

1 **The best plant-guarding ants in extrafloral nectaried plants and myrmecophytes**
2 **according to baiting tests**

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18

19 **ABSTRACT**

20 Extrafloral nectaried plants and myrmecophytes offer resources to ants that may engage in
21 protective mutualisms. The role of different ant species in herbivore deterrence has long been
22 analyzed by using herbivore baits, and ants are regarded as effective plant guards if they
23 attack and/or remove the baits (mostly termites) from plants. Here, we conducted a
24 comparative investigation on which ants display aggression toward baits, which ants are
25 better plant guards, and which plants (extrafloral nectaried plants or myrmecophytes) are
26 better defended by ants. Data from the literature revealed that baiting studies have been
27 performed on 37 extrafloral nectaried plant species and 19 myrmecophytes, and have
28 involved over 30 genera of ants. Extrafloral nectaried plants and myrmecophytes rely on
29 specific ant fauna to defend them from herbivores. In extrafloral nectaried plants,
30 *Camponotus* and *Crematogaster* were regarded as the best plant protectors, as they attacked
31 baits in nearly all plants. In myrmecophytes, *Azteca*, *Pheidole* and *Pseudomyrmex* were the
32 most important bait attackers. Myrmecophytes were better protected by ants, as all baits were
33 attacked; in extrafloral nectaried plants, some ants failed to attack the baits. Plants can be
34 patrolled by several different ants, but there is a core of ants that excel in protection, and this

35 varies according to plant type (extrafloral nectaried plants and myrmecophytes). With this
36 knowledge, it may be possible to label different ants as effective plant guards, to anticipate
37 their effects on plant performance and even to understand their potential role as biological
38 control agents.

39

40 Keywords: *Azteca*; *Camponotus*; centrality, extrafloral nectar; herbivore bait; myrmecophyte

41 **1. Introduction**

42

43 Mutualistic interactions between plants and ants are shaped by the provision of shelter
44 and/or food resources by plants (e.g., nesting sites, extrafloral nectar) in exchange for
45 ecological services by ants, who act as bodyguards by defending the plants from herbivores
46 (Cagnolo and Tavella, 2015; Oliveira et al., 2021). Plants that offer resources to ants are
47 represented by myrmecophytes and extrafloral nectary (EFN) bearing plants. Myrmecophytes
48 usually provide ants with shelter, domatia and/or food resources (Dejean et al., 2017), while
49 EFN plants usually provide only extrafloral nectar, a carbohydrate-rich solution (Nogueira et
50 al., 2015). The interaction between ants and myrmecophytes is often obligate and involves a
51 few ant species associated with each plant species while EFN plants sustain a facultative
52 interaction with ants, meaning non-specialization between both parties (Blüthgen et al., 2007;
53 Fiala et al., 1999). This gradient between facultative and obligatory interactions can therefore
54 impact the results of the protection mutualism (Fiala et al., 1994) .

55 Given the richness of ants found in plants, it is not surprising that some are neither
56 aggressive nor exert a role as bodyguards (Leal et al., 2006). This results in two contrasting
57 scenarios. On the one hand, there is a core of ants that maintains a beneficial relationship with
58 plants; on the other hand, there are ants that benefit from plant resources but do not offer a
59 countermeasure (Nogueira et al., 2012). In addition, in some cases, ants may even negatively
60 influence plant performance or fail to deter herbivores (Melati and Leal, 2018). Evaluating ant
61 protection effectiveness, therefore, is essential to better understand which ants establish
62 mutual interactions with plants, potentially reducing the damage inflicted by herbivores and
63 increasing the reproductive success of plants (Raupp et al., 2020).

64 To investigate ant protection effectiveness, scientists often use herbivore baits to
65 analyze ant aggressiveness (Calixto et al., 2021). Baiting tests start with the introduction of a
66 bait, e.g., termites or caterpillars, on the plant, and ant behavior is recorded within a given

67 period (Oliveira et al., 2021). Protection is considered successful whenever ants attack or
68 remove the bait (Fagundes et al., 2017). This procedure has provided important insights to
69 explain why some ants perform better than others in herbivore deterrence and plant protection
70 (Lach and Hoffmann, 2011). For instance, *Pseudomyrmex* ants were labeled as effective plant
71 guards because they attacked more bait and were more alert than other ant species that co-
72 occurred on the same plants (Oliveira et al., 1987). However, to our knowledge, there is no
73 extensive comparative investigation on which ants display aggressive behavior toward
74 herbivore baits, which ants are better plant guards, and which plants (myrmecophytes or EFN
75 plants) are better defended by ants. A close examination of ant aggressiveness might permit
76 us to label different ants as effective plant guards, to anticipate their effects on plant
77 performance, and to understand their potential role as biological control agents (Kenne et al.,
78 2000; Schatz et al., 1997).

79 In this study, we examined the literature regarding baiting tests, and we retrieved
80 information on the ant species examined, the ant behavior (attack), and the types of study
81 plants (EFN plants and myrmecophytes) where tests were performed. With this data, we
82 investigated (*i*) whether some ants were bait-tested more than others and (*ii*) if ant attack was
83 related with plant type. This might give us an indication of which ants are more common in
84 myrmecophilous plants and whether and where they attack most baits (Fagundes et al., 2017;
85 Leal et al., 2006).

86 Furthermore, in line with the degree of ant protection exerted by each ant that foraged
87 on a plant population (Fagundes et al., 2017; Tillberg, 2004), we (*iii*) investigated the ants that
88 were regarded as better protectors in EFN plants and myrmecophytes. Within the bait-tested
89 ant community, some ants may excel and be regarded as better bodyguards; thus we
90 hypothesized that a core of ants would outstand in terms of attacking baits (Cruz et al., 2018;
91 Leal et al., 2006).

92 To conclude we (*iv*) analyzed which EFN-plants and myrmecophytes were defended
93 by only one ant genus, named here as sole defenders (the reasons for using ant genera are
94 clarified in the Methods). We expected that more species of myrmecophytes would rely on
95 sole defenders for protection because of the close and specific association between ants and
96 myrmecophytes in comparison with EFN plants, where more ants are assumed to interact with
97 plants (Blüthgen et al., 2007; Cagnolo and Tavella, 2015).

98

99 **2. Materials and Methods**

100 **2.1. Literature search**

101

102 The search for literature of baiting tests was made using the words “ants, baits,
103 termites, extrafloral nectar, myrmecophytes, myrmecophily, ant aggressiveness” and
104 variations. The Google Scholar (<https://scholar.google.com.br/>) was the only internet
105 searching tool used, as it reunites literature from all publishers and usually permits the direct
106 download of the paper, either from the publishers’ website, or from other platforms such as
107 the Research Gate or the personal webpage of scientists. Our search covered papers published
108 as early as 1981 (first study that examined ant attack toward baits) until December 2021.
109 After a thorough search, we came across dozens of studies which were downloaded and read.
110 During the reading of papers, we also scrutinized the references list to gather additional
111 literature. We also came in touch with scientist to request studies that were not fully available
112 on the internet.

113

114 **2.2. Criteria to standardize the data**

115

116 To standardize the research, only the papers that fulfilled the following criteria were
117 included in our analyses: (i) published papers, as this literature type undergoes peer review;
118 (ii) papers in English, for permitting the checking of data by anyone in the scientific
119 community; (iii) studies that covered arboreal ants; (iv) studies conducted in the field; (v)
120 papers in which the methods clearly specified the use of animal baits, such as termites and
121 other insects; (vi) papers with taxonomic identification (at least genus) of the study ants and
122 plants; (vii) studies in which the information of the ants associated with each plant could be
123 retrieved; (viii) papers that indicated which ants engaged in aggressive behavior toward baits.
124 On some occasions authors showed only a subset of ants that demonstrated aggressive
125 behavior; according to these authors, these ants were regarded as the most important in the
126 community. This data was included in our research.

127

128 **2.3. Data collection**

129

130 Each paper included in the analyses was named and number labeled. The following
131 information was retrieved and systematically organized: the country where the fieldwork took
132 place, the taxonomic information of ants and plants, the baits used (e.g., termite, caterpillar,
133 grasshopper), the type of study plant (‘EFN’ – plants that produce extrafloral nectar – Del-

134 Claro et al. 2016; and ‘myrmecophytic’ – plants that shelter ants and may have or not have
135 food resources – Dejean et al. 2018), and whether ants attacked the baits.

136 An ‘attack’ consists of the aggression of ants, which varies from species (biting,
137 stinging, spraying liquids), but we also considered the molestation of ants as attack, as this is
138 also an antiherbivore mechanism (Alves-Silva and Del-Claro, 2014; Fuente and Marquis,
139 1999). Furthermore, for small ants, it is difficult to determine if they bite the baits; in some
140 instances, the authors did not explicitly mention that ants really bit the baits, but this was
141 implied in the study (Leal et al., 2006; Vidal et al., 2016).

142 The repertoire of ant behavior toward baits is extensive (Dejean et al., 2009). For
143 instance, recruiting and bait removal from plants might also provide evidence of plant
144 protection. Recruitment involves the arrival of other nestmates at the place where the bait is
145 being attacked (Pacelhe et al., 2019). Removal consists of removing the bait from the plant by
146 either taking it to the nest, throwing it away, killing it or making it abandon the plant (Alves-
147 Silva and Del-Claro, 2016; Vantaux et al., 2007). Despite the importance of both behaviors in
148 herbivore deterrence, scientists rarely provide such information; in addition, these behaviors
149 are not subjected to tests (such as ant attacks toward baits), but rather sporadically noted in
150 the field. In fact, studies rarely specify whether ants display recruitment and removal of baits.
151 This may derail detailed comparisons and even bias conclusions. However, the attack (or not)
152 of ants on baits is always specified. Thus, to keep the data as appropriate as possible to allow
153 statistical comparisons and to increase our understanding of ant behavior, we examined only
154 ant attacks.

