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1 Biological interactions involving the myrmecophyte *Hirtella physophora* and its associates
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11

12 Running head : A myrmecophyte and its associates

13

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17

18 Keywords: ant-plant associations, cleptobiosis, fungi, myrmecotrophy, reduviids, wasps

19

20 In ecological communities, several species interact with one another to regulate their
21 abundance. For example, mutualisms benefit all species involved, commensalism benefits one
22 species but not the other, competition (for a resource) lowers the fitness of all species
23 involved, whereas for predation, herbivory and parasitism one species is negatively affected
24 by the other. We present the biological interactions between the myrmecophyte *Hirtella*
25 *physophora* (i.e., a plant sheltering ants in hollow structures) and other organisms. Two
26 *Allomerus* ants build gallery-shaped traps on this plant using hairs from its stems and the
27 mycelium of an Ascomycota fungus to capture prey. They also use their refuse to provide
28 their host plant and the fungi with nutrients (myrmecotrophy). Social wasps build their nests
29 under the *Hirtella* leaves to benefit from the protection provided by *Allomerus* against army
30 ants. Several insects can rob parts of *Allomerus* prey (cleptobiosis), but can be captured in
31 turn. Finally, the reduviid *Zelus annulosus*, which foils attacks by *Allomerus* due to a sticky
32 substance that covers its egg masses and coats its legs, mostly attacks prey in groups and then
33 shares them. Their populations are regulated by parasitoid wasps. Consequently, almost all
34 possible biological interactions were noted in this study.

35

36 ADDITIONAL KEYWORDS: ant-plant associations, cleptobiosis, fungi, myrmecotrophy,
37 reduviids, wasps

38

39

INTRODUCTION

40
41
42 The species in an ecosystem, which first need to be adapted to the abiotic conditions and their
43 changes over time, interact with each other. These interactions, whether short- or long-term,
44 have outcomes which are positive, negative or neutral (“+, -, 0”). Antagonistic interactions,
45 negative for both parties (-, -), such as competition, have frequently been included among the
46 main driving forces behind evolution as in Darwin’s (1858) “struggle for life” and Van
47 Valen’s (1973) “Red Queen hypothesis” (see also Boero, 2015; Strotz *et al.*, 2018).
48 Exploitative interactions or enemy-victim interactions (+, -), which include predation,
49 cleptobiosis, parasitism, parasitoidism and herbivory, can be triggered by semiochemicals
50 such as allelopathy and herbivore-induced plant volatiles. Mutualisms (i.e., reciprocal benefits
51 for interacting species; +, +), which can be obligate or facultative as in biotic pollination, have
52 been interpreted as central to the diversification of life (Thompson, 2006; Leigh, 2010;
53 Aguilée *et al.*, 2018). They have also been noted as promoting species persistence, ecological
54 diversity, resistance to invaders and climatic changes, and their disappearance can lead to the
55 collapse of an ecosystem (Qian & Akçay, 2020; Morales-Linares *et al.*, 2021a,b; Dejean *et*
56 *al.*, 2022a). Furthermore, the relationships where one partner is unaffected and the other is
57 positively affected (commensalism: +, 0) or disadvantaged (amensalism 0, +), relatively
58 frequent, enhance community stability (Mougi & Kondoh, 2012; Mougi, 2016). Finally,
59 neutralism (0, 0) is where two species interact without affecting each other (Matthews &
60 Whittaker, 2014).

61 Biological relationships are context dependent, illustrating that changing the strengths
62 of the interactions permits the passage from one situation to another because the signs “+, -,
63 0” are related to these strengths (e.g., the “interaction compass”; Holland & DeAngelis,

64 2009). Thus, a mutualistic interaction could become commensalistic depending on the
65 ecological circumstances (Mougi, 2016).

66 Most published studies dealing with interspecific relationships are limited to two or
67 three interacting species, whereas the network approach provides quantitative information on
68 the interactions between numerous partners (Dáttilo *et al.*, 2013; Dejean *et al.*, 2018; Delmas
69 *et al.*, 2019). Another approach consists of the compilation of “classic” experimental studies
70 dealing with several interacting organisms that provides information on how a focal species is
71 adapted to its environment and how several other species interact with it. This is what we aim
72 to show here. We focus on the myrmecophyte, or plant sheltering a limited number of ants in
73 hollow structures called domatia, *Hirtella physophora* (Chrysobalanaceae). We explore its
74 obligatory association with the ant species *Allomerus decemarticulatus* and *A. octoarticulatus*,
75 a fungus the ants use to build gallery-shaped traps, and the different insect taxa that interact
76 with the ant-plant mutualism.

77

78 THE GENUS *HIRTELLA*

79

80 Plants of the genus *Hirtella* include 105 Neotropical species, mostly understory shrubs and
81 treelets, although one species has been noted in Zanzibar and another in Madagascar (Prance,
82 2021). Most of them bear extrafloral nectaries (EFNs; i.e., glands rewarding aggressive
83 arthropods, mostly ants, for protection from herbivores; Marazzi, Bronstein & Koptur, 2013)
84 on the undersurface of the leaves. Individuals from the section “Myrmecophila”, which bear
85 domatia at the base of the lamina, mostly shelter ants of the genus *Allomerus*, *Azteca*, or
86 *Solenopsis*. Bisexual flowers are slightly zygomorphic with five sepals and imbricate petals.
87 The monocarpellary ovary is usually inserted at the mouth of the receptacle-tube, the filiform
88 style being longer than the length of the calyx and the receptacle-tube, the same being true for

