feeding on ripe figs, more than recorded

51 for any other plants [1]. Figs are also fed upon by a wide range of invertebrate species,

52 including wasps, flies, beetles and moths, which in turn support a diverse parasitoid fauna

53 [2]. Mites and nematodes are also found in some figs [3-5] as well as microorganisms such

54 as fungi [6] and protistans [11].

55 Female pollinator fig wasps (Agaonidae) enter figs in order to lay their eggs inside the

56 ovules that line their inner surface [7]. Their larvae develop inside the ovules, which

57 are galled by the females at the time that eggs are laid [8]. This is in contrast to most

58 other fig wasps (non-pollinating fig wasps -NPFW) that usually do not enter figs to

59 oviposit, but lay their eggs into the ovules while standing on the outer surface of the figs

60 The entry of fig wasp pollinators into figs allows them to be used as vectors for transport

61 between figs by a variety of smaller less intrinsically mobile organisms, including

62 microorganisms, mites and nematodes [9-11].

63 Fig trees display two contrasting breeding systems. Monoecious fig trees have trees

64 where individual figs that contain both female and male flowers and the female flowers

65 support the development of both seeds and pollinator fig wasp offspring. Dioecious fig
66 tree species have individuals that either produce ‘male’ figs that support development of
67 fig wasp offspring and pollen or have ‘female’ figs that reproduce via seeds [12, 13].
68 Adult female pollinator fig wasps that enter receptive female figs cannot reproduce and
69 often do not re-emerge from the first fig they enter. Even if they do re-emerge, they have
70 lost their wings and cannot fly away in search of figs on other trees. Reproduction in
71 dioecious fig trees is maintained because of mutual mimicry between the sexes, which
72 results in pollinator females failing to distinguish male from female figs prior to entry
73 [14] and because once they are inside the figs the pollinators continue to behave as if they
74 were in male figs [15]. The inability of pollinators to distinguish between male and
75 female figs means that any animals that are transported between figs of a dioecious fig
76 tree are routinely at risk of being taken inside a female fig, from which there will be no
77 subsequent generation of fig wasps to act as vectors and at certain seasons most of the
78 figs available for entry may be female. Perhaps reflecting this significant potential source
79 of mortality, published records suggest that phoretic mites are only associated with
80 monoecious fig tree hosts [5]. In contrast, nematodes are associated routinely with both
81 monoecious and dioecious fig trees species [11].

82 Fig trees and their pollinators have a long history of mutualistic association,

83 extending for tens of millions of years [16] and Dominican amber fossils show that
84 pollinator fig wasps have been also transporting nematodes between figs for much of this
85 period [17]. Today, nematodes are recorded throughout the distributional and
86 taxonomic range of fig trees [18-21]. The nematodes in figs belong to several different
87 families, suggesting multiple independent colonisations of figs, followed in some cases
88 by extensive radiations [22]. One fig tree species may support several different species
89 of nematodes and up to eight species of nematodes have been recorded from a single fig
90 of *F. racemosa* L. in Indonesia [11]. Nematodes develop and reproduce inside the figs
91 and offspring are ready to attach themselves again to female pollinators when the new
92 generation of adult pollinators are ready to leave the figs [3, 10]. Female pollinators are
93 chosen preferentially by nematodes, because males and most female NPFW do not enter
94 the figs to oviposit so cannot act as vectors in the same way as female pollinators [23,
95 24]. Transfer of nematodes into figs via the ovipositors of NPFW has not been
96 confirmed [5, 10, 25]. *Schistonchus caprifici* Gasperrini, a nematode that reproduces in
97 male figs of *Ficus carica* L., has nonetheless been recorded as also entering NPFW
98 females, although there is no evidence that they ever manage to enter the figs and
99 reproduce [25]. Nematodes waiting to attach to female pollinators may be scattered
100 around the interior of the figs, or be aggregated in male flowers [19]. The latter is a

101 response to the active pollen collection behaviour of some female pollinators [26]. These
102 females seek out the male flowers and move pollen from them into their pollen pockets,
103 and collect the nematodes while doing so.

104 The feeding behaviour of most fig nematodes is unknown, but is clearly diverse
105 [27, 28]. The presence of stylets is indicative of plant-feeding, but different species vary
106 in their preferred feeding sites within the figs [28]. Nematodes use their stylets to
107 puncture plant cells, to withdraw food and also to secrete proteins and metabolites that
108 aid the nematode in feeding on the plants [29]. Among species that lack stylets, some
109 feed on the decaying corpses of pollinator females, and some may also start feeding on
110 the females before they have died [30]. Fig trees belonging to subgenus *Sycomorus*
111 often have their figs partly filled by a liquid at times during their development [31] and
112 these species appear to support particularly rich nematode faunas. Free-swimming
113 nematodes in these figs may be predatory on other nematodes or feed on the protists
114 that are often present at high densities in the fig liquid [11].

115 Males and females of dioecious plants can differ in their attractiveness and
116 suitability for plant-feeding invertebrates [32]. Figs of both tree sexes fill with liquid
117 in *Sycomorus* figs, but only male figs contain male flowers, and although the figs of both
118 male and female plants contain female flowers, their contrasting floral development (with

119 galled ovules or seeds, respectively) mean they offer differing resources to any nematodes
120 that have been carried inside. In this paper, we address the following questions in
121 relation to the nematodes associated with a dioecious fig tree, *F. hispida*, in Indonesia: (i)
122 How many nematode species are present locally, and how abundant are they? (ii) What
123 are their life cycles? And (iii) Are they capable of developing and reproducing inside both
124 male and female fig trees?