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156 **2.4. Ants who engage in aggressive behavior**

157

158 Our data cover ant genera rather than species, and this is justified. Ants were identified
159 to species level in approximately 2/3 of baiting tests, but this number varied according to
160 plant types; for instance, only 58% of the ants in myrmecophytes were identified to species
161 level, especially due to the difficulty of dealing with small ants, such as *Azteca* and
162 *Crematogaster*. Thus, the use of ant genera is substantiated by the difficulty of some authors
163 in identifying some ants at the species level (Koch et al., 2016). Many ant species were
164 observed in the field and classified into genera but were not collected and subjected to further
165 taxonomic identification. In some cases, new species were classified as ‘sp. nov’ (and
166 variations) and no specific name was provided.

167 By using ant genera, we also avoided considering ant species that were misidentified
168 to the species level or species that belong to a group of species (e.g., *Camponotus* aff.
169 *blandus*, *Pseudomyrmex* gr. *pallidus*). We also noted misspelling in specific names, which
170 indicates minimal taxonomic attention to the species level in some cases. To conclude, the
171 approach of focusing on ant genera to classify ants as effective plant guards is valid when we
172 consider that arboreal species in many genera present conservative behavior when it comes to
173 foraging, patrolling and aggressiveness toward baits (Bächtold and Del-Claro, 2018;
174 Fagundes et al., 2017; Oliveira et al., 1987).

175

176 **2.5. Data analyses**

177

178 We found 56 papers that fulfilled our study criteria and we managed to retrieve 344
179 records of ants that were bait-tested. Exploratory analyses revealed the presence of 33 ant
180 genera, however the most frequent ants belonged to nine genera, namely *Camponotus*,
181 *Crematogaster*, *Pseudomyrmex*, *Pheidole*, *Cephalotes*, *Azteca*, *Ectatomma*, *Solenopsis* and
182 *Brachymyrmex* (Supplementary material 1), which together were present in 85% of papers (n
183 = 48), 85% of baiting tests ($n = 292$) and at least in one plant type (EFN and/or
184 myrmecophytes). Our analyses will cover the data of these nine genera of ants only ($n = 292$,
185 the statistical unit of our study), as it permits the use of statistical analyses and comparisons.

186

187 **2.6. Statistical analyses**

188

189 Goodness of fit chi-squared tests were used to check if one ant genus was tested more
190 than others; we compared the frequency of each ant in baiting tests, in the occurrence of plant
191 species, plant families, plant types (EFN and myrmecophytes) and in the papers that bait-
192 tested ants (objective *i*). Two-way Anova tests were conducted to analyze if ant attacks were
193 related with the ant genera and plant-types (objective *ii*).

194 To investigate the importance of each ant as plant-guard (objective *iii*), we built
195 ecological networks with pairwise interactions between ants and the plants where they
196 attacked baits. The ecological network was defined by the connections between plants and
197 ants, and it is considered a holistic approach to investigate ecological communities (e.g.,
198 pollination, predator-prey interactions, mutualisms) and how species interact with their
199 partners (Trøjelsgaard and Olesen, 2016). Both ants and plants were regarded as nodes, and
200 the interactions between them were labelled as links (Guimarães, 2020).

201 We built adjacency matrices where values of one or zero were assigned to cells with or
202 without interactions between ants and plant species, respectively. According to Palacio et al.,
203 (2016 and references therein) binary data (presence and absence of interactions between
204 species) are appropriate to calculate centrality indices (Mello et al., 2015) and results do not
205 differ in comparison to weighted data (i.e., how often a species *i* interact with species *j*); in
206 addition binary data may identify central nodes as good as weighted data does. With the
207 organization of the matrix and further analyses of data we could see the plants defended by
208 each ant, possible overlaps of different ants in a single plant species, and which plants relied
209 on only one ant genus for defense (sole defenders). A total of two matrices were built, one for
210 EFN-plants and other for myrmecophytes.

211 We calculated the degree, which shows the number of plant species where ants were
212 observed to attack baits (Cagnolo and Tavella, 2015) and we also estimated the centrality
213 values for ants in each network. Centrality indices have been incorporated in ecological
214 studies to determine the important species in communities (Farine and Whitehead, 2015;
215 Genrich et al., 2017; Martín González et al., 2010; Poulin et al., 2013). Collectively, the
216 centrality indexes not only consider the quantity of interactions between parties (degree
217 distribution), but also the indirect connections of a species with the whole plant community
218 (Delmas et al. 2019; Silva et al. 2020). Thus, a value of the relative importance of a species in
219 a community is provided (Borrett, 2013; Martín González et al., 2010).

220 In our study we used the betweenness (BC) and the eigenvalue (EC) centrality
221 indexes. The first gives more weight to ants that have higher degree (i.e., ants that attack baits
222 in many plant species) and if removed cause the rupture of the network (Olesen et al., 2007).
223 In this context, ants with high BC are better plant-guards, because they attack baits in several
224 plant species, may act alone in a few plant species, and if removed may leave plants either
225 unattended or patrolled by so-called less aggressive ants (by less aggressive we mean those
226 ants that occur in a few plant species and are thus not frequent in the plant community)
227 (Sazima et al., 2010; Trøjelsgaard and Olesen, 2016).

228 The EC gives weight to overlaps between ant species in plants. Ants that occur in
229 many plant species will have higher EC, however, the less frequent ants that co-occur with the
230 most frequent ants will also have higher EC (Borgatti, 2005; Morand et al., 2020; Silva et al.,
231 2020). This index indicates the contribution of ants to the plant community and also shows the
232 specialization, as ants with the lowest EC will be rare in the community and will not share
233 plants with frequent ants.

234 The metrics used in plant-animal networks are almost limitless (Delmas et al., 2019;
235 Guimarães, 2020), but we used only those that were suitable to address our objectives. For
236 instance, the closeness centrality (the occurrence of a species i in the same niches/plants of
237 other species) is also used in some studies (Mello et al., 2015), but it positively relates with
238 degree and BC (Sazima et al., 2010). In addition, we calculated the metrics for ants only; the
239 species of plants were not incorporated into the metrics as we were interested in the functional
240 groups they belonged to (i.e., EFN-plants or myrmecophytes), rather than on particular plant
241 species. Our objectives are in accordance with the definition of microscopic metrics of the
242 network as we focused on the importance of species (in our case, the different ants) rather
243 than the overall network structure (Trøjelsgaard and Olesen, 2016). All centrality indices were
244 calculated with the *igraph* package in R statistical software (Csardi and Nepusz, 2006).

245 To conclude, we investigated which plant species sustained only one ant genus for
246 protection. Most plants were patrolled by more than one ant genus, whereas others relied on
247 only one genus of ants as a plant-guard. We separated the ants that attacked baits on only one
248 plant species and these ants were regarded as sole protectors. We then used the Fisher's exact
249 test to examine which plant type (EFN-plants and myrmecophytes) had more sole defending
250 ants (objective *iv*).

251 We are aware that some interactions in the plant-ant network may be forbidden links,
252 i.e., interactions that are likely to not occur (Kiziridis et al., 2020). Given the spatial scale of
253 our data (information retrieved from 56 papers that bait-tested ants in eight countries), the
254 forbidden links were expected to occur. We are fully aware of the issues related with
255 forbidden links (Jordano, 2016). Nonetheless, the purpose of our network was to show that
256 among the pool of ants, some genera are regarded as more important than others in plant
257 defense; and if that particular ant (genera) is removed, the plant can be occupied by ants with
258 similar aggressiveness, or not. In addition, the most important ants in our work are
259 widespread in the world and can overlap in several plant species (shown in Results). To
260 conclude we did not consider particular plant species, but rather if plants either belonged to
261 the EFN or the myrmecophyte group.

262 We provide an overview of the characteristics of baiting studies, such as where
263 investigations were performed, the bait types used and the study plants, but without statistical
264 tests. Supplementary materials bring figures with more detail of the data.

265 All the analyses and figures were made in R statistical software version 4.1.3.

266

267 **3. Results**

268 3.1. Overview of baiting studies

269

270 The baiting studies which were performed with the most frequent ants (*Camponotus*,
271 *Crematogaster*, *Pseudomyrmex*, *Pheidole*, *Azteca*, *Ectatomma*, *Solenopsis*, *Cephalotes* and
272 *Brachymyrmex*) were conducted in eight countries, half of them in Brazil (50% of the total, n
273 = 24 studies); 60% of the baiting tests were also made in Brazil ($n = 174$). Termites were used
274 more often (81% of tests) than the other bait types, such as caterpillars, beetles, orthopterans,
275 flies, and hemipterans (Supplementary material 2). The plants were represented by 56 species
276 in 37 genera and 26 families, especially Fabaceae (30% of plants species, $n = 17$ species)
277 (Supplementary material 3). A total of 37 EFN-plant species and 19 myrmecophytes were
278 studied (Supplementary material 4).

279

280 3.2. Ants in baiting tests

281

282 Both *Camponotus* and *Crematogaster* were significantly more frequent in baiting
283 tests, plant species, plant families, and in the number of studies that had these ants under
284 investigation (Table 1). Their frequency in EFN-plants was also significantly higher in
285 comparison to the other ants examined (Table 1). In myrmecophytes, there was no difference
286 in the frequency of ants.