89 the stamens (Fig. 1A). *Hirtella* spp. are mostly pollinated by lepidopterans, occasionally by
90 stingless bees, whereas *H. rugosa* is pollinated by hummingbirds (Prance, 2021). Also,
91 pollinators are protected from guest ant aggressiveness thanks to a spatial and temporal
92 segregation (Malé *et al.*, 2015). In common with other Chrysobalanaceae, the fruits are
93 drupes that are generally bird-dispersed (Fig. 1B) (Prance, 2021). Finally, *Hirtella* are
94 associated with arboreal mycorrhizae that dominate in the nutrient-poor soils of tropical
95 rainforests where they form a symbiotic association with many higher plants facilitating their
96 nutrient uptake, abiotic stress tolerance and growth *via* nitrogen fixation and the production of
97 stimulating substances (Kottke *et al.*, 2004).

98 *Hirtella physophora*, the focal taxon of this review, forms long-lived understorey
99 treelets whose lifespan (up to ≈ 350 years) is much longer than that of its associated *A.*
100 *decemarticulatus* colonies (up to ≈ 21 years) (Orivel *et al.*, 2011). Because of the position of
101 the EFNs on the undersurface of the leaves, the domatia that result from the curling of the
102 lamina margin on either side of the petiolar insertion bear EFNs on their inner surface,
103 providing food to all members of the incipient colonies until some workers become foragers
104 (Leroy *et al.*, 2008). This permits a “claustral” colony foundation with repeated
105 provisioning by founding queens, which thus have no need to forage for food to raise the
106 first brood and be exposed to predators as would be the case in a semi-claustral foundation
107 where the queen forages for food to feed the first larvae. In typical claustral foundation the
108 queens raise their brood by metabolizing reserves and alary muscles (Hölldobler & Wilson,
109 1990).

110

111 THE SPECIFICITY OF THE *HIRTELLA PHYSOPHORA-ALLOMERUS*112 *DECEMARTICULATUS* ASSOCIATION

113

114 MECHANISMS BEHIND THE SPECIFICITY OF THE ANT-MYRMECOPHYTE ASSOCIATIONS

115

116 It has been shown that the winged queens of arboreal ants prefer certain tree species when
117 building their nests. Indeed, this selection occurs during larval life *via* “pre-imaginal learning”
118 and the first days of adult life *via* “early learning”. Because these types of learning are related
119 to a “sensitive period” after which the influence of the environment ceases, the preference is
120 acquired *via* a process akin to imprinting. Note that these ants can be experimentally
121 conditioned to a non-attractive plant by breeding them in contact with this plant during larval
122 and early learning, as sometimes occurs in nature (Djiéto-Lordon & Dejean, 1999a; see also
123 Gibernau *et al.*, 2007; Rocha *et al.*, 2020; Dejean *et al.*, 2022b).

124 Almost all plant-ants are specifically associated with only one myrmecophyte, as is the
125 case for *Allomerus* spp. and *H. physophora*. Because the future queens are bred inside the
126 domatia (leaf as well as caulinary domatia), they are in contact with the host plant tissues until
127 swarming; in other words, during the whole of their larval life and a large part of their adult
128 life. Thus, host plant selection results from both pre-imaginal and early learning in such a way
129 that mated queens specifically look for their associate myrmecophyte to found their colonies
130 (Djiéto-Lordon & Dejean, 1999b; Inui *et al.*, 2001; Edwards *et al.*, 2006; Grangier *et al.*,
131 2009).

132

133 BEHAVIOUR OF *ALLOMERUS DECEMARTICULATUS* FOUNDING QUEENS IN FRENCH GUIANA

134

135 After swarming, *A. decemarticulatus* queens identify *H. physophora* by responding to
136 chemical and visual cues (but not *A. octoarticulatus*). The queens of both species use the
137 trichomes lining the domatium as construction material to obstruct its entrance prior to
138 claustral foundation (Grangier *et al.*, 2009; Orivel *et al.*, 2017).

139 In the Petit Saut area, two studies showed that 99% and 95.5% of the *H. physophora*
140 surveyed sheltered an *A. decemarticulatus* colony, illustrating the specificity of the
141 association (Solano *et al.*, 2003; Grangier *et al.*, 2009; Dejean *et al.*, 2018). Yet, in other areas
142 of French Guiana, *A. octoarticulatus* occupy 100% of the *H. physophora* (Kaw Mountain),
143 and both species are present at the *Montagne des singes* (Orivel *et al.*, 2017).

144

145 *ALLOMERUS* AS PREDATORS PROVIDING HOST PLANT PROTECTION

146

147 BUILDING A TRAP TO CAPTURE PREY

148

149 *Allomerus decemarticulatus* workers build gallery-shaped traps along the stems of their host
150 *Hirtella* to collectively ambush prey. To build the galleries, workers first cut the trichomes
151 along the sections of plant stems interconnecting the leaves, clearing a path. Temporarily
152 bound together with a compound regurgitated by the ants, these cut trichomes are manipulated
153 to form the vault of the gallery that is supported by adjacent, uncut trichomes functioning as
154 pillars. In this vault, workers punch small holes, with diameters slightly larger than their
155 heads, to allow them to enter and exit. This structure is then reinforced by the mycelium of a
156 sooty mould (i.e., Ascomycota, Chaetothyriale). Fungal growth then starts around the holes
157 and spreads rapidly to the rest of the structure into which the workers incorporate pieces of
158 prey captured by the colony, sooty moulds developing from insect debris and probably a
159 sugary substance provided by the ants (Dejean *et al.*, 2005).