125

126 **Materials and methods**

127

128 **Study species and site**

129 *Ficus hispida* is a fig trees belonging to subgenus Sycomorus and is functionally
130 dioecious with distinct male and female individuals. The species is distributed throughout
131 India, Nepal, Laos, Thailand, Malaysia, southern China [33], Sri Lanka, Myanmar, New
132 Guinea, Australia, Andaman island [34], and also Indonesia [28]. *F. hispida* is a shrub
133 or moderate-sized free standing tree up to 13 metres tall, with spreading branches. Like
134 many other dioecious fig trees, *F. hispida* sometimes shows asynchronous fruiting within
135 a plant, with different phases of figs found at the same time on the same tree. Pollinating
136 wasp that enter figs from female trees will pollinate the flowers, but are unable to lay eggs
137 because of stigma structure and because the figs contain only long-styled female flowers

138 that prevent the wasps' ovipositors from reaching the ovules. Therefore, mature female
139 *F. hispida* figs contain seeds, but no pollinator offspring. The pollinating wasps that enter
140 figs of male trees can lay eggs in the female flowers, where the fig wasp offspring develop,
141 but no seeds are produced. Male flowers inside male figs mature at the same time as the
142 pollinator offspring, allowing them to transport pollen to other trees. The newly emerged
143 female pollinators disperse to find new receptive figs and start another developmental
144 cycle if they enter figs on a male tree [7, 35, 36].

145 Figs of *F. hispida* are borne in long clusters at the base of the tree, as well as on the
146 branches [7]. Fig development has been characterised by Galil and Eisikowich [38] with
147 modifications by Valdeyron and Lloys [39] for dioecious figs. Phase A figs are pre-
148 receptive. Pollinators (and nematodes) enter during B phase. Fig wasp offspring (male
149 trees) and seeds (female trees) develop and mature during C phase, along with any
150 nematodes reproducing inside the figs. In D phase the next generation of male
151 pollinators emerge to mate with females that are still in their galls. The females then leave
152 their galls, actively collect pollen into pollen baskets and emerge through an exit hole in
153 the fig wall cut by the males (the start of E phase). Figs from female trees do not have a
154 D phase, as no pollinator wasps develop there. Once the seeds in female figs are mature,
155 the figs ripen and become attractive to seed dispersers (E-Phase). Like many other fig

156 trees belonging to subgenus *Sycomorus*, C phase figs of *F. hispida* often contain
157 noticeable amounts of liquid [40]. *C. solmsi marchali* is the only pollinator recorded
158 from *F. hispida* in Indonesia [28], but *F. hispida* is host to different *Ceratosolen*
159 pollinators elsewhere within its wide geographical range. Its larval development was
160 described by [41].

161 The phenology and contents of *F. hispida* figs were monitored in the northern
162 part of Sumatra Island in Aceh Province, Indonesia for six months from March to August
163 2018. The trees were growing along roads in mountainous areas of Leupung District,
164 about 25 km (95° 15' 34.92" E; 05° 22' 55.68" N) from the provincial capital, Banda
165 Aceh. Roadside *F. hispida* are common in the study area, usually growing in clumps
166 of several individuals with both female and male trees present together. The region has
167 a tropical climate that supports rainforest vegetation, with fairly constant average
168 temperatures throughout the year and little diurnal variation. Meteorological
169 information for the area was obtained from Blang Bintang Station, the closest
170 Meteorological Station under the Indonesian Meteorological and Geophysical Agency,
171 located about 30 km from the study area. There was little seasonal variation in daily
172 temperatures during the six months of study. The average daily temperature during
173 the study was 28.22 ± 0.09 °C (mean \pm SE), with average minimum temperature was

174 24.18 \pm 0.09 $^{\circ}$ C (mean \pm SE) and maximum was 32.26 \pm 0.15 (mean \pm SE). Monthly
175 minimum and maximum temperatures are quite stable, with only slight variation between
176 the six months of study. During the six months of the study, March was the driest month
177 with no rain at all, while May was the wettest month with 15 days of rain that accumulated
178 361.7 mm of precipitation.

179 Figs were present on both sexes of *F. hispida* more or less continuously.
180 Asynchronous fruiting often resulted in a variety of developmental stages being present
181 at any one time. Reasonably discrete ‘cohorts’ were nonetheless present, allowing the
182 development of marked groups of figs to be followed. Sampling followed the complete
183 development of each cohort. In some cases, mature D and E phase figs were present on
184 the trees when new A- phase figs first appeared, providing opportunities for some cycling
185 of fig wasp populations on individual male trees. Fig cohorts were sampled at weekly
186 intervals from three male trees and three female trees (Table 1).

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193 **Table 1. Table 1. Location of the *Ficus hispida* trees sampled**

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| No | Tree code | Tree sex | Location coordinates | |
|----|-----------|----------|----------------------|-----------------|
| | | | (East) | (North) |
| 1 | H1 | male | 95° 14' 30.21'' | 05° 20' 39.33'' |
| 2 | H2 | male | 95°14' 37.02'' | 05° 20' 39.48'' |
| 3 | H3 | male | 95°14' 29.00'' | 05° 20' 55.30'' |
| 4 | H4 | female | 95°14' 27.20'' | 05° 20' 58.38'' |
| 5 | H5 | female | 95°14' 27.20'' | 05° 21' 10.10'' |
| 6 | H6 | female | 95°14' 57.20'' | 05° 21' 29.36'' |

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196

197 **Sampling procedures and fig extractions**

198 The development times for each cohort were calculated from when the first A phase
199 figs were recorded until the first E phase fig was present. Ten figs were sampled
200 haphazardly from each cohort of each tree from A phase through to E phase (when wasp
201 offspring had left the male figs, and female figs were soft and ready to be eaten by
202 frugivores). The developmental stage, colour, and diameter of each fig were recorded.
203 Later the same day, the contents of each fig, including any liquid if present, were placed

204 individually in a Baermann extraction funnel, using a method adapted from Sriwati,
205 Takemoto and Futai [42] as modified by Jauharlina [11]. Each fig was cut into six to
206 eight pieces and placed onto a layer of fine fabric and immersed in 60 ml of distilled
207 water. Water held within the funnel ensured that the fig pieces remained under water.
208 After 24 hours, the liquid below the funnel was placed in 20 ml reaction tubes and left
209 undisturbed for 3 hours. The upper part of the liquid was then removed without
210 disturbing the lower liquid using a small pipette, and then discarded. Occasional checks
211 confirmed that it did not contain nematodes. One ml extracts from the remaining 5 ml in
212 the bottom of the tubes were placed on a one ml capacity nemacytometer glass slide
213 (counting slide) and observed under a microscope. Any nematodes present were
214 counted and adults were identified. Observations were repeated on the rest of the extract,
215 giving five counts from each fig.