287

288 **Table 1** Frequency data of the most common ants in baiting studies. Superscript lowercase
289 letters indicate statistically significant differences, according to chi-squared tests.

| Ants | Baiting tests | Plant species | Plant families | EFN plants | Myrmecophytes | Papers |
|----------------------|-----------------------------------|----------------------------------|--------------------------------|----------------------------------|--------------------------------|---------------------------------|
| <i>Camponotus</i> | 98 ^a | 37 ^a | 18 | 33 ^a | 4 | 29 ^a |
| <i>Crematogaster</i> | 47 ^b | 30 ^{ab} | 15 | 25 ^{ab} | 5 | 23 ^{ab} |
| <i>Pseudomyrmex</i> | 41 ^c | 23 ^{ac} | 8 | 18 ^a | 5 | 14 ^a |
| <i>Cephalotes</i> | 23 ^d | 16 ^b | 8 | 14 ^b | 2 | 12 ^{ab} |
| <i>Pheidole</i> | 23 ^d | 14 ^c | 6 | 9 ^c | 5 | 12 ^{ab} |
| <i>Azteca</i> | 18 ^d | 9 ^c | 6 | 2 ^c | 7 | 12 ^{ab} |
| <i>Ectatomma</i> | 16 ^d | 10 ^c | 5 | 8 ^c | 2 | 8 ^d |
| <i>Solenopsis</i> | 14 ^d | 10 ^c | 7 | 9 ^c | 1 | 8 ^d |
| <i>Brachymyrmex</i> | 12 ^d | 10 ^c | 6 | 9 ^c | 1 | 10 ^b |
| Chi-squared | $\chi^2 = 184.88$ $P < 0.0001$ | $\chi^2 = 46.52$ $P < 0.0001$ | $\chi^2 = 18.86$ $P < 0.05$ | $\chi^2 = 53.35$ $P < 0.0001$ | $\chi^2 = 10.18$ $P > 0.05$ | $\chi^2 = 28.51$ $P < 0.001$ |

290

291

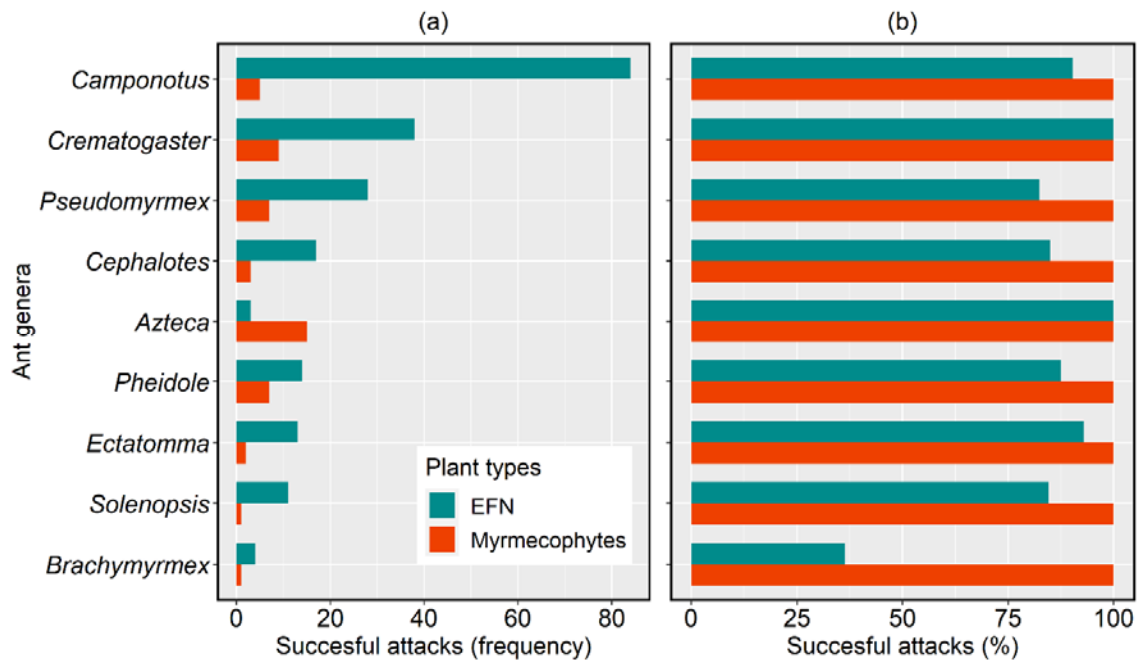
292

293 3.3. Ant attacks

294

295 EFN-plants were studied more often than myrmecophytes ($n = 242$ and 50 tests,
 296 respectively) (Figure 1a). The *Camponotus* was by far more frequent in EFN-plants than in
 297 myrmecophytes; *Azteca* was the only ant that was more frequent in myrmecophytes. In
 298 myrmecophytes, all baits were attacked; in EFN-plants, attacks were successful in 88% of
 299 tests ($n = 212$ tests). All ants attacked baits in both plant types (Figure 1b). Ant attacks were
 300 significantly related with plant types, but not ant genera (Table 2).

301



302

303 **Fig. 1.** Ant genera that engaged in bait attack (frequency – a; successful attacks – b). EFN-
 304 plants sustained more baiting tests, with *Camponotus* and *Crematogaster* being frequently
 305 bait-tested. Myrmecophytes had 100% of baits attacked by ants.

306

307 **Table 2** Anova indicating whether the frequency of attacks (log-transformed data) was
 308 influenced by the ant genera or plant type (i.e., EFN and myrmecophytes).

309

| Successful attack of ants | | | | | |
|---------------------------|----|--------|---------|---------|---------------|
| Variables | Df | Sum Sq | Mean Sq | F-value | P-value |
| Ant genera | 8 | 9.75 | 1.12 | 1.52 | 0.2824 |
| Plant type | 2 | 7.99 | 7.99 | 9.99 | 0.0134 |
| Residuals | 8 | 6.40 | 0.80 | | |

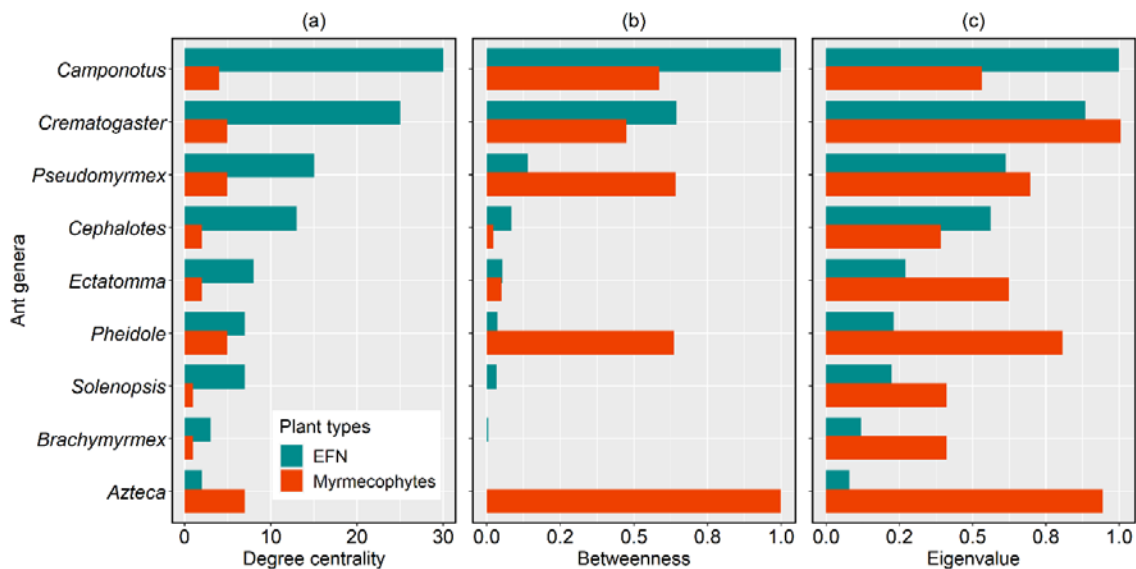
310

311 3.4. Important ants in the network

312

313 *Camponotus* and *Crematogaster* attacked baits in most plants within the EFN-plant
314 community and had the highest degree (Figures 2a and 3a). These ants had also the highest
315 BC and EC, indicating both that they were central to the network stability and they were
316 connected to plants with other ants (Figures 2b, 3a, 3b). The BC was almost negligible for
317 *Azteca*, *Brachymyrmex*, *Solenopsis* and *Pheidole*, indicating null to low importance in
318 attacking baits in the EFN plant community; their EC shows that they shared a few plants
319 with the most influential nodes in the EFN network (Figures 2c and 3b).

320 The *Azteca* had the lowest degree, BC and EC in EFN plants, but they stood out in
321 myrmecophytes with the highest degree, maximum BC and the second highest EC (Figures 2
322 and 3). This demonstrates how important were the *Azteca* in herbivore deterrence in
323 myrmecophytes. The *Azteca* were followed by *Pheidole*, *Pseudomyrmex* and *Crematogaster*
324 (Figures 2 and 3). Other ants (*Solenopsis*, *Brachymyrmex*, *Cephalotes* and *Ectatomma*)
325 occurred in few myrmecophytes and their occurrence overlapped with the most
326 aggressive/frequent ants; thus their BC and EC were low in myrmecophytes (Figures 2 and 3).
327