160 When preparing to ambush prey, the workers hide, mandibles wide open, under the holes
161 in the galleries. As soon as an insect walks or lands on the gallery, its leg extremities are
162 seized by the closest workers that then pull it backward into their holes where they keep a
163 tight grasp on it (Fig. 1A). After this very rapid sequence, numerous recruited workers exit the

164 galleries through holes situated around the prey to bite and sting it and grasp free legs,
165 antennae or wings, pulling them into holes. Nestmate recruitment stops when the prey is
166 completely immobilized. Then, the workers holding the extremity of the seized appendages
167 weave in and out of holes further and further away until the prey is progressively stretched
168 against the gallery. Killed prey are slowly transported, the workers moving simultaneously
169 from hole to hole, toward the closest domatium where they carve up the prey. Depending on
170 prey size and the hardness of its cuticle, stretching and dismembering takes from 10 minutes
171 to several hours. Thanks to their trap, workers can capture prey up to 1800 times their weight
172 (Dejean *et al.*, 2005).

173 The width of the extremity of a prey's leg is an important parameter of prey capture
174 success because, when wider than the diameter of the gallery's holes, the workers fail to keep
175 a good hold on the prey. Also, quick-running flies and fast-reacting locusts frequently escape.
176 Many large prey do escape but often shed their trapped legs through autotomy, that, being
177 heavier than a dozen workers, represent a profitable reward for the ants. The capture of
178 swarming, winged termite or ant females also permits the capture of several males attracted
179 by their sex pheromones (Dejean *et al.*, 2005).

180 This predatory behaviour, based on the construction of a gallery-shaped trap, was also
181 noted for *A. octoarticulatus* on *H. physophora*, *A. octoarticulatus* var. *demerarae*, the plant-
182 ant associated with *Cordia nodosa*, as well as the dolichoderine ant *Azteca brevis* whose
183 galleries are pierced with more widely spaced holes (Dejean *et al.*, 2005; Orivel *et al.*, 2017;
184 Schmidt & Dejean, 2018).

185

186

PREDATION BY WORKERS PATROLLING THE HOST PLANT FOLIAGE

187

188 *Allomerus decemarticulatus* and *A. octoarticulatus* workers also patrol their host *Hirtella*
189 foliage, attacking insects that land on it and capturing them. After detection, patrolling
190 workers seize the encountered insects by a leg and pull backwards. In reaction, the potential
191 prey pulls in the opposite direction. The situation changes with the arrival of new workers
192 likely attracted by a pheromone that seize in turn a prey leg and pull backward, thus, the
193 prey is spread-eagled. The workers that arrive later return to their nest to recruit more
194 nestmates that then either participate in stretching the prey, stinging it, or patrolling around
195 the scene (Dejean *et al.*, 2001; Orivel *et al.*, 2017).

196

197

DIRECT AND INDIRECT DEFENCE OF THE HOST PLANT

198

199 The predatory behaviour described above is well adapted to the protection of the host plant
200 even when alien insects fight back and escape (aggression) because the *A. decemarticulatus*
201 workers generally patrol the most vulnerable and valuable parts of the host plant. The old
202 leaves, less patrolled, are nevertheless protected by chemicals. Thus, *H. physophora*
203 individuals benefit from constant defence by *A. decemarticulatus* workers that complement
204 plant traits such as trichomes, leaf toughness and toxic compounds. Furthermore, these
205 workers are responsible for an indirect “inducible” defence of their host plants as, when
206 patrolling their foliage, they rapidly recruit nestmates to foliar wounds, something preventing
207 further attacks by defoliators (Grangier *et al.*, 2008). Indirect “inducible” defence occurs
208 when plants react to herbivory through the production of volatiles attracting the natural
209 enemies of herbivorous insects (Tollrian & Harvell, 1999).

210

211

WHAT ANT SPECIES IS THE MOST BENEFICIAL FOR *HIRTELLA PHYSOPHORA*?

212

213 Surveys conducted in five areas of primary forest in French Guiana show that, although
214 mainly associated with *A. decemarticulatus*, in some areas *H. physophora* can shelter *A.*
215 *octoarticulatus* (i.e., a species different from the cryptic *A. octoarticulatus* var. *demerarae*
216 associated with *Cordia*; see Orivel *et al.*, 2017). These two *Allomerus* species build gallery-
217 shaped traps under the stems of their host plants, but the percentage of host plant stems
218 covered by galleries and the proportion of holes occupied by ambushing workers are lower
219 for *A. octoarticulatus* than for *A. decemarticulatus*-associated plants. The *H. physophora*
220 sheltering *A. octoarticulatus* were less prone to phytophagy and produced significantly more
221 and larger leaves than those inhabited by *A. decemarticulatus*, whereas the volume of the
222 domatia as well as the flowering and fruiting success of the plants were similar. As such, for
223 colonies of similar sizes, the number of *A. octoarticulatus* workers patrolling the leaves was
224 higher than for *A. decemarticulatus*, so that the time needed by *A. octoarticulatus* workers
225 to find an intruder on the host plant foliage was shorter and less variable than for *A.*
226 *decemarticulatus*. (Orivel *et al.*, 2017).

227 Both *Allomerus* species also provide their host plant with nutrients (myrmecotrophy;
228 see details below) and a ^{15}N labelling experiment resulted in a significant increase in $\delta^{15}\text{N}$
229 with no difference in ^{15}N translocation between these two ant species, showing a similar
230 functioning (Orivel *et al.*, 2017).