216

217

218 **Result**

219

220 **Fruiting phenology of *F. hispida***

221 Over the six-month sampling period, a total of 430 figs were collected. The
222 development of figs from A-phase to E-phase lasted for seven to eight weeks for male

223 trees and for seven weeks for female trees. The figs from male trees were 1.59 ± 0.03
224 cm in diameter (mean \pm SE, N = 30 figs) during A phase prior to pollinator entry, and
225 reached 3.39 ± 0.06 cm in diameter (mean \pm SE, N = 30 figs) at maturity. The figs from
226 female trees were roughly the same size as male figs at similar stages of development
227 (1.47 ± 0.07 cm, mean \pm SE, N = 30, at A phase, and 3.55 ± 0.02 cm, mean \pm SE, N = 30,
228 at E phase). Figs from A, B, and C-phases were green in colour and had a hard texture
229 with abundant white latex. When the figs developed into D-phase (male trees) and late
230 C-phase (female trees), they became a little softer and developed a yellowish colour.
231 The amount of latex also decreased.

232 B-phase was the shortest phase during the development of figs. It lasted only 2-3
233 days. At this stage the ostiole became a bit loose to allow the pollinating wasps to enter.
234 The number of pollinators that entered the B-phase figs ranged from 1 - 3 wasps per fig,
235 with an average of 1.30 ± 0.1 (Mean \pm SE, N = 30 figs) on male trees and 1.27 ± 0.1
236 (Mean \pm SE, N= 30 figs) on female trees. Average number of entries did not vary
237 between the sexes (glmer, $z = 0.11$, $P = 0.90$). One pollinating wasp per fig was the
238 most common occurrence in both male and female trees of *F. hispida*, representing 76.6 %
239 and 80 % of the total figs respectively (Table 2).

240

241

242

243 **Table 2. Frequency of pollinating fig wasps entering the B-phase figs on male and**
244 **female trees of *Ficus hispida* (N=30 figs from each tree sex)**

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| Tree sex | Frequency of pollinating wasps entering figs (%) | | |
|----------|--|------|-----|
| | 1 | 2 | 3 |
| Male | 76.6 | 16.7 | 6.7 |
| Female | 80.0 | 13.3 | 6.7 |

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248 **Fig nematodes and their life cycle**

249

250 A sub-sample of 185 figs (91 from male and 94 from female trees) had their
251 contents extracted in the laboratory to examine nematode development inside the figs.
252 Nematodes were recorded in all fig samples from male trees (100 % occupancy) and in
253 most of the figs from female trees (91.18 % Occupancy). Three species of nematodes,
254 a bacteria feeder, *Caenorhabditis* sp. (Rhabditidae), and two plant parasites, *Ficophagus*
255 cf. *centerae* and *Martininema baculum* (Aphelenchoididae), were recorded, and all were
256 found in figs from both male and female trees. *F.* cf. *centerae* and *M. baculum* were

257 previously identified based on molecular profiles (Sriwati et al., 2017). The other species,
258 *Caenorhabditis* sp. is typologically and phylogenetically close to another fresh fig
259 associate, *C. inopinata* (Kanzaki et al., 2018), but its detailed taxonomic description and
260 molecular phylogenetic status will be presented elsewhere. After being carried into the
261 B phase figs, the nematodes were present within the central lumen throughout fig
262 development (including the liquid if present). Old pollinating wasps that had transported
263 the nematodes appeared to be quickly abandoned shortly after entry, and there was no
264 indication that the corpses of the vectors continued to provide nutritional resources.
265 73.3% of 30 B-phase figs from male trees had pollinators with nematodes still in physical
266 contact, ranging 1-2 nematodes per wasp with an average of 1.09 ± 0.06 nematodes per
267 fig wasp (Mean \pm SE, N = 24 pollinators). On female trees, 56.7 % of 30 B-phase figs
268 had pollinators with nematodes, ranging 1-2 nematodes per wasp with an average of 1.18
269 ± 0.09 (Mean \pm SE, N = 17 pollinators). All nematodes transported by the wasps into
270 the B-phase figs were at pre-adult or juvenile stages.

271 The number of nematodes found inside B phase figs ranged from 3-26 per fig on
272 male trees with an average of 10.20 ± 1.68 (Mean \pm SE, N = 15 figs) and 0-20 on female
273 trees with an average of 8.44 ± 1.57 (Mean \pm SE, N = 18 figs). There was no significant
274 difference between fig sexes in mean numbers of nematodes inside B phase figs (glmer,

275 $z = 0.459$, $P = 0.759$) (Fig 1). At D-phase, when the new generation of fig wasps were
276 ready to leave male figs, immature nematodes attached themselves to the female wasps
277 and were carried out of the figs. The life cycle of fig nematodes transferred by
278 pollinating wasps into figs of *F. hispida* trees is summarized in Fig 2.

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280

281 **Fig 1. The number of nematodes found inside B-phase figs on male and female**
282 **trees of *Ficus hispida*, shortly after the death of fig wasp vectors** (Mean \pm SE, N = 15
283 figs for male trees, N = 18 figs for female trees).

284

285 **Fig 2. Life cycles of nematodes associated with pollinating wasps on male and**
286 **female dioecious fig trees of *Ficus hispida* based on routine observation on figs**
287 **morphology and extract.**

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289

290 All three nematode species produced offspring inside both figs from male and
291 female trees. In male trees, peak populations of nematodes were in D-phase figs, when
292 the new generation of wasps was ready to leave (Fig 3). In female trees, where wasps
293 could not reproduce, all the nematodes that developed inside the figs had no means of

294 dispersal. Peak nematode populations in female figs were at late C-phase (equivalent in
295 timing to D- phase in male figs) and the time when they would have been seeking out fig
296 wasp vectors, if any had been present (Fig 4). The peak population sizes in figs of both
297 male and female trees were not significantly different from each other (glmer, $z = -0.837$,
298 $P = 0.402$) showing that nematodes could reproduce successfully in figs of female trees
299 of *F. hispida*.

300

301 **Fig 3. Nematode populations per fig (all species and stages) during fig development**

302 **on male trees of *F. hispida*.** Counts were obtained from extractions of whole figs
303 (Mean \pm SE, N = 5-6 figs for each phase, error bars represent standard errors of the means).