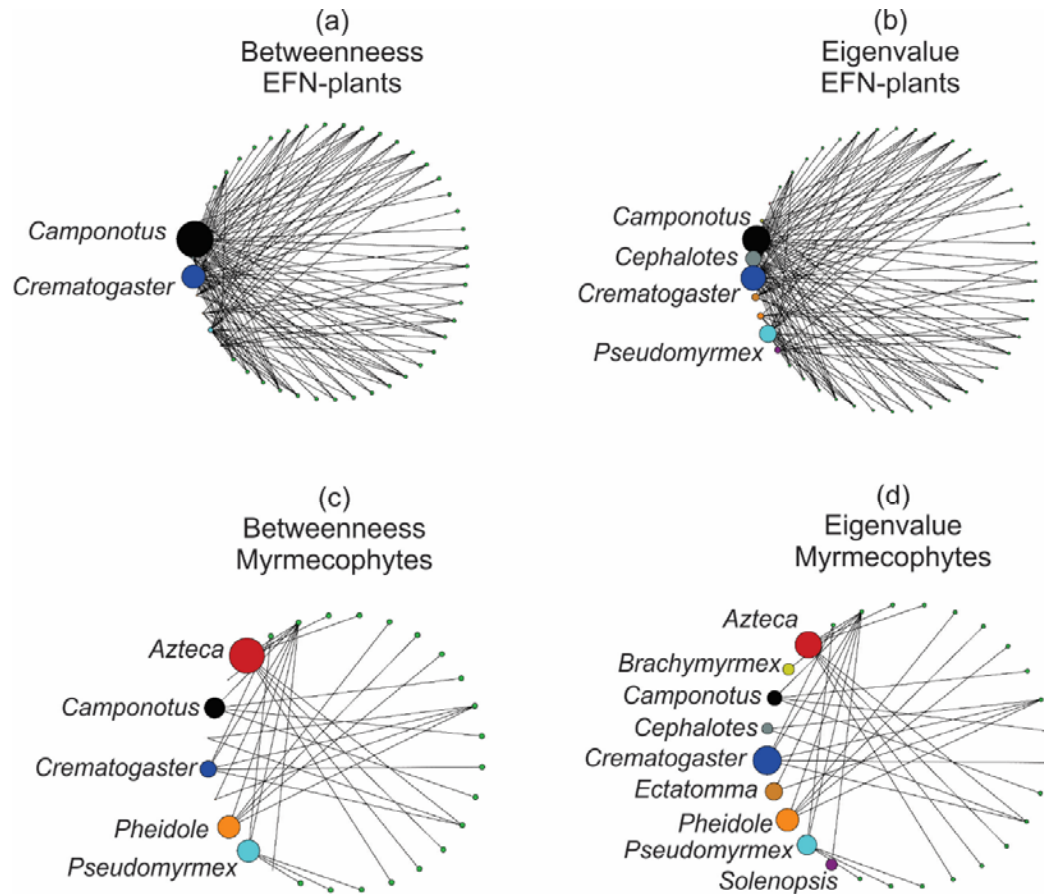


328

329 **Fig. 2.** Measures of the relative importance of ants according to baiting tests. (a) Degree
330 centrality which shows the number plants where each ant was bait-tested; (b) betweenness
331 centrality (BC), that indicates ants that were central to the stability of the network; (c)
332 eigenvalue centrality (EC) that measures co-occurrence of ants in the plant community.

333 Values of BC and EC are relativized (0 to 1, lowest and highest value for a species,
334 respectively) to permit a better visual comparison among plant communities.

335



336

337 **Fig. 3.** Ants that engaged in aggressive behavior to baits in (a, b) plants with extrafloral
338 nectaries and (c, d) myrmecophytes. The small green circles represent the different plant
339 species (names not shown). The size of circles indicates the relative importance of each ant
340 according to the betweenness (BC) and eigenvalue (EC) centrality indexes.

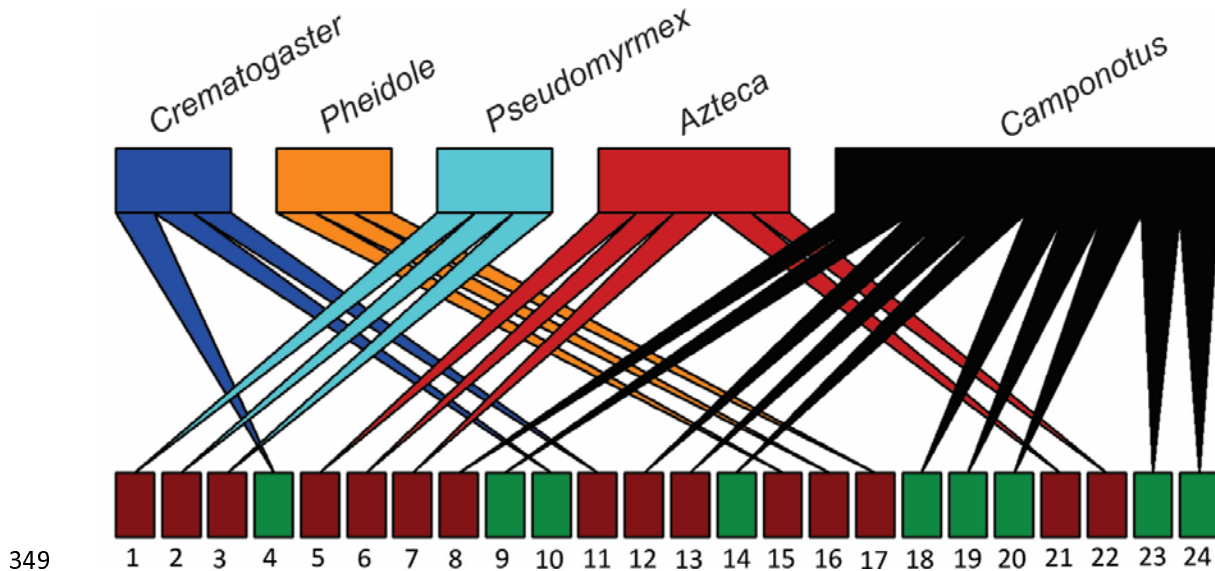
341

342 3.5. Sole defenders

343 Out of the plants examined, nine EFN-plants and 15 myrmecophytes relied on only
344 one ant genus for defense (Fisher's exact test, $p < 0.01$). The *Azteca*, *Pheidole* and

345 *Pseudomyrmex* were the sole defenders of myrmecophytes ($n = 5, 3$ and 3 plants,
346 respectively), while the *Camponotus* and *Crematogaster* were the sole defenders of some
347 myrmecophytes and EFN-plants (Figure 4).

348



351 **Fig. 4.** Bipartite network showing the ants that were sole defenders of EFN-plants (green) and
352 myrmecophytes (dark red). Plant names: 1 *Acacia collinsii*, 2 *Acacia cornigera*, 3 *Acacia*
353 *hindsii*, 4 *Cassia javanica*, 5 *Cecropia obtusa*, 6 *Cecropia obtusifolia*, 7 *Cecropia*
354 *pachystachya*, 8 *Korthalsia furtadoana*, 9 *Lafoensia pacari*, 10 *Leea aculeata*, 11 *Macaranga*
355 *banca*, 12 *Macaranga puncticulata*, 13 *Nepenthes bicalcarata*, 14 *Ouratea spectabilis*, 15
356 *Piper fimbriulatum*, 16 *Piper sagittifolium*, 17 *Piper* sp., 18 *Qualea multiflora*, 19
357 *Stachytarpheta glabra*, 20 *Stryphnodendron polyphyllum*, 21 *Tetrathylacium macrophyllum*,
358 22 *Tococa formicaria*, 23 *Triumfetta semitriloba*, 24 *Vachellia constricta*.

359

360 4. Discussion

361 4.1. Overview

362

363 Each plant type had a core of the most important ants in terms of bait attack, thus
364 corroborating our hypothesis. With all results combined, *Camponotus* and *Crematogaster*
365 excelled in EFN plants, and *Azteca*, *Pheidole* and *Pseudomyrmex* were the main bait attackers
366 in myrmecophytes. The importance of these five ants was highlighted by their high centrality
367 indices, indicating that they are frequent bait attackers in the plant community and that their

368 absence could make plants unattended or attended by less frequent ants. These ants were also
369 the sole defenders of some plants, indicating that without them, plants might rely on no ants
370 as partners.

371

372 **4.2. Core of the most important ants**

373

374 *Camponotus* and *Crematogaster* were the most bait-tested ants and had the highest
375 degree in many cases, except in myrmecophytes, where *Azteca* was dominant. In plant–ant
376 relationships, there is usually a core of ants that dominate the interactions with plants; thus, it
377 is expected that the most frequent ants receive higher importance in networks (Dáttilo et al.,
378 2013; Lange et al., 2013; Silva et al., 2020). The centrality indices used in our study
379 (betweenness and eigenvalue) not only considered the degree (how many plants ants interact
380 with) but also the specific connections between ants and plants (Mello et al., 2015; Silva et
381 al., 2020). More weight was given to ants that, whenever removed, might disrupt the network,
382 leave plants unattended or be attended by less frequent ants (Genrich et al., 2017). Thus,
383 species degree may not be related to other centrality values in some cases. For instance, both
384 *Pseudomyrmex* and *Crematogaster* occurred in five myrmecophytes, but the former had
385 higher BC; thus, *Pseudomyrmex* was more important to myrmecophytes than *Crematogaster*.
386 This occurred both because *Pseudomyrmex* was present in myrmecophytes that had no other
387 ants (sole defender of three plants), and because these ants shared only two plants with other
388 ants. In contrast, *Crematogaster* attacked baits in plants that supported four other ants; that is
389 why the EC was higher for *Crematogaster*, in comparison to *Pseudomyrmex*. Thus, in the
390 absence of *Crematogaster*, plants can be patrolled by more ants than plants deprived of
391 *Pseudomyrmex*. Interpretation of the different centrality indices (see Delmas et al., 2019;
392 Palacio et al., 2016; Poulin et al., 2013) is paramount to understand the relative importance of
393 each node for the stability of the network.

394 *Azteca*, *Pheidole* and *Pseudomyrmex* had the highest degree and BC values in
395 myrmecophytes. Due to their small size, these ants can shelter and breed inside domatia and
396 rapidly respond to disturbances in plants (Goheen and Palmer, 2010; Pringle et al., 2014). The
397 specificity of this interaction makes *Azteca*, *Pheidole* and *Pseudomyrmex* the most important
398 in myrmecophytes (Oliveira et al., 1987; Schmidt and Dejean, 2018). Since they are
399 commonplace in myrmecophytes, they are likely to attack more baits. In contrast, EFN plants
400 sustain a non-specific community of ants that do not shelter on plants but rather nest on the
401 soil and forage on plants for food resources (Blüthgen et al., 2006). Even so, the ants in

402 genera *Camponotus* and *Crematogaster* are constant in samplings (Burger et al., 2021;
403 Koptur, 1984; Oliveira et al., 2021); these ants were more bait-tested, attacked more baits and
404 received high BC values.