231 Therefore, although *A. octoarticulatus* provides more benefits to its host plant, this does
232 not result in a competitive advantage. Ant biological traits such as the higher production of
233 winged queens particularly attracted by *H. physophora* volatiles (i.e., not shown for *A.*
234 *octoarticulatus*), the faster development of its colonies and its potential to build large gallery-
235 shaped traps might explain the supremacy of *A. decemarticulatus* (Orivel *et al.*, 2017).

236

237

238

239

CHARACTERISTICS OF THE FUNGUS ASSOCIATED WITH *ALLOMERUS* ANTS

240

241 Due to their ecological characteristics, slow growth rates and the presence of contaminants,
242 the Chaetothyriales associated with structures built by tropical plant-ants are difficult to
243 isolate with standard procedures. An easy-to-use protocol was fine-tuned, based on the use of
244 wet cotton as a selective medium to stimulate the growth of these fungi while minimizing the
245 growth of contaminants. Pure cultures of mycelia were obtained from nuclei that are
246 concentrated either in young hyphae or in the tips of older hyphae (Ruiz-González *et al.*,
247 2013).

248

Allomerus decemarticulatus workers are tasked with manipulating, growing and cleaning
249 the mycelium of the fungus used to reinforce the gallery-shaped trap. A molecular analysis
250 showed the specificity of this fungus as only one monophyletic group of closely related
251 haplotypes of an ascomycete species from the order Chaetothyriales, known as
252 *Trimmatostroma cordae*, was isolated from the galleries. One haplotype dominates and the
253 presence of other haplotypes varies among areas as well as among ant colonies (Ruiz-
254 González *et al.*, 2011, 2019; see Lauth *et al.*, 2013 for 11 microsatellite markers isolated from
255 the two *Allomerus* species). Furthermore, this fungus has also been noted in the gallery-
256 shaped traps of *Azteca brevis* (Mayer & Voglmayer, 2009).

257

258

THE FUNCTIONING OF THE *ALLOMERUS-TRIMMATOSTROMA CORDAE* ASSOCIATION

259

260 In the most studied, obligate food-based ant-fungus mutualism involving *Attina* ants
261 (Myrmicinae; tribe Attini) and Basidiomycota, mostly Agaricaceae, the workers have been
262 shown to eliminate parasitic fungi by way of the antifungal actinomycete bacteria growing on

263 their cuticle (Schultz, 2021). Thus, we looked for such a defence mechanism against parasitic
264 fungi in the non-nutritional *Allomerus*-fungi mutualism.

265 A molecular biology approach based on the sequence of the 16S rRNA or Internal
266 TranscribedSpacer (ITS) regions of microorganisms associated with four plant-ants, including
267 three *Allomerus* species, showed the presence of five bacterial and two fungal phyla in the
268 host myrmecophyte domatia. Among them, Proteobacteria (known as ant symbionts) and
269 Actinobacteria dominated, with the ants shaping the composition of the microbial
270 communities inside the domatia. Because these taxa were found in all domatia sampled, this
271 bacterial community is likely specific (Ruiz-González *et al.*, 2019).

272 *Allomerus* spp. were the only ants associated with Actinobacteria species. The bacteria
273 might play a role in their defence of *T. cordae*, the mutualist fungus they use to build the
274 gallery-shaped traps (Ruiz-González *et al.*, 2019). Certain other bacteria, as yet unidentified,
275 might be involved in their defence of the plant from diseases and substrate degradation.
276 Furthermore, nitrogen-fixing bacteria from the genera *Burkholderia*, *Curtobacterium*,
277 *Enterobacter*, *Escherichia*, *Pantoea*, and *Rhizobium* were noted, just as they have been for
278 *Pseudomyrmex* plant-ants (Eilmus & Heil, 2009).

279

280 HOST PLANT PROVISIONING BY THE ANT AND ITS ASSOCIATED FUNGUS

281

282 GENERAL SITUATION: MYRMECOTROPHY

283

284 In addition to biotic protection from defoliators, pathogens and competing plants, some plant-
285 ants also provide their host myrmecophytes with nutrients, something identified from studies
286 using stable isotopes. This process, called “myrmecotrophy” by Beattie (1989), probably
287 evolved in response to the absence of soil for epiphytes and nutrient-poor soils for tropical

288 phanerophytes. As ant wastes and faeces accumulate in the host plant domatia, humid
289 conditions favour their degradation by fungi and/or bacteria, thus releasing nutrients that are
290 absorbed by the host plant through specific tissues such as the walls of the domatia, rhizomes,
291 protuberances and roots (Chomicki & Renner, 2017; Dejean *et al.*, 2021 and papers cited
292 therein).

293

294 THE CASE OF *HIRTELLA PHYSOPHORA* AND ITS ASSOCIATED ANTS

295

296 To identify if the ant *A. decemarticulatus* and/or the fungus *T. cordae* can provide the
297 myrmecophyte *H. physophora* with nutrients, an experiment was conducted where all three
298 partners received an aqueous honey solution enriched with ^{15}N . Then, we alternatively removed
299 the ants, the fungus or both. For the ants, this solution was placed on small plastic discs to avoid
300 any contact with plant tissues; the solution was deposited on the gallery-shaped trap, and, for
301 the plant, the solution was injected into a domatium (Leroy *et al.*, 2011).