304 Fig phases follow the terminology of Galil & Eisikowich [38].

305

306 **Fig 4. Nematode populations per fig (all species and stages) during fig development**

307 **on female trees of *F. hispida*.** Counts were obtained from extractions of whole figs
308 (Mean \pm SE, N = 5-8 figs for each phase, error bars represent standard errors of the means).

309 Figs from female trees do not have a D-phase as there is no fig wasps develop inside the
310 figs. Phases follow the terminology of Galil & Eisikowich [38].

311

312

313 Male fig wasps emerge before their females and seek out galls that contain the
314 females for mating. Nematodes were seen on these males. Examination of the new
315 generation of adult female fig wasps in early D phase figs, before holes had been chewed
316 into the walls of their galls by the males, found no nematodes were present. This changed
317 once the male pollinators chewed holes into the galls in order to mate, and the nematodes
318 could gain entry. Similarly, there were no nematodes recorded from inside the anthers
319 of male flowers. Judging from the presence of adult nematodes and immatures
320 throughout all but the earliest phases of fig development, in both male and female figs, it
321 is likely that both male and female figs supported more than one generation of each of the
322 three nematode species during the time taken for one generation of fig wasps to develop.

323

324

325 **Discussion**

326 Mature individuals of *F. hispida* in equatorial North Sumatra fruited almost
327 continuously, with one fig cohort merging into another all year round. On male trees this
328 sometimes resulted in sufficiently unsynchronized fruiting for fig wasp populations to
329 cycle on one male tree, as seen in some other dioecious fig tree species [43, 44].
330 Asynchronous fruiting should reduce pollinator mortalities associated with flight between
331 trees [41], and thereby increase the trees' ability to maintain local populations of their

332 pollinators and the nematodes they carry [44]. It may also increase the likelihood of
333 pollinator females entering male figs, and increase the numbers of females entering
334 individual male figs, both of which will be advantageous for any nematodes they are
335 carrying.

336 Two of the nematode species found in this study were the same species as those
337 described earlier from the same area [45]) Previously the two nematodes species from
338 family Aphelenchoididae were morphologically identified as *Schistonchus centerae* and
339 *S. guangzhouensis* [11], however further molecular identification showed that these two
340 species were *Ficophagus* cf. *centerae* and *Martininema baculum* [45].
341 Aphelenchoididae species are known to be phytophagous and feed on flowers inside the
342 figs. They have a stylet, a stomatal structure used to feed on plant tissues [20, 45].
343 During C phase, many nematodes were also seen swimming in those figs where liquid
344 was present. Adults and juveniles of the two aphelenchoidid species from *F. hispida*
345 sought out only adult female pollinators, which became available to the nematodes after
346 pollinator males had chewed holes into the females' galls for mating. A New World
347 *Schistonchus* sp. associated with *F. laevigata* contacts adult pollinators in the same way
348 [27].

349 Rhabditid nematodes have been recorded previously from figs of *F. septica* in

350 Taiwan [46]. Recently, the species *Caenorhabditis inopinata* has been observed inside
351 the figs of *F. septica* in Japan [47, 48] and the presence of *Caenorhabditis* nematodes in
352 *F. hispida* figs is a new record. Its ecology in the figs of *F. hispida* is still unclear, but
353 it was transferred between figs exclusively as juvenile dauer larvae. Other
354 *Caenorhabditis* species are colonizers of nutrient and microorganism-rich organic
355 material [9, 50]. The *Caenorhabditis* species from *F. hispida* appears not to be
356 facultatively necromenic (feeding on the vector's cadaver after the fig wasp dies) and
357 given that there is more than one generation within the figs, other food sources are clearly
358 used. Protistans are routinely present within the lumen of the figs and they are one
359 potential food source, as are the larvae of the aphelenchoidid species.

360 The life cycles of the nematodes found in *F. hispida* were similar to those reported
361 earlier in other fig tree species [5, 11, 51-53], but this study has revealed that nematodes
362 can develop and reproduce inside figs from female trees of *F. hispida* despite the absent
363 of fig wasp offspring. Studies of nematodes in dioecious fig trees have focused on male
364 trees, from which a new generation of fig wasps can develop [20, 35]. Nematodes
365 developed and reproduced within figs on female trees in a similar way, but they perished
366 once the figs were mature and either eaten by vertebrates or fell to the ground.
367 Resources absent from figs on female trees (galled ovules and male flowers) were clearly

368 not required by the nematodes, but both pollinating fig wasps and the nematodes they
369 carry are frequent victims of their host plant's reproductive system.

370 Despite the high occupancy rates and large numbers of nematodes within the figs
371 of *F. hispida* they had no obvious effect on the development of pollinating wasps inside
372 the galls on male trees and the development of seeds on female trees. In male figs, the
373 reproductive success of the pollinators is important for the survival of future generations
374 of nematodes because reduced pollinator reproductive success translates into fewer
375 vectors for the later generations of nematodes [30]. Even among the nematode species
376 that feed on dead or dying pollinators after they enter new figs there is little evidence that
377 they cause significant harm to the living fig wasps [54]. This apparent absence of a
378 negative impact on the pollinators is advantageous to the nematodes, and they contribute
379 to the exceptional biodiversity centered on figs without impinging on the core mutualism
380 on which that diversity depends.

381

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383

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388

389 **Reference**

390

391 1. Shanahan M, So S, Compton SG, Corlett R. Fig-eating by vertebrate frugivores: a
392 global review. *Biological Reviews*. 2001;76(4):529-72.

393 2. Chen Y, Compton SG, Liu M, Chen XY. Fig trees at the northern limit of their range:
394 the distributions of cryptic pollinators indicate multiple glacial refugia. *Molecular*
395 *Ecology*. 2012;21(7):1687-701.

396 3. Compton SG. One way to be a fig. *African Entomology*. 1993;1(2):151-8.

397 4. Pereira RAS, Semir J, Menezes Junior AdO. Pollination and other biotic interactions
398 in figs of *Ficus eximia* Schott (Moraceae). *Revista Brasileira de Botanica*.
399 2000;23(2):217-24.