405

406 **4.3. Effective plant guards?**

407

408 Ecologists tend to be cautious in classifying a given ant as an effective plant guard
409 because the aggressive behavior and protection efficiency of each ant species should be seen
410 as a continuous rather than ordinal scale (Ness, 2006). In addition, the benefits ants provide to
411 plants depend on two undissociated factors: quality and quantity. The former refers to the type
412 of benefits received by plants, which in our case is bait attack, a proxy for herbivore
413 deterrence (Calixto et al., 2021; Oliveira et al., 1987; Philpott et al., 2008) and the latter is the
414 frequency of interactions between ants and plants (Koch et al., 2018; Ness, 2006). The
415 combination of attack and frequency permitted us to draw a hierarchy of the importance of
416 ants, with *Camponotus*, *Crematogaster* (EFN plants), *Azteca*, *Pheidole* and *Pseudomyrmex*
417 (myrmecophytes) as the top plant defenders. In fact, some plants were observed to depend
418 upon these ants only (the sole defenders), which is evidence of their importance as
419 bodyguards in the plant community.

420 Intrinsic ant behavior and frequency in the plant community might be a good
421 indication to label ants as effective plant guards. For instance, *Ectatomma* ants are aggressive
422 and possess a privileged weaponry (body size, large mandibles and sting) that subdues several
423 types of herbivores (Bächtold and Alves-Silva, 2013; Del-Claro and Marquis, 2015; Robbins,
424 1991; Schatz et al., 1997), but they are not regularly found on plants (Bächtold et al. 2016). In
425 contrast, *Camponotus* and *Azteca* are shorter than *Ectatomma* and do not possess large
426 mandibles or stings, but they consistently forage on many plant species (Blüthgen et al., 2000;
427 Lange et al., 2019; Monique et al., 2022). Thus, they might exert a relatively higher role in
428 herbivore deterrence in the plant community than infrequent ants (Calixto et al., 2021;
429 Frederickson, 2005).

430 One may assume that the classification of ants as effective plant guards is premature.
431 In fact, ideally all ants should be tested equally and then scientists could compare the attack
432 rates among the ant community. Nonetheless, we do not believe that the different sample size
433 of bait-tested ants is a drawback, as our dataset comprises a microcosm of plant–ant studies.
434 The frequency of bait-tested ants in the literature reflects the occurrence of these ants in the
435 field (Del-Claro and Marquis, 2015; Fagundes et al., 2017; Lange et al., 2013). *Camponotus*

436 and *Crematogaster* are the most sampled species in the EFN plant community (Ribeiro et al.,
437 2018) and the *Azteca*–myrmecophyte system is the most studied (Dejean et al., 2009). Our
438 data covers papers from as far back as 40 years ago, and *Camponotus*, *Crematogaster* and
439 *Azteca* have been consistently sampled ever since, indicating both that these ants are
440 widespread in the field and that plant–ant interactions have temporal stability, with the core
441 ant species occupying the same plants over time (e.g., *Camponotus* – Oliveira 1997; Koch et
442 al. 2016; *Crematogaster* – Guimarães et al. 2006; Franco and Cogni 2013; *Azteca* – Tillberg
443 2004; Pringle et al. 2014). According to Díaz-Castelazo et al. (2010), generalist ants maintain
444 the stability of the network and remain over time, even with the inclusion of other ants in the
445 system. In this context, the results found here reflect what we actually see in the field in terms
446 of plant occupation by ants.

447

448 **5. Conclusions**

449

450 The importance of ants, such as *Camponotus*, *Crematogaster* (EFN plants), *Azteca*,
451 *Pseudomyrmex* and *Pheidole* (myrmecophytes) for herbivore deterrence is highlighted by
452 their frequency on plants, their intrinsic aggressive behavior, the bait attack and their sole
453 occurrence on some plant species. Forthcoming approaches may gather important evidence to
454 rank ants according to their capacity to defend plants from herbivores. We also advocate for
455 established protocols to evaluate and compare ant aggressiveness and determine which
456 species perform better than others.

457

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461

462 **References**

463

- 464 Alves-Silva, E., Del-Claro, K., 2016. On the inability of ants to protect their plant partners
465 and the effect of herbivores on different stages of plant reproduction. *Austral Ecol.* 41,
466 263–272. <https://doi.org/10.1111/aec.12307>
- 467 Alves-Silva, E., Del-Claro, K., 2014. Fire triggers the activity of extrafloral nectaries, but ants
468 fail to protect the plant against herbivores in a neotropical savanna. *Arthropod. Plant.*
469 *Interact.* 8, 233–240. <https://doi.org/10.1007/s11829-014-9301-8>

- 470 Bächtold, A., Alves-Silva, E., 2013. Behavioral strategy of a lycaenid (Lepidoptera)
471 caterpillar against aggressive ants in a Brazilian savanna. *Acta Ethol.* 16, 83–90.
- 472 Bächtold, A., Del-Claro, K., 2018. Ant-partners play a minor role on occurrence of the
473 myrmecophilous butterfly *Leptotes cassius* in its host plant. *Arthropod. Plant. Interact.*
474 12, 377–384. <https://doi.org/10.1007/s11829-017-9586-5>
- 475 Blüthgen, N., Verhaagh, M., Goitía, W., Jaffé, K., Morawetz, W., Barthlott, W., 2000. How
476 plants shape the ant community in the Amazonian rainforest canopy: the key role of
477 extrafloral nectaries and homopteran honeydew. *Oecologia* 125, 229–40.
478 <https://doi.org/10.1007/s004420000449>
- 479 Blüthgen, Nico, Menzel, F., Blüthgen, Nils, 2006. Measuring specialization in species
480 interaction networks. *BMC Ecol.* 6, 1–12. <https://doi.org/10.1186/1472-6785-6-9>
- 481 Blüthgen, Nico, Menzel, F., Hovestadt, T., Fiala, B., Blüthgen, Nils, 2007. Specialization,
482 constraints, and conflicting interests in mutualistic networks. *Curr. Biol.* 17, 341–346.
483 <https://doi.org/10.1016/j.cub.2006.12.039>
- 484 Borgatti, S.P., 2005. Centrality and network flow. *Soc. Networks* 27, 55–71.
485 <https://doi.org/10.1016/J.SOCNET.2004.11.008>
- 486 Borrett, S.R., 2013. Throughflow centrality is a global indicator of the functional importance
487 of species in ecosystems. *Ecol. Indic.* 32, 182–196.
488 <https://doi.org/10.1016/J.ECOLIND.2013.03.014>
- 489 Burger, H.F., Vondráčková, K., Skłodowski, M., Koid, Q., Dent, D.H., Wallace, K., Fayle,
490 T.M., 2021. Protection from herbivores varies among ant genera for the myrmecophilic
491 plant *Leea aculeata* in Malaysian Borneo. *Asian Myrmecology* 14, 1–16.
492 <https://doi.org/10.20362/am.01>
- 493 Cagnolo, L., Tavella, J., 2015. The network structure of myrmecophilic interactions. *Ecol.*
494 *Entomol.* 40, 553–561. <https://doi.org/10.1111/EEN.12229>
- 495 Calixto, E.S., Lange, D., Moreira, X., Del-Claro, K., 2021. Plant species specificity of ant-
496 plant mutualistic interactions: Differential predation of termites by *Camponotus crassus*
497 on five species of extrafloral nectaries plants. *Biotropica* 53, 1406–1414.
498 <https://doi.org/10.1111/btp.12991>
- 499 Cruz, N.G., Cristaldo, P.F., Bacci, L., Almeida, C.S., Camacho, G.P., Santana, A.S., Ribeiro,
500 E.J.M., Oliveira, A.P., Santos, A.A., Araújo, A.P.A., 2018. Variation in the composition
501 and activity of ants on defense of host plant *Turnera subulata* (Turneraceae): strong
502 response to simulated herbivore attacks and to herbivore's baits. *Arthropod. Plant.*
503 *Interact.* 12, 113–121. <https://doi.org/10.1007/s11829-017-9559-8>

- 504 Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research.
- 505 Dáttilo, W., Guimarães, P.R., Izzo, T.J., 2013. Spatial structure of ant-plant mutualistic
506 networks. *Oikos* 122, 1643–1648. <https://doi.org/10.1111/j.1600-0706.2013.00562.x>
- 507 Dejean, A., Azémar, F., Petitclerc, F., Delabie, J.H.C., Corbara, B., Leroy, C., Céréghino, R.,
508 Compin, A., 2018. Highly modular pattern in ant-plant interactions involving specialized
509 and non-specialized myrmecophytes. *Sci. Nat.* 105, 1–8. [https://doi.org/10.1007/s00114-](https://doi.org/10.1007/s00114-018-1570-0)
510 018-1570-0
- 511 Dejean, A., Grangier, J., Leroy, C., Orivel, J., 2009. Predation and aggressiveness in host
512 plant protection: A generalization using ants from the genus *Azteca*. *Naturwissenschaften*
513 96, 57–63. <https://doi.org/10.1007/s00114-008-0448-y>
- 514 Dejean, A., Petitclerc, F., Compin, A., Azémar, F., Corbara, B., Delabie, J.H.C., Leroy, C.,
515 2017. Hollow internodes permit a neotropical understory plant to shelter multiple
516 mutualistic ant species, obtaining protection and nutrient provisioning (myrmecotrophy).
517 *Am. Nat.* 190, E124–E131. <https://doi.org/10.1086/693782>
- 518 Del-Claro, K., Marquis, R.J., 2015. Ant species identity has a greater effect than fire on the
519 outcome of an ant protection system in Brazilian Cerrado. *Biotropica* 47, 459–467.
520 <https://doi.org/10.1111/btp.12227>
- 521 Del-Claro, K., Rico-Gray, V., Torezan-Silingardi, H.M., Alves-Silva, E., Fagundes, R.,
522 Lange, D., Dáttilo, W., Vilela, A.A., Aguirre, A., Rodriguez-Morales, D., 2016. Loss and
523 gains in ant-plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies.
524 *Insectes Soc.* 63, 207–221. <https://doi.org/10.1007/s00040-016-0466-2>
- 525 Delmas, E., Besson, M., Brice, M.-H., Burkle, L.A., Dalla Riva, G. V., Fortin, M.-J., Gravel,
526 D., Guimarães, P.R., Hembry, D.H., Newman, E.A., Olesen, J.M., Pires, M.M., Yeakel,
527 J.D., Poisot, T., 2019. Analysing ecological networks of species interactions. *Biol. Rev.*
528 94, 16–39. <https://doi.org/10.1111/brv.12433>
- 529 Díaz-Castelazo, C., Guimarães, P.R., Jordano, P., Thompson, J., Marquis, R., Rico-Gray, V.,
530 2010. Changes of a mutualistic network over time: reanalysis over a 10□ year period.
531 *Ecology* 91, 793–801. <https://doi.org/10.1890/08-1883.1>
- 532 Fagundes, R., Dáttilo, W., Ribeiro, S.P., Rico-Gray, V., Jordano, P., Del-Claro, K., 2017.
533 Differences among ant species in plant protection are related to production of extrafloral
534 nectar and degree of leaf herbivory. *Biol. J. Linn. Soc.* 122, 71–83.
535 <https://doi.org/10.1093/biolinnean/blx059>
- 536 Farine, D.R., Whitehead, H., 2015. Constructing, conducting and interpreting animal social
537 network analysis. *J. Anim. Ecol.* 84, 1144–1163. <https://doi.org/10.1111/1365->