302 Providing each partner with a ^{15}N -enriched honey solution resulted in a significant
303 increase in $\delta^{15}\text{N}$ in all cases, indicating that the solution can be absorbed by them.

304 The removal of the fungus also resulted in a significant increase in $\delta^{15}\text{N}$ in the host plant
305 leaves, demonstrating that the plant can obtain nitrogen directly from ant wastes and faeces.
306 Removing the ants while provisioning the fungus with the ^{15}N -enriched solution led to a
307 significant increase in the $\delta^{15}\text{N}$ in the plant tissue, illustrating that this mutualist fungus also
308 participates in providing *H. physophora* with nutrients. Finally, a non-significant increase in
309 $\delta^{15}\text{N}$ was observed when the ^{15}N -enriched solution was injected directly inside the domatia of
310 plants from which both the ants and the fungus had been removed as compared to the removal
311 of only the ants. Thus, in this tri-partite association, both the ant *A. decemarticulatus* and the

312 fungus *T. cordae* participate in the nutrient provisioning of their host myrmecophyte, *H.*
313 *physophora* (Leroy *et al.*, 2011).

314 There are two ways to explain how nitrogen passes from the mutualist fungus to its host
315 myrmecophyte tissues. First, a histological investigation has shown that the inner surfaces of
316 old domatia, scarified by the ants, were covered with hyphae that penetrate inside the mesophyll
317 of these domatia. This was not the case for young domatia, even when their inner epidermis
318 was scarified by the ants (Leroy *et al.*, 2011). Second, optical microscopy and transmission
319 electron microscopy showed the presence of hyphae inside the cellular compartment and
320 perforating the walls of the parenchyma cells of the host myrmecophyte stem tissue in contact
321 with the gallery-shaped trap. Furthermore, the ^{15}N provisioning of the fungus in the galleries
322 examined *via* nanoscale secondary-ion mass spectrometry isotopic imaging resulted in the
323 significant enrichment of the ^{15}N signature of the host plant leaves. Nanoscale secondary-ion
324 mass spectrometry imaging proved that nitrogen was transferred from the fungus to the host
325 plant stem tissues (Leroy *et al.*, 2017).

326 Therefore, the mutualist fungus is connected to the host myrmecophyte stem tissues and
327 actively transfers nitrogen to these tissues in both old domatia and along the gallery-shaped
328 traps that run along the *H. physophora* stems.

329

330 THE FUNCTIONING OF THE MYRMECOPHYTE-ANT MUTUALISM

331

332 THE EFFICACIOUSNESS OF THE PREDATION

333

334 To look for the long-term impact of predation on both the ants and their host plant, we
335 conducted a 9-month-long field experiment on 41 control and 31 experimental tall *H.*
336 *physophora* trees selected for their similar height, trunk diameter at their base and number of

337 leaves. Also, 25 trees that had lost their associated ant colonies were monitored to verify their
338 fate. The colonies on experimental trees were over-provisioned twice a week with large
339 insects captured at a light trap, whereas the colonies on control trees were left to capture prey
340 naturally (i.e., one to two items per week) (Dejean *et al.*, 2013a).

341 At the end of the survey, the rates of defoliation of experimental and control *H.*
342 *physophora* trees were very similar and much lower than for trees having lost their guest ant
343 colony showing the importance of *A. decemarticulatus* in protecting their host tree from pest
344 insects. Over-provisioned *A. decemarticulatus* colonies produced significantly more winged
345 females than control colonies, whereas the numbers of workers and males produced were not
346 significantly different between experimental and control colonies. Thus, more prey captured
347 enhances colony fitness with a female-biased sex ratio. Finally, experimental trees showed
348 higher $\delta^{15}\text{N}$ values than control ones, confirming myrmecotrophy, and produced significantly
349 more leaves, flowers and fruits than control trees (Dejean *et al.*, 2013a).

350 Thus, both vegetative growth and reproductive investment were enhanced by over-
351 provisioning the ant colonies, the higher production of leaves providing the ants with a greater
352 possibility of lodging in leaf domatia. Therefore, both biotic protection and myrmecotrophy
353 favour host plant growth and fitness as well as the possibility of lodging guest ants (Dejean *et*
354 *al.*, 2013a).

355

356 EVOLUTIONARY CONFLICTS OF INTEREST BETWEEN MYRMECOPHYTES AND THEIR GUEST ANTS:

357

358 In mutualisms, vertical transmission occurs when hosts transmit symbionts to their offspring,
359 whereas in horizontal transmission the association needs to be re-established every
360 generation, but these associations are stable thanks to reciprocal by-product benefits (Leeks,
361 dos Santos & West, 2019; Leimar & Hammerstein, 2010). This is the case for *Allomerus-*

362 *Hirtella* associations where the biotic protection provided by ants is due to their territoriality
363 and/or predatory behaviour, whereas myrmecotrophy is related to their wastes and faeces (i.e.,
364 cases of by-product benefits); on the plant side, the domatia are structural for the
365 myrmecophyte (Orivel *et al.*, 2017). Yet, as with any horizontally transmitted association,
366 each partner tries to obtain the maximum profit at minimum cost, inducing potential
367 parasitism. In addition to by-product benefits, the high frequency of horizontally transmitted
368 mutualisms in nature suggests the existence of mechanisms of regulation.