400 5. Jauharlina J, Lindquist EE, Quinnell RJ, Robertson HG, Compton SG. Fig wasps as
401 vectors of mites and nematodes. *African Entomology*. 2012;20(1):101-10.

402 6. Lachaise D. Niche separation of African *Lissocephala* within *Ficus* drosophilid
403 community. *Oecologia*. 1977;31(2):201-14.

404 7. Galil J. Fig biology. *Endeavour*. 1977;1(2):52-6.

- 405 8. Yu H, Compton SG. Moving your sons to safety: galls containing male fig wasps
406 expand into the centre of figs, away from enemies. *Plos One*. 2012;7(1).
- 407 9. Michailides TJ, Morgan DP, Subbarao KV. Fig endospesis: an old disease still a
408 dilemma for California growers. *Plant Disease*. 1996;80:828-40.
- 409 10. Krishnan A, Muralidharan S, Sharma L, Borges RM. A hitchhiker's guide to a
410 crowded syconium: how do fig nematodes find the right ride? *Functional Ecology*.
411 2010;24(4):741-9.
- 412 11. Jauharlina J. *Fig Trees and Fig Wasps: Their Interactions with Non-mutualists*.
413 Leeds: University of Leeds; 2014.
- 414 12. Janzen DH. How many parents do the wasps from a fig have. *Biotropica*.
415 1979;11(2):127-9.
- 416 13. Verkerke W. Structure and function of the fig. *Experientia*. 1989;45(7):612-22.
- 417 14. Grafen A, Godfray HCJ. Vicarious selection explains some paradoxes in dioecious
418 fig pollinator systems. *Proceedings of The Royal Society B Biological Sciences*.
419 1991;245(1312):73-6.
- 420 15. Raja S, Suleman N, Compton SG. Why do fig wasps pollinate female figs? *Symbiosis*.
421 2008;45(1-3):25-8.
- 422 16. Compton SG, Ball AD, Collinson ME, Hayes P, Rasnitsyn AP, Ross AJ. Ancient fig

- 423 wasps indicate at least 34 Myr of stasis in their mutualism with fig trees. *Biology*
424 *Letters*. 2010;6(6):838-42.
- 425 17. Poinar GJ, Poinar R. *The Amber Forest: A Reconstruction of a Vanished World*. :
426 Princeton University Press.; 1999. 292 pp. p.
- 427 18. Poinar GOJ, Herre EA. Speciation and adaptive radiation in the fig wasp nematode
428 *Parasitodiplogaster* Diplogasteridae Rhabditida in Panama. *Revue de Nematologie*.
429 1991;14(3):361-74.
- 430 19. Vovlas N, Inserra RN, Greco N. *Schistonchus caprifici* parasitizing caprifig (*Ficus*
431 *carica sylvestris*) florets and the relationship with its fig wasp (*Blastophaga psenes*)
432 vector. *Nematologica*. 1992;38(2):215-26.
- 433 20. Kanzaki N, Giblin-Davis RM, Davies KA, Center BJ. *Teratodiplogaster martini* n.
434 sp. and *Parasitodiplogaster doliostoma* n. sp. (Nematoda: Diplogastridae) from the
435 syconia of *Ficus* species from Africa. *Nematology*. 2012;14:529-46.
- 436 21. Wöhr M, Greeff JM, Kanzaki N, Ye W, Giblin-Davis RM. Molecular and
437 morphological observations on *Parasitodiplogaster sycophilon* Poinar, 1979
438 (Nematoda: Diplogastrina) associated with *Ficus burkei* in Africa *Nematology*
439 2014;16:453-62.
- 440 22. Davies KA, Ye W, Kanzaki N, Bartholomaeus F, Zeng Y, Giblin-Davis RM. A

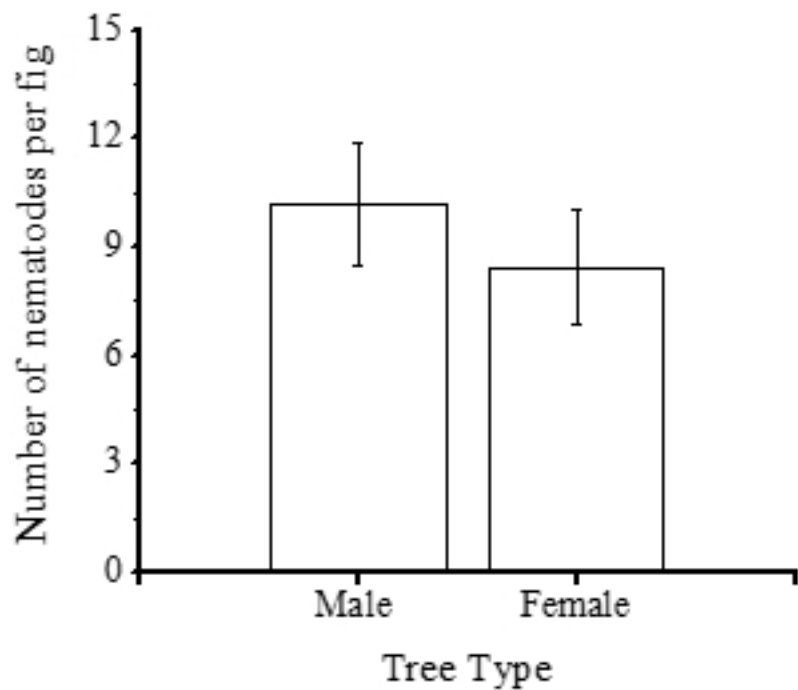
- 441 review of the taxonomy, phylogeny, distribution and co-evolution of *Schistonchus*
442 Cobb, 1927 with proposal of *Ficophagus* n. gen. and *Martininema* n. gen.
443 (Nematoda: Aphelenchoididae) *Nematology*. 2015;17:1-69.
- 444 23. Kerdelhue C, Rossi JP, Rasplus JY. Comparative community ecology studies on old
445 world figs and fig wasps. *Ecology*. 2000;81(10):2832-49.
- 446 24. Proffit M, Schatz B, Borges RM, Hossaert-Mckey M. Chemical mediation and niche
447 partitioning in non-pollinating fig-wasp communities. *Journal of Animal Ecology*.
448 2007;76(2):296-303.
- 449 25. Vovlas N, Larizza A. Relationship of *Schistonchus caprifici* (Aphelenchoididae)
450 with fig inflorescences, the fig pollinator *Blastophaga psenes*, and its cleptoparasite
451 *Philotrypesis caricae*. *Fundamental and Applied Nematology*. 1996;19(5):443-8.
- 452 26. Kjellberg F, Jouselin E, Bronstein JL, Patel A, Yokoyama J, Rasplus JY. Pollination
453 mode in fig wasps: the predictive power of correlated traits. *Proceedings of the Royal*
454 *Society of London Series B-Biological Sciences*. 2001;268(1472):1113-21.
- 455 27. Center BJ, Giblin-Davis RM, Herre EA, Chung-Schickler GC. Histological
456 comparisons of parasitism by *Schistonchus* spp. (Nemata : Aphelenchoididae) in
457 neotropical *Ficus* spp. *Journal of Nematology*. 1999;31(4):393-406.
- 458 28. Davies KA, Bartholomaeus F, Kanzaki N, Ye W, Giblin-Davis RM. Three new