- 538 2656.12418
- 539 Fiala, B., Grunsky, H., Maschwitz, U., Linsenmair, K.E., 1994. Diversity of ant-plant
540 interactions: protective efficacy in *Macaranga* species with different degrees of ant
541 association. *Oecologia* 97, 186–192. <https://doi.org/10.1007/BF00323148/METRICS>
- 542 Fiala, B., Jakob, A., Maschwitz, U., Linsenmair, K.E., 1999. Diversity, evolutionary
543 specialization and geographic distribution of a mutualistic ant-plant complex:
544 *Macaranga* and *Crematogaster* in South East Asia. *Biol. J. Linn. Soc.* 66, 305–331.
545 <https://doi.org/10.1111/j.1095-8312.1999.tb01893.x>
- 546 Franco, M.S., Cogni, R., 2013. Common-garden experiments reveal geographical variation in
547 the interaction among *Crotalaria pallida* (Leguminosae: Papilionideae), *Utetheisa*
548 *ornatrix* L. (Lepidoptera: Arctiidae), and extrafloral nectary visiting ants. *Neotrop.*
549 *Entomol.* 42, 223–229. <https://doi.org/10.1007/s13744-013-0114-8>
- 550 Frederickson, M.E., 2005. Ant species confer different partner benefits on two neotropical
551 myrmecophytes. *Oecologia* 2005 1433 143, 387–395. [https://doi.org/10.1007/S00442-](https://doi.org/10.1007/S00442-004-1817-7)
552 [004-1817-7](https://doi.org/10.1007/S00442-004-1817-7)
- 553 Fuente, M.A.S., Marquis, R.J., 1999. The role of ant-tended extrafloral nectaries in the
554 protection and benefit of a Neotropical rainforest tree. *Oecologia* 118, 192–202.
555 <https://doi.org/10.1007/s004420050718>
- 556 Genrich, C.M., Mello, M.A.R., Silveira, F.A.O., Bronstein, J.L., Paglia, A.P., 2017. Duality
557 of interaction outcomes in a plant–frugivore multilayer network. *Oikos* 126, 361–368.
558 <https://doi.org/10.1111/OIK.03825>
- 559 Goheen, J.R., Palmer, T.M., 2010. Defensive plant-ants stabilize megaherbivore-driven
560 landscape change in an African savanna. *Curr. Biol.* 20, 1768–1772.
561 <https://doi.org/10.1016/J.CUB.2010.08.015>
- 562 Guimarães, P.R., 2020. The structure of ecological networks across levels of organization.
563 *Annu. Rev. Ecol. Evol. Syst.* 51, 433–460. [https://doi.org/10.1146/annurev-ecolsys-](https://doi.org/10.1146/annurev-ecolsys-012220-120819)
564 [012220-120819](https://doi.org/10.1146/annurev-ecolsys-012220-120819)
- 565 Guimarães, P.R., Raimundo, R.L.G., Bottcher, C., Silva, R.R., Trigo, J.R., 2006. Extrafloral
566 nectaries as a deterrent mechanism against seed predators in the chemically protected
567 weed *Crotalaria pallida* (Leguminosae). *Austral Ecol.* 31, 776–782.
568 <https://doi.org/10.1111/j.1442-9993.2006.01639.x>
- 569 Jordano, P., 2016. Sampling networks of ecological interactions. *Funct. Ecol.* 30, 1883–1893.
570 <https://doi.org/10.1111/1365-2435.12763/SUPPINFO>
- 571 Kenne, M., Schatz, B., Durand, J.L., Dejean, A., 2000. Hunting strategy of a generalist ant

- 572 species proposed as a biological control agent against termites. *Entomol. Exp. Appl.* 94,
573 31–40. <https://doi.org/10.1046/j.1570-7458.2000.00601.x>
- 574 Kiziridis, D.A., Boddy, L., Eastwood, D.C., Yuan, C., Fowler, M.S., 2020. Incorporating
575 alternative interaction modes, forbidden links and trait-based mechanisms increases the
576 minimum trait dimensionality of ecological networks. *Methods Ecol. Evol.* 11, 1663–
577 1672. <https://doi.org/10.1111/2041-210X.13493>
- 578 Koch, E.B.A., Camarota, F., Vasconcelos, H.L., 2016. Plant ontogeny as a conditionality
579 factor in the protective effect of ants on a neotropical tree. *Biotropica* 48, 198–205.
580 <https://doi.org/10.1111/btp.12264>
- 581 Koch, E.B.A., Dáttilo, W., Camarota, F., Vasconcelos, H.L., 2018. From species to
582 individuals: does the variation in ant–plant networks scale result in structural and
583 functional changes? *Popul. Ecol.* 60, 309–318. <https://doi.org/10.1007/S10144-018-0634-5/FIGURES/5>
- 584
- 585 Koptur, S., 1984. Experimental evidence for defense of *Inga* (Mimosoideae) saplings by ants.
586 *Ecology* 65, 1787–1793. <https://doi.org/10.2307/1937775>
- 587 Lach, L., Hoffmann, B.D., 2011. Are invasive ants better plant-defense mutualists? A
588 comparison of foliage patrolling and herbivory in sites with invasive yellow crazy ants
589 and native weaver ants. *Oikos* 120, 9–16. <https://doi.org/10.1111/J.1600-0706.2010.18803.X>
- 590
- 591 Lange, D., Calixto, E.S., Rosa, B.B., Sales, T.A., Del-Claro, K., 2019. Natural history and
592 ecology of foraging of the *Camponotus crassus* Mayr, 1862 (Hymenoptera: Formicidae).
593 *J. Nat. Hist.* 53, 1737–1749. <https://doi.org/10.1080/00222933.2019.1660430>
- 594 Lange, D., Dáttilo, W., Del-Claro, K., 2013. Influence of extrafloral nectary phenology on
595 ant-plant mutualistic networks in a neotropical savanna. *Ecol. Entomol.* 38, 463–469.
596 <https://doi.org/10.1111/een.12036>
- 597 Leal, I.R., Fischer, E., Kost, C., Tabarelli, M., Wirth, R., 2006. Ant protection against
598 herbivores and nectar thieves in *Passiflora coccinea* flowers. *Ecoscience* 13, 431–438.
599 [https://doi.org/10.2980/1195-6860\(2006\)13\[431:APAHAN\]2.0.CO;2](https://doi.org/10.2980/1195-6860(2006)13[431:APAHAN]2.0.CO;2)
- 600 Martín González, A.M., Dalsgaard, B., Olesen, J.M., 2010. Centrality measures and the
601 importance of generalist species in pollination networks. *Ecol. Complex.* 7, 36–43.
602 <https://doi.org/10.1016/j.ecocom.2009.03.008>
- 603 Melati, B.G., Leal, L.C., 2018. Aggressive bodyguards are not always the best: Preferential
604 interaction with more aggressive ant species reduces reproductive success of plant
605 bearing extrafloral nectaries. *PLoS One* 13, 1–13.

- 606 <https://doi.org/10.1371/journal.pone.0199764>
- 607 Mello, M.A.R., Rodrigues, F.A., Costa, L. da F., Kissling, W.D., Şekercioğlu, Ç.H.,
608 Marquitti, F.M.D., Kalko, E.K.V., 2015. Keystone species in seed dispersal networks are
609 mainly determined by dietary specialization. *Oikos* 124, 1031–1039.
610 <https://doi.org/10.1111/oik.01613>
- 611 Monique, K., de Souza, G.R., Calixto, E.S., Silva, E.A., 2022. Temporal variation in the
612 effect of ants on the fitness of myrmecophilic plants: seasonal effect surpasses periodic
613 benefits. *Sci. Nat.* 109, 1–9. <https://doi.org/10.1007/s00114-022-01805-w>
- 614 Morand, S., Chaisiri, K., Kritiyakan, A., Kumlert, R., 2020. Disease ecology of rickettsial
615 species: A data science approach. *Trop. Med. Infect. Dis.* 5, 64.
616 <https://doi.org/10.3390/tropicalmed5020064>
- 617 Ness, J.H., 2006. A mutualism's indirect costs: The most aggressive plant bodyguards also
618 deter pollinators. *Oikos* 113, 506–514. <https://doi.org/10.1111/j.2006.0030-1299.14143.x>
- 619 Nogueira, A., Guimarães, E., Machado, S., Lohmann, L., 2012. Do extrafloral nectaries
620 present a defensive role against herbivores in two species of the family Bignoniaceae in a
621 Neotropical savannas? *Plant Ecol.* 213, 289–301. [https://doi.org/10.1007/s11258-011-](https://doi.org/10.1007/s11258-011-9974-3)
622 [9974-3](https://doi.org/10.1007/s11258-011-9974-3)
- 623 Nogueira, A., Rey, P.J., Alcántara, J.M., Feitosa, R.M., Lohmann, L.G., 2015. Geographic
624 mosaic of plant evolution: Extrafloral nectary variation mediated by ant and herbivore
625 assemblages. *PLoS One* 10. <https://doi.org/10.1371/journal.pone.0123806>
- 626 Olesen, J.M., Bascompte, J., Dupont, Y.L., Jordano, P., 2007. The modularity of pollination
627 networks. *Proc. Natl. Acad. Sci.* 104, 19891–19896.
628 <https://doi.org/10.1073/pnas.0706375104>
- 629 Oliveira, F.M.P., Câmara, T., Durval, J.I.F., Oliveira, C.L.S., Arnan, X., Andersen, A.N.,
630 Ribeiro, E.M.S., Leal, I.R., 2021. Plant protection services mediated by extrafloral
631 nectaries decline with aridity but are not influenced by chronic anthropogenic
632 disturbance in Brazilian Caatinga. *J. Ecol.* 109, 260–272. [https://doi.org/10.1111/1365-](https://doi.org/10.1111/1365-2745.13469)
633 [2745.13469](https://doi.org/10.1111/1365-2745.13469)
- 634 Oliveira, P.S., 1997. The ecological function of extrafloral nectaries: herbivore deterrence by
635 visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Funct.*
636 *Ecol.* 11, 323–330. <https://doi.org/10.1046/j.1365-2435.1997.00087.x>
- 637 Oliveira, P.S., Oliveira-Filho, A.T., Cintra, R., 1987. Ant foraging on ant-inhabited *Triplaris*
638 (polygonaceae) in western Brazil: A field experiment using live termite-baits. *J. Trop.*
639 *Ecol.* 3, 193–200. <https://doi.org/10.1017/S0266467400002066>