369 To test if the ants partially castrate *H. physophora*, Malé *et al.* (2012) conducted a 3-
370 week-long field experiment on 23 *H. physophora* individuals with young, budding
371 inflorescences. Using rings of Tanglefoot®, *A. decemarticulatus* workers were excluded from
372 one inflorescence per plant whereas the other inflorescences were accessible. The impact of
373 ant activity on fruit production was assessed by comparing the fruit-to-flower ratios between
374 ant-patrolled and ant-excluded inflorescences. Because the workers cut and chew the buds,
375 the number of buds on ant-patrolled inflorescences decreased greatly; this was not the case for
376 ant-excluded inflorescences. Consequently, ant-patrolled inflorescences produced
377 significantly fewer flowers and fruits than ant-excluded ones, showing that ant activity
378 negatively affects *H. physophora* fitness via partial castration through the elimination of a part
379 of the buds (Malé *et al.*, 2012).

380 To verify if the plant retaliates against the ants, Malé *et al.* (2014) conducted a 17-month-
381 long field experiment on 54 flowering *H. physophora* sheltering well-developed *A.*
382 *decemarticulatus* colonies. Three groups of 18 trees of similar size, number of branches,
383 leaves and inflorescences were selected for the following treatments: (1) the inflorescences
384 were cut off at an early stage (“total castration”); (2) untreated plants were submitted to
385 natural castration (“intermediate castration”); and (3) ants were excluded from the
386 inflorescences using rings of Tanglefoot® (“no castration”). As a result, the non-castrated

387 plants produced fewer leaves than did totally or partially castrated ones. The surface area of
388 the blades of new leaves was not affected by this experiment, but the domatia of new leaves
389 on totally castrated plants were significantly smaller and so contained fewer EFNs than those
390 on pre-treatment plants or the two other treatments (intermediate and non-castrated plants).
391 Thus, these small domatia sheltered fewer ant larvae than “normal” domatia and, sometimes,
392 even no larvae at all, illustrating that the plant is able to retaliate against virulent castrating ant
393 colonies by producing smaller domatia (Malé *et al.*, 2014).

394 Malé *et al.* (2016) sampled 394 *H. physophora* individuals and 377 *A. decemarticulatus*
395 colonies from 14 locations in French Guiana and genotyped them to examine the putative role
396 of population structure in the stability of this mutualism. The dispersal distances were short,
397 those of the plant higher than those of the ant, resulting in the local transmission of the
398 association and, thus, inbred populations. As a result, the plant populations probably have a
399 greater gene flow than do the ants. Thus, castrating colonies that are too virulent face the risk
400 of local extinction due to the lack of *H. physophora* offspring. Consequently, such low spatial
401 distances in these populations favour the stability of this mutualistic relationship by
402 influencing the reciprocal local adaptation of the partners. The local transmission of this ant-
403 myrmecophyte association enables partial alignments of the partners’ interests as the plant
404 retaliation mechanisms are locally adapted to the castration behaviour of their guest ants
405 (Malé *et al.*, 2016).

406 Another case of retaliation in evolutionary conflicts of interest between mutualistic
407 species was reported by Izzo & Vasconcelos (2002) between *H. myrmecophila* and *A.*
408 *octoarticulatus*. This ant species provides protection from defoliators, but it also severs the
409 inflorescences situated on relatively young branches (including those with domatia), acting as
410 a castrating parasite. On the other hand, the plant discards domatia from old leaves that are
411 rarely or never patrolled by the workers (protected through chemicals), and so bear

412 inflorescences that then produce flowers and fruits. The experimental removal of ants resulted
413 in flower and fruit production on both young and old branches, demonstrating the determinant
414 role of guest ants in the destruction of the inflorescences (Izzo & Vasconcelos, 2002).

415

416 THE MYRMECOPHYTE-ANT ASSOCIATION AS A PROTECTIVE NESTING SITE
417 FOR SOME WASP SPECIES *VIS-À-VIS* ARMY ANTS

418

419 GENERAL SITUATION

420

421 Arboreal-nesting social wasps are under pressure from ant predation that is considered the
422 main driving force behind the evolution of wasp nest architecture in the Neotropics (London
423 & Jeanne, 2000). To protect their colonies, non-swarming Polistinae build a nest with a
424 pedicel onto which they deposit substances repellent to ants, whereas most swarming species
425 build an envelope to protect the combs, facilitating nest defence wherein workers actively
426 remove ants (Jeanne, 1996; Grangier & Lester, 2011). Wasps also select sites where ant
427 attacks are unlikely or difficult (e.g., on man-made structures). Yet, these defences are futile
428 against columns of army ants that force wasps to abscond and build a new nest elsewhere
429 (O'Donnell & Jeanne, 1990). Because many arboreal ants are aggressive toward army ants,
430 some wasp species install their nests near the nest of arboreal ants (Herre, Windsor & Foster,
431 1986; Corbara *et al.*, 2009; Souza, Pires & Prezoto, 2013) (Fig. 3A-E). These ants defend the
432 access to their host tree by attacking army ants at its base, causing the columns to deviate
433 (Chadab-Crepet & Rettenmeyer, 1982; Dejean *et al.*, 2014).

434

435 WASPS NESTING UNDER *HIRTELLA PHYSOPHORA* LEAVES

436

437 A field survey conducted by Ruiz-González *et al.* (2010) in the understory of Guianese
438 rainforests showed that the nests of the non-social wasp *Nitela constructor* (Hymenoptera:
439 Crabronidae) were only recorded in association with *H. physophora* sheltering an *A.*
440 *decemarticulatus* colony. These wasp nests are present at a low density as a survey showed
441 the presence of nine *N. constructor* nests on 832 *H. physophora*. The wasp nests are bottle-
442 shaped and anchored on the central vein of the leaf 0.5–2 cm from the domatia occupied by
443 the ants (see Fig. 3F). They are built from the hyphae of the endophytic fungus
444 *Ramichloridium strelitziae* (Ascomycota: Capnodiales) that also develops on some EFNs of
445 the host plant and are assembled together with strands of spider silk collected from the
446 surroundings (Ruiz-González *et al.*, 2010).