- 459 species of *Schistonchus* (Aphelenchoididae) from the *Ficus* subgenus *Sycomorus*
460 (Moraceae) in northern Australia. *Nematology*. 2013;15:347-62.
- 461 29. Lambert K, Bekal S. Introduction to Plant-Parasitic Nematodes. The Plant Health
462 Instructor DOI: 101094/PHI-I-2002-1218-01.2002.
- 463 30. Herre EA. Factors affecting the evolution of virulence: Nematode parasites of fig
464 wasps as a case study. *Parasitology*. 1995;111:S179-S91.
- 465 31. Compton SG, McLaren FAC. Respiratory adaptations in some male fig wasps.
466 Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen Series
467 C-Biological and Medical Sciences. 1989;92(1):57-71.
- 468 32. Tsuji K, Sota S. Florivores on the dioecious shrub *Eurya japonica* and the preferences
469 and performances of two polyphagous geometrid moths on male and female plants.
470 *Entomological Science*. 2013;16:291–7.
- 471 33. Yang DR, Peng YQ, Song QS, Zhang GM, Wang RW, Zhao TZ, et al. Pollination
472 biology of *Ficus hispida* in the tropical rainforests of Xishuangbanna, China. *Acta*
473 *Botanica Sinica*. 2002;44(5):519-26.
- 474 34. Ali M, Chaudhary N. *Ficus hispida* Linn.: A review of its pharmacognostic and
475 ethnomedicinal properties. *Pharmacognosy reviews*. 2011;5(9):96-102.
- 476 35. Weiblen GD, Yu DW, West SA. Pollination and parasitism in functionally dioecious

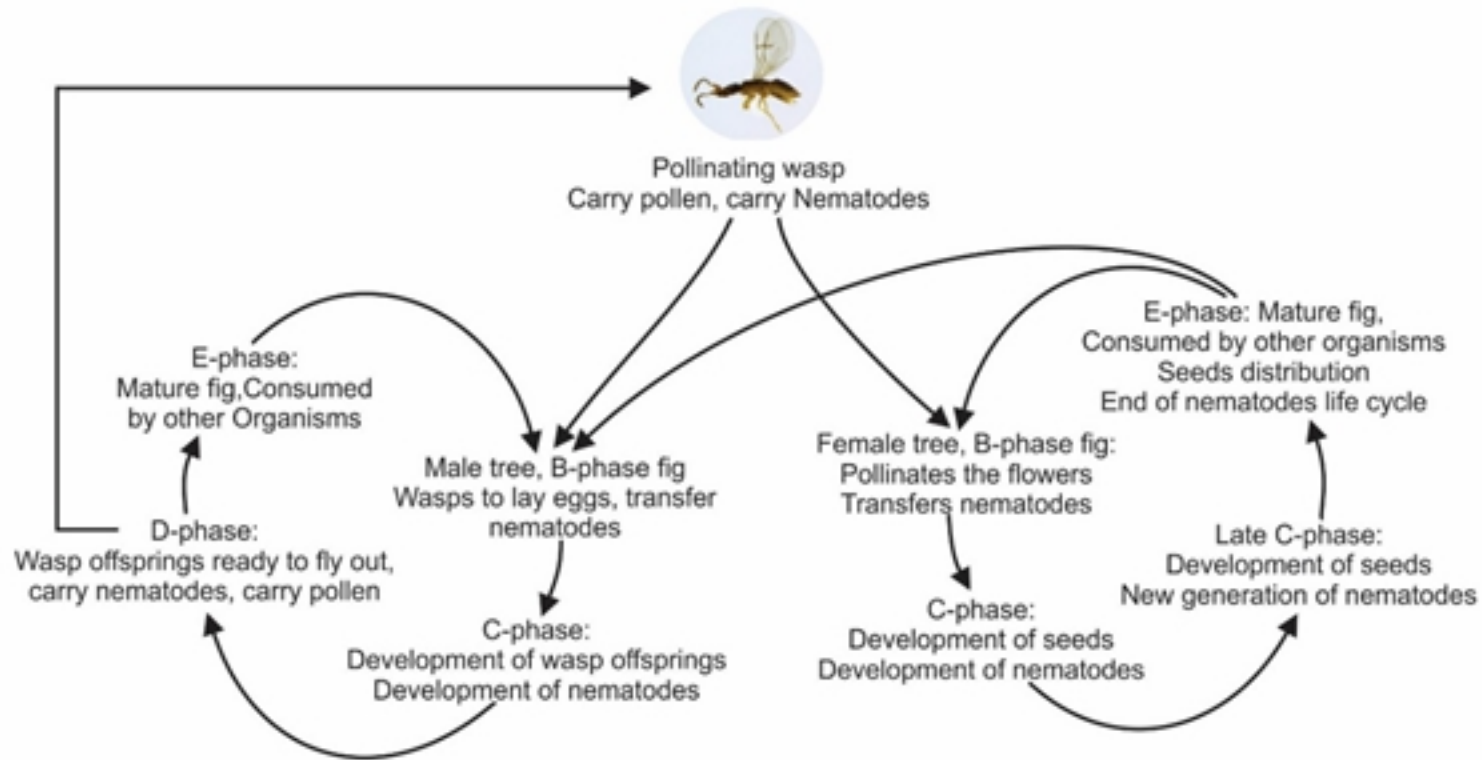
- 477 figs. Proceedings of the Royal Society of London Series B-Biological Sciences.
478 2001;268(1467):651-9.
- 479 36. Harrison RD, Yamamura N. A few more hypotheses for the evolution of dioecy in
480 figs (*Ficus*, Moraceae). *Oikos*. 2003;100(3):628-35.
- 481 37. Lee SH, Ng ABC, Ong KH, O'Dempsey O, Tan HTW. The satatus and distribution
482 of *Ficus hispida* L. f. (Moraceae) in Singapore. *Nature in Singapore*. 2013;6:85-90.
- 483 38. Galil J, Eisikowich D. Flowering cycles and fruit types in *Ficus sycomorus* in Israel.
484 *New Phytologist*. 1968;67:745-58.
- 485 39. Valdeyron G, Lloyd DG. Sex-differences and flowering phenology in the common
486 fig, *Ficus carica* L. *Evolution*. 1979;33(2):673-85.
- 487 40. Berg CC, Corner EJH. Moraceae-*Ficus*. Flora Malesiana Series I (Seed Plants).
488 Volume 17, Part 2 National Herbarium of the Netherlands, Leiden. 2005.
- 489 41. Jia XC, Yao JY, Chen YZ, Cook JM, Crozier RH. The phenology and potential for
490 self-pollination of two Australian monoecious fig species. *Symbiosis*. 2008;45(1-
491 3):91-6.
- 492 42. Sriwati R, Takemoto S, Futai K. Seasonal changes in the nematode fauna in pine
493 trees killed by the pinewood nematode, *Bursaphelenchus xylophilus*. *Japanese*
494 *Journal of Nematology*. 2006;36(2):87-100.