- 640 Oliveira, P. S., Silva, A.F., Martins, A.B., 1987. Ant foraging on extrafloral nectaries of
641 *Qualea grandiflora* (Vochysiaceae) in cerrado vegetation: ants as potential antiherbivore
642 agents. *Oecologia* 74, 228–230. <https://doi.org/10.1007/BF00379363>
- 643 Pacelhe, F.T., Costa, F. V., Neves, F.S., Bronstein, J., Mello, M.A.R., 2019. Nectar quality
644 affects ant aggressiveness and biotic defense provided to plants. *Biotropica* 51, 196–204.
645 <https://doi.org/10.1111/btp.12625>
- 646 Palacio, R.D., Valderrama-Ardila, C., Kattan, G.H., 2016. Generalist species have a central
647 role in a highly diverse plant–frugivore network. *Biotropica* 48, 349–355.
648 <https://doi.org/10.1111/BTP.12290>
- 649 Philpott, S.M., Perfecto, I., Vandermeer, J., 2008. Effects of predatory ants on lower trophic
650 levels across a gradient of coffee management complexity. *J. Anim. Ecol.* 77, 505–511.
651 <https://doi.org/10.1111/j.1365-2656.2008.01358.x>
- 652 Poulin, R., Krasnov, B.R., Pilosof, S., Thielges, D.W., 2013. Phylogeny determines the role
653 of helminth parasites in intertidal food webs. *J. Anim. Ecol.* 82, 1265–1275.
654 <https://doi.org/10.1111/1365-2656.12101>
- 655 Pringle, E.G., Novo, A., Ableson, I., Barbehenn, R. V., Vannette, R.L., 2014. Plant-derived
656 differences in the composition of aphid honeydew and their effects on colonies of
657 aphid-tending ants. *Ecol. Evol.* 4, 4065–4079. <https://doi.org/10.1002/ece3.1277>
- 658 Raupp, P.P., Gonçalves, R.V., Calixto, E.S., Anjos, D. V., 2020. Contrasting effects of
659 herbivore damage type on extrafloral nectar production and ant attendance. *Acta*
660 *Oecologica* 108. <https://doi.org/10.1016/j.actao.2020.103638>
- 661 Ribeiro, L.F., Solar, R.R.C., Muscardi, D.C., Schoereder, J.H., Andersen, A.N., 2018.
662 Extrafloral nectar as a driver of arboreal ant communities at the site-scale in Brazilian
663 savanna. *Austral Ecol.* 43, 672–680. <https://doi.org/10.1111/aec.12612>
- 664 Robbins, R.K., 1991. Cost and evolution of a facultative mutualism between ants and lycaenid
665 larvae (Lepidoptera). *Oikos* 62, 363–369. <https://doi.org/10.2307/3545502>
- 666 Sazima, C., Guimarães, P.R., dos Reis, S.F., Sazima, I., 2010. What makes a species central in
667 a cleaning mutualism network? *Oikos* 119, 1319–1325. <https://doi.org/10.1111/j.1600-0706.2009.18222.x>
- 669 Schatz, B., Lachaud, J.-P., Beugnon, G., 1997. Graded recruitment and hunting strategies
670 linked to prey weight and size in the ponerine ant *Ectatomma ruidum*. *Behav. Ecol.*
671 *Sociobiol.* 40, 337–349. <https://doi.org/10.1007/s002650050350>
- 672 Schmidt, M., Dejean, A., 2018. A dolichoderine ant that constructs traps to ambush prey
673 collectively: Convergent evolution with a myrmicine genus. *Biol. J. Linn. Soc.* 124, 41–

- 674 46. <https://doi.org/10.1093/biolinnean/bly028>
- 675 Silva, E.A., Anjos, D., Bächtold, A., Lange, D., Maruyama, P.K., Del-Claro, K., Mody, K.,
676 2020. To what extent is clearcutting vegetation detrimental to the interactions between
677 ants and Bignoniaceae in a Brazilian savanna? *J. Insect Conserv.* 24, 103–114.
678 <https://doi.org/10.1007/s10841-020-00216-4>
- 679 Tillberg, C. V., 2004. Friend or foe? A behavioral and stable isotopic investigation of an ant-
680 plant symbiosis. *Oecologia* 140, 506–515. [https://doi.org/10.1007/S00442-004-1601-](https://doi.org/10.1007/S00442-004-1601-8/TABLES/3)
681 [8/TABLES/3](https://doi.org/10.1007/S00442-004-1601-8/TABLES/3)
- 682 Trøjelsgaard, K., Olesen, J.M., 2016. Ecological networks in motion: micro- and macroscopic
683 variability across scales. *Funct. Ecol.* 30, 1926–1935. [https://doi.org/10.1111/1365-](https://doi.org/10.1111/1365-2435.12710)
684 [2435.12710](https://doi.org/10.1111/1365-2435.12710)
- 685 Vantaux, A., Dejean, A., Dor, A., Orivel, J., 2007. Parasitism versus mutualism in the ant-
686 garden parabiosis between *Camponotus femoratus* and *Crematogaster levior*. *Insectes*
687 *Soc.* 54, 95–99. <https://doi.org/10.1007/s00040-007-0914-0>
- 688 Vidal, M.C., Sendoya, S.F., Oliveira, P.S., 2016. Mutualism exploitation: Predatory
689 drosophilid larvae sugar-trap ants and jeopardize facultative ant-plant mutualism.
690 *Ecology* 97, 1650–1657. <https://doi.org/10.1002/ecy.1441>

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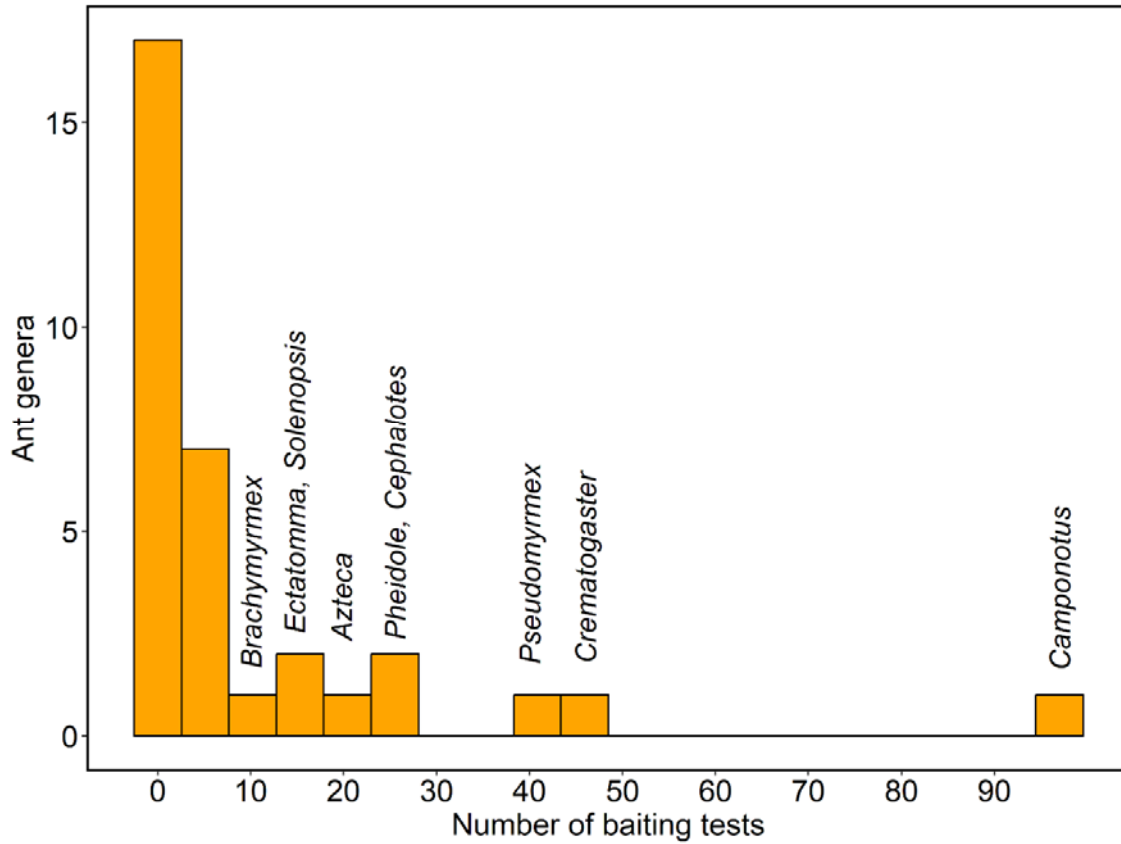
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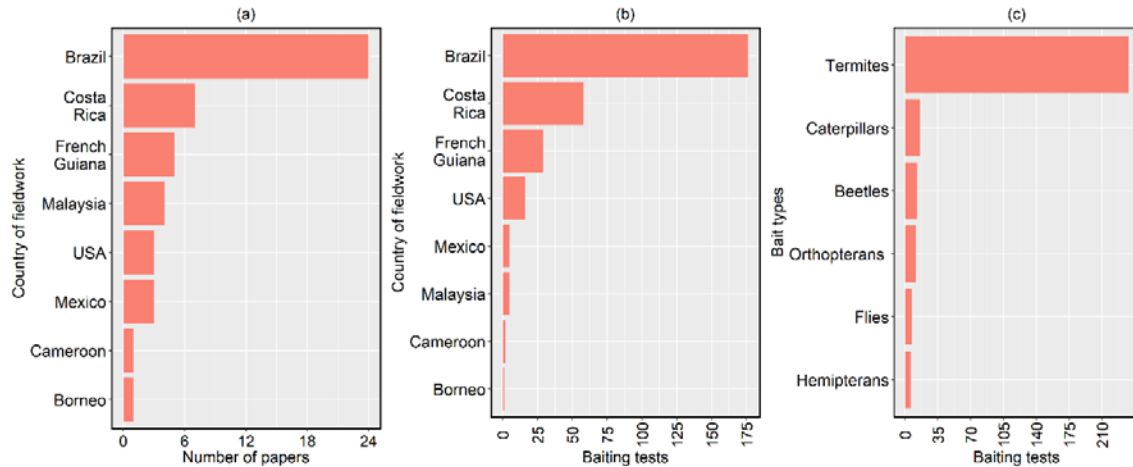
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706 **Supplementary material 1.** Histogram showing the frequency each ant studied in baiting
707 studies. The first two columns refer to several other genera of ants. Data were retrieved from
708 56 published papers.
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723 **Supplementary material 2.** Overview of some characteristics of baiting studies that
724 investigated ant aggressiveness. (a) Number of papers conducted in each country; (b) number
725 of times ants were subjected to baiting tests in each country; (c) types of baits used in baiting
726 tests. These data are not followed by statistical tests.