447 Also, social wasps can build their nest on *Hirtella*. This was noted in a survey conducted
448 by Dejean *et al.* (2012) in the understory of Guianese rainforests on 545 *H. physophora*
449 associated with an *A. decemarticulatus* colony. Indeed, 87 of them (15.9%) sheltered a social
450 wasp nest belonging to one of nine polistine species (i.e., one nest: *Polistes pacificus*, *Polybia*
451 *affinis* and *Polybia occidentalis*; three nests: *Mischocyttarus synoecus* and *M. oecothrix*; four
452 nests: *M. punctatus* and *Angiopolybia pallens*; 19 nests: *M. adolphi*; and 51 nests for *M.*
453 *lecointei lecointei*) (Fig. 3A-E).

454

455 ENEMIES OF THE ASSOCIATION

456

457 DEFOLIATION DURING THE LIFE CYCLE OF *HIRTELLA PHYSOPHORA*

458

459 Grangier *et al.* (2008) conducted an 8-month-long field experiment based on ant-exclusion
460 using pyrethrum on 30 experimental *H. physophora* individuals; 30 control plants remained
461 untouched. Four developmental leaf stages were distinguished to determine the amount of

462 herbivory. Among those produced during the experiment, we noted stage 1 (i.e., “juvenile
463 leaves”: the youngest, less than 5-cm-long, incompletely formed domatia), and stage 2 (i.e.,
464 “expanding leaves”: fully developed domatia, but immature blade). Those already present
465 corresponded to stage 3 (i.e., “young leaves”: mature but tender, light green, horizontal, 15-
466 25-cm long), and stage 4 (i.e., “old leaves”: non-senescent up to 30-cm long, stiff, dark green,
467 horizontal).

468 Ant-exclusion had no effect on herbivory on stages 1 and 4 leaves, but stages 2 and 3
469 leaves from ant-excluded plants were highly damaged (Grangier *et al.*, 2008; see also Orivel
470 *et al.*, 2011).

471 Defoliation was carried out by locusts, tettigonids, and adult chrysomelid beetles, but
472 rarely caterpillars, and only one lepidopteran (Notodontidae) was noted (Fig. 4). Although
473 leaf-cutting ants are the main defoliators in the Neotropics, they rarely attack *H. physophora*
474 devoid of associate ants likely because their scouts first encounter chemically defended old
475 leaves (situated at the base of the trees), and so do not recruit nestmates.

476 This ant protection from defoliators is enhanced by an indirect “inducible” defence as
477 patrolling workers rapidly recruit nestmates to foliar wounds, preventing further attacks by
478 defoliators. Furthermore, control plants produced more new leaves than ant-excluded plants
479 showing, here too, the importance of ant presence for this myrmecophyte (Grangier *et al.*,
480 2008; see also Dejean *et al.*, 2013a for *H. physophora* highly defoliated after the natural loss
481 of the guest ants).

482

483 CLEPTOBIOSIS BY *MELLIPONA* AND SOCIAL WASPS: WHEN ROBBING PREY CAN TURN FATAL

484

485 Cleptobiosis, or the robbing of food or nesting materials from homo- or heterospecific taxa,
486 can be advantageous when it is possible to procure the coveted items. Prey robbing permits

487 predatory species to save the time and energy necessary to search for and subdue prey while
488 avoiding being injured by prey defences (Breed, Cook & Krasnec, 2012).

489 Because *A. decemarticulatus* workers spend a very long time between capturing an insect
490 and its complete dismantling, predatory flying insects can gain an advantage by stealing such
491 prey. Thus, the ants can lose pieces or entire prey, but may have the opportunity to capture the
492 cleptobionts in turn. Thus, Dejean *et al.* (2012) conducted an experiment consisting of
493 furnishing ca. 2.5-cm-long immature female grasshoppers to *Allomerus* colonies on 158 *H.*
494 *physophora*. In all cases, these grasshoppers were immediately captured by the workers
495 ambushing them in their gallery-shaped traps. The cleptobionts recorded included the workers
496 of a stingless bee species (*Trigona* sp.), six social wasp species (i.e., *Angiopolybia pallens*,
497 *Agelaia myrmecophila*, *Ag. cajennensis*, *Ag. pallipes*, *Ag. fulvofasciata*, and *Ag. testacea*),
498 reduviid heteropterans and flies. Stingless bees and social wasps cut off pieces of prey and
499 robbed them (Fig. 3B), reduviids fed on the prey haemolymph and flies licked the fluids that
500 leaked out from prey spread-eagled by the ants. Because these robbers often placed the
501 extremity of a leg on the edge of the gallery-shaped trap, they were at risk of being caught by
502 *Allomerus* workers.

503 For social wasps, we found a relationship between their size and rate of their capture. Small
504 wasps (i.e., *Ag. myrmecophila* and *An. pallens*) were easily captured whereas the larger ones
505 always escaped. All reduviids were captured, while *Trigona* stingless bees and flies often
506 escaped (Fig. 3C-D). Thus, the balance sheet for the ants was positive *vis-à-vis* the reduviids
507 and four out of the six social wasp species as the total weight of the captured individuals
508 exceeded that of the prey parts robbed. Thus, the capture of a prey item frequently permits the
509 ants to catch other prey behaving as cleptobionts (Dejean *et al.*, 2012).