- 495 43. Patel A. Variation in a mutualism: Phenology and the maintenance of gynodioecy in
496 two Indian fig species. *Journal of Ecology*. 1996;84(5):667-80.
- 497 44. Kuaraksa C, Elliott S, Hossaert-Mckey M. The phenology of dioecious *Ficus* spp.
498 tree species and its importance for forest restoration projects. *Forest Ecology and*
499 *Management*. 2012;265:82-93.
- 500 45. Sriwati R, Takeuchi-Kaneko Y, Jauharlina J, Kanzaki N. Aphelenchoidid nematodes
501 associated with two dominant *Ficus* species in Aceh, Indonesia. *Nematology*.
502 2017;19:323-31.
- 503 46. Kanzaki N, Tanaka R, Giblin-Davis RM, Ragsdale EJ, Nguyen CN, Li HF, et al. A
504 preliminary survey of fig-associated nematodes in the Asian subtropics. *Journal of*
505 *Nematology*. 2012;44(4):470-.
- 506 47. Kanzaki N, Tsai IJ, Tanaka R, Hunt VL, Liu D, Tsuyama K, et al. Biology and
507 genome of a newly discovered sibling species of *Caenorhabditis elegans*. *Nature*
508 *Communications*. 2018;9(1):3216.
- 509 48. Woodruff GC, Phillips PC. Field studies reveal a close relative of *C. elegans* thrives
510 in the fresh figs of *Ficus septica* and disperses on its *Ceratosolen* pollinating wasps.
511 *BMC ecology*. 2018;18(1):26-.
- 512 49. Okumura E, Ishikawa Y, Tanaka R, Yoshiga T. Propagation of *Caenorhabditis*

- 513 *japonica* in the Nest of Its Carrier Insect, *Parastrachia japonensis*. Zoological
514 Science. 2013;30(3):174-7.
- 515 50. Kiontke K, Sudhaus W. Ecology of Caenorhabditis species. In: Wormbook, editor.
516 The C elegans Research Community: Wormbook,
517 doi10.1895/wormbook.1.37.1,http://www.wormbook.org.2006.
- 518 51. Anand LR. Studies on the association of a new nematode species *Schistonchus*
519 *osmani* sp. n. (Aphlenchoidea Nickle, 1971), a wasp and fig *Ficus recemosa*. Uttar
520 Pradesh Journal of Zoology. 2002;22(3):281-3.
- 521 52. Anand LR. Association of a nematode *Ceratosolenus racemosa* gen. n. sp.n
522 (Cylindrocorporidae: Rhabditida Goodey, 1939) a wasp (*Ceratosolen sp.n.*) and fig
523 *Ficus racemosa*. Uttar Pradesh Journal of Zoology. 2005;25(1):59-62.
- 524 53. Davies K, Bartholomaeus F, Ye W, Kanzaki N, Giblin-Davis R. *Schistonchus*
525 (Aphelenchoididae) from *Ficus* (Moraceae) in Australia, with description of *S.*
526 *aculeata* sp n. Nematology. 2010;12:935-58.
- 527 54. Van Goor J, Piatscheck F, Houston DD, Nason JD. Figs, pollinators, and parasites:
528 A longitudinal study of the effects of nematode infection on fig wasp fitness. Acta
529 Oecologica. 2018;90:140-50.
- 530