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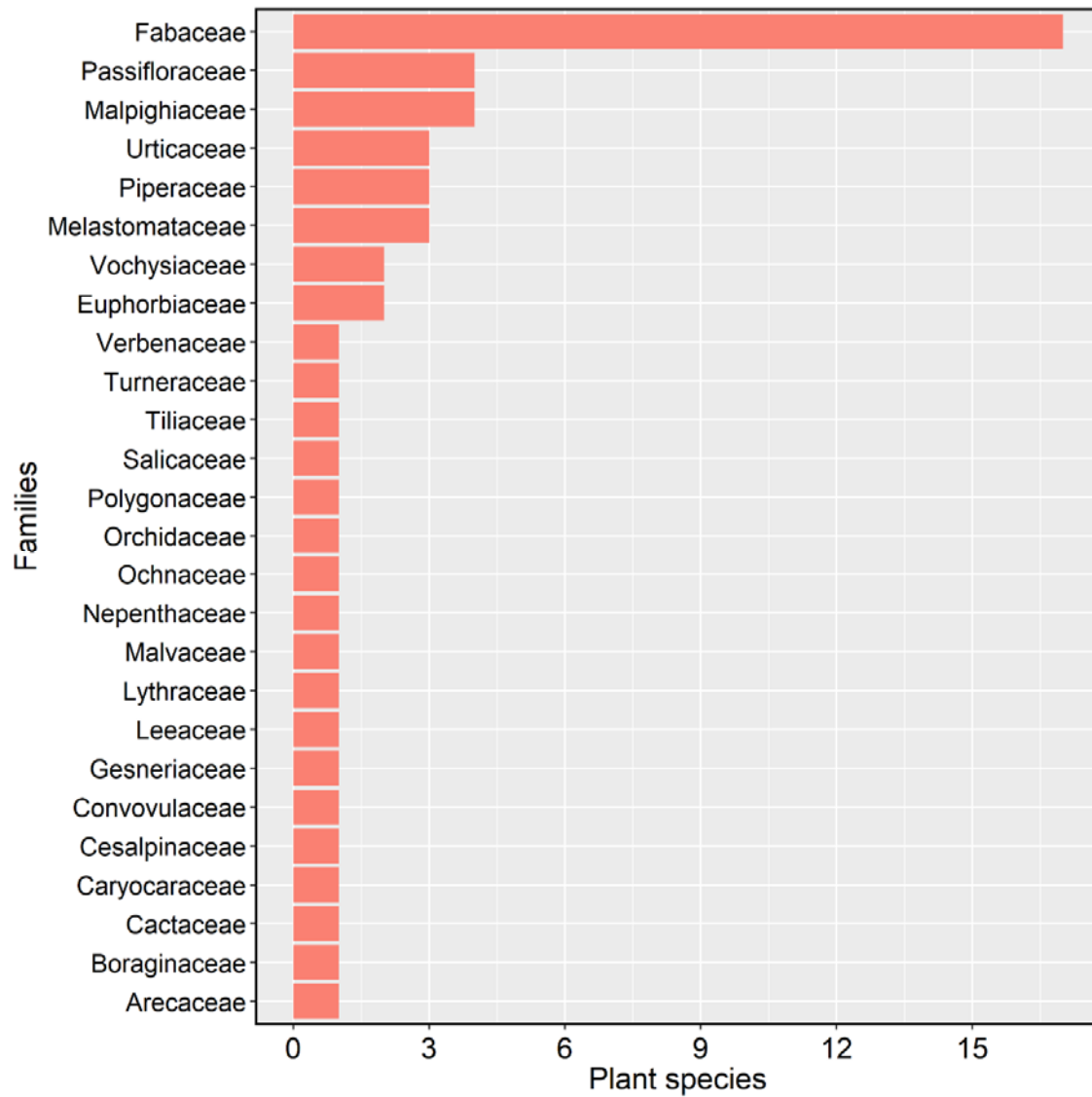
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747 **Supplementary material 3.** Frequency of species in each plant family, that was used in
748 baiting tests.
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759 **Supplementary material 4.** Plant species according to functional groups. EFN – plants with
760 extrafloral nectaries.

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| Plant species | Plant type |
|------------------------------------|--------------|
| <i>Banisteriopsis campestris</i> | EFN |
| <i>Banisteriopsis malifolia</i> | EFN |
| <i>Bionia coriacea</i> | EFN |
| <i>Caryocar brasiliense</i> | EFN |
| <i>Cassia fasciculata</i> | EFN |
| <i>Cassia javanica</i> | EFN |
| <i>Chamaecrista desvauxi</i> | EFN |
| <i>Chamaecrista mucronata</i> | EFN |
| <i>Codonanthe calcarata</i> | EFN |
| <i>Crotalaria micans</i> | EFN |
| <i>Crotalaria pallida</i> | EFN |
| <i>Epidendrum denticulatum</i> | EFN |
| <i>Ferocactus wislizeni</i> | EFN |
| <i>Heteropterys umbelata</i> | EFN |
| <i>Hibiscus pemambucensis</i> | EFN |
| <i>Inga densiflora</i> | EFN |
| <i>Inga marginata</i> | EFN |
| <i>Inga punctata</i> | EFN |
| <i>Ipomoea pandurata</i> | EFN |
| <i>Lafoensia pacari</i> | EFN |
| <i>Leea aculeata</i> | EFN |
| <i>Ouratea spectabilis</i> | EFN |
| <i>Passiflora auriculata</i> | EFN |
| <i>Passiflora biflora</i> | EFN |
| <i>Passiflora coccinea</i> | EFN |
| <i>Passiflora oerstedii</i> | EFN |
| <i>Peixotoa tomentosa</i> | EFN |
| <i>Pityrocarpa moniliformis</i> | EFN |
| <i>Qualea grandiflora</i> | EFN |
| <i>Qualea multiflora</i> | EFN |
| <i>Senna pendula</i> | EFN |
| <i>Senna remiformis</i> | EFN |
| <i>Stachytarpheta glabra</i> | EFN |
| <i>Stryphnodendron polyphyllum</i> | EFN |
| <i>Triumfetta semitriloba</i> | EFN |
| <i>Turnera subulata</i> | EFN |
| <i>Vachellia constricta</i> | EFN |
| <i>Acacia collinsii</i> | myrmecophyte |
| <i>Acacia cornigera</i> | myrmecophyte |
| <i>Acacia hindsii</i> | myrmecophyte |
| <i>Cecropia obtusa</i> | myrmecophyte |

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|------------------------------------|--------------|
| <i>Cecropia obtusifolia</i> | myrmecophyte |
| <i>Cecropia pachystachya</i> | myrmecophyte |
| <i>Cordia alliodora</i> | myrmecophyte |
| <i>Korthalsia furtadoana</i> | myrmecophyte |
| <i>Macaranga banca</i> | myrmecophyte |
| <i>Macaranga puncticulata</i> | myrmecophyte |
| <i>Maieta guianensis</i> | myrmecophyte |
| <i>Nepenthes bicalcarata</i> | myrmecophyte |
| <i>Piper fimbriulatum</i> | myrmecophyte |
| <i>Piper sagittifolium</i> | myrmecophyte |
| <i>Piper</i> sp. | myrmecophyte |
| <i>Tetrathylacium macrophyllum</i> | myrmecophyte |
| <i>Tococa formicaria</i> | myrmecophyte |
| <i>Tococa guianensis</i> | myrmecophyte |
| <i>Triplaris surinamensis</i> | myrmecophyte |

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