Figure



Figure

Number of nematodes per fig

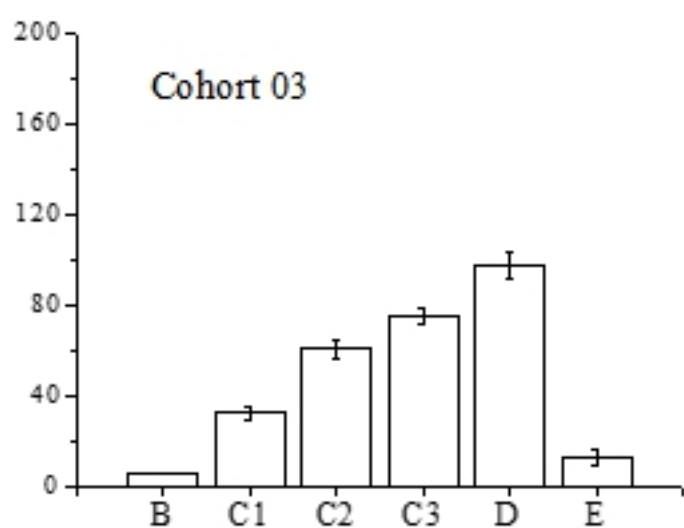
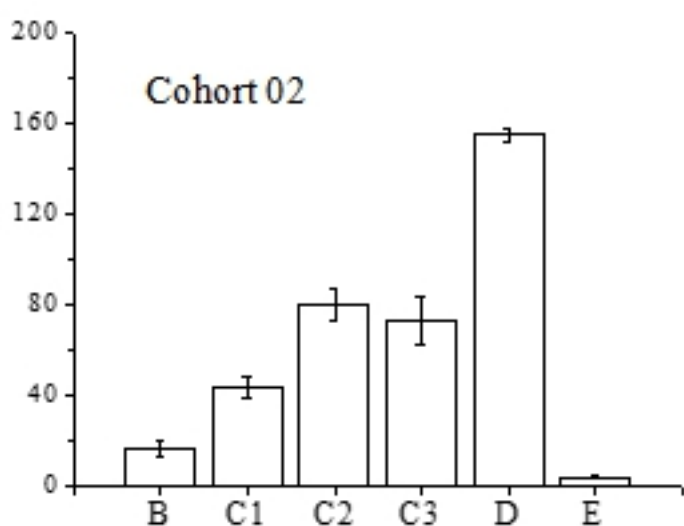
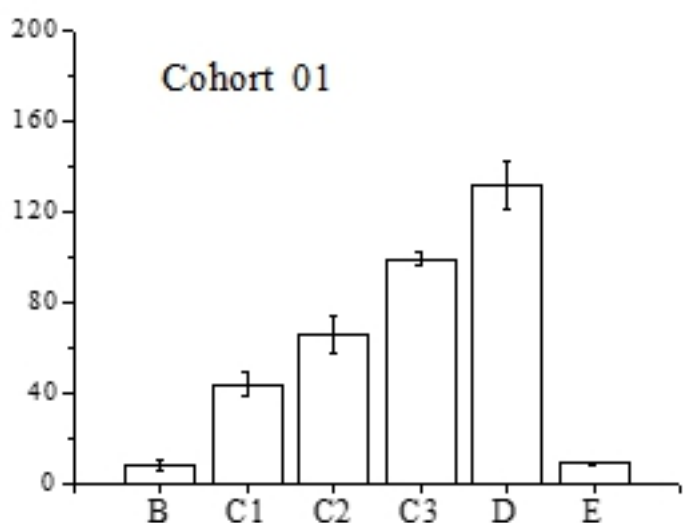
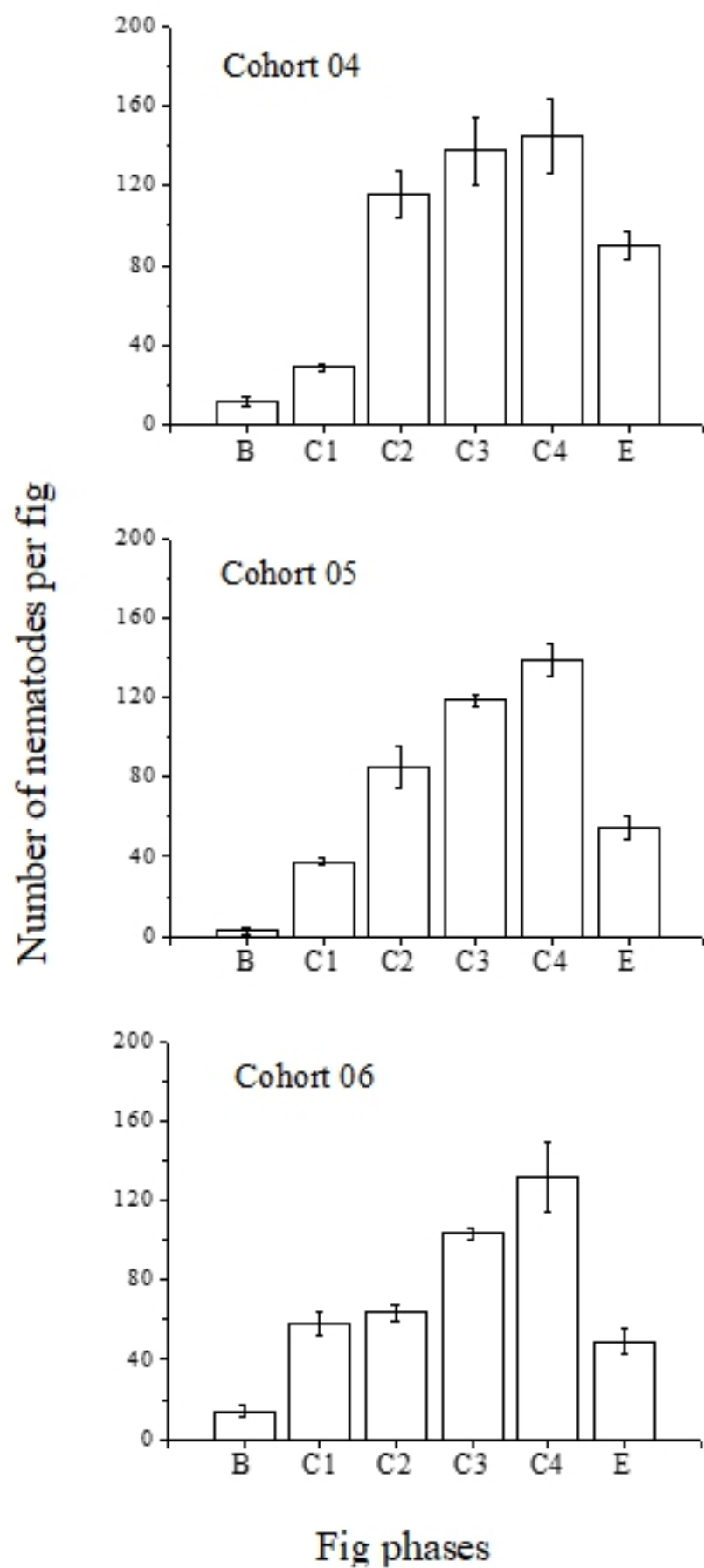


Fig phases

Figure



Figure