510

511

512
513 *Hirtella physophora* frequently shelters the egg clusters (protected by a sticky substance),
514 nymphs and adults of the assassin bug *Zelus annulosus* (Reduviidae: Harpactorinae). *Zelus*
515 nymphs and adults seem adapted to living with *Allomerus* ants as they are tolerated walking
516 on the host plant foliage while avoiding the gallery-shaped traps when moving from one leaf
517 to another (Revel *et al.*, 2010). Nymphs never feed on EFNs nor suck the sap from the leaves
518 or stems, something noted for some other Harpactorinae (Weirauch *et al.*, 2021; Gil-Santana
519 & Keller, 2022). Rather, they prey on 48 flying or jumping insect taxa that venture onto their
520 host plant foliage, including winged termites, cockroaches, orthopterans, dipterans and
521 coleopterans. *Allomerus decemarticulatus* workers are only attacked when they attempt to bite
522 a *Zelus* leg that is covered with a sticky substance (Revel *et al.*, 2010). Indeed, in common
523 with most Harpactorinae, this substance, secreted by dermal glands or gathered from certain
524 plants, provides protection from the ants while improving prey capture success (Zhang &
525 Weirauch, 2013).

526 The first instar *Zelus* nymphs usually remain grouped under the leaf where they hatched,
527 whereas the second and third instars devote several hours a day to the upper side of the leaf
528 where they sit motionless with their forelegs pointing upward (“group ambushing”). The
529 nymphs nearly always attack simultaneously and then share the caught prey especially when
530 they are large. Fourth and fifth instar nymphs usually ambush solitarily. For the rest of the
531 day, at night or during rainfall, nymphs of all stages remain grouped under a leaf. The second
532 instar nymphs capture only small, fragile prey, while larger instars attack almost all insects
533 that try to land on their leaf, including items much larger than them (Fig. 4A-C). Cases of
534 cleptobiosis were noted where the nymphs fed on prey captured by *Allomerus* on their trap
535 including flies, social wasps, and stingless bees (Fig. 3E) (Dejean *et al.*, 2013b).

536 An ambushing nymph generally lifts and spreads its forelegs. It detects prey from a
537 distance, approaching it while lifting its forelegs higher, then slamming them down onto the
538 prey, which becomes stuck on the sticky setae and, extending its rostrum, bites the seized prey
539 in an inter-segmental region, paralyzing it (Fig. 4A). The nymph then lifts the prey impaled
540 on the extremity of the rostrum and uses its forelegs to rotate the prey while injecting saliva
541 and sucking out digested matter from different inter-segmental areas. This process is repeated
542 several times until the emptied prey is discarded (Revel *et al.*, 2010; Dejean *et al.*, 2013b).

543 In nature, nymphs are siblings emerging from the same egg cluster. Nevertheless,
544 individuals gathered from different geographical areas to form mixed groups in the laboratory
545 behave exactly as do siblings: they share prey. Presumably, this sharing fosters tolerance
546 among offspring from two or more females that lay eggs on the same plant (Dejean *et al.*,
547 2013b).

548 In insects, egg clutches favour larval/nymphal aggregation which frequently permits early
549 instars to organize group defence (Jolivet, 2008). Group hunting associated with food sharing,
550 known in other Harpactorinae, brings fitness benefits to the participants through increased per
551 capita food intake while minimizing the cost of capture and increasing the rate of successful
552 captures with less risk of injury or being preyed upon in turn (Jackson, Salm & Nelson, 2010;
553 Bailey, Myatt & Wilson, 2013). Although protected by a sticky substance, *Z. annulosus* eggs
554 can be parasitized by tiny parasitoid Hymenoptera (AD, pers. obs), something noted for other
555 *Zelus* species, the parasitoids belonging to the genus *Telenomus* (Scelionidae) (Ralston,
556 1977).

557

558

CONCLUSION

559

560 In this review we compiled information from experimental studies dealing with several
 561 interacting organisms, all associated with only one plant species, the myrmecophyte *Hirtella*
 562 *physophora* (Chrysobalanaceae) showing that most of the interactions recorded are known
 563 (see Table 1).

564

565 Table 1. The different interspecific relationships noted in this review.

Cases noted in this review		
Competition (-,-)	Between two <i>Allomerus</i> species known to be symbiotically associated with <i>H. physophora</i>	This review
Neutralism (0,0)	exists <i>vis-à-vis</i> the host plant when <i>Zelus</i> individuals capture non-herbivorous insects or between <i>Zelus</i> and <i>Allomerus</i> that in general ignore each other	This review
Mutualism (+,+)	In the tripartite association between <i>H. physophora</i> - <i>Allomerus</i> - <i>Trimmatostroma</i> , the plant benefits from nutrients provided by both the ants and the fungus.	This review
Obligate	In the <i>Trimmatostroma</i> -ant association, the ant uses the hyphae of this fungus to reinforce their traps Also, <i>Hirtella</i> are associated with arboreal mycorrhizae that provide nutrients	This review Kottke <i>et al.</i> (2004)
Facultative	Biotic pollination by lepidopterans	This review
Predation (+,-)	By <i>Allomerus</i> ants by patrolling or using a trap for collective ambush <i>Zelus</i> nymphs conduct individual and collective ambush and share prey.	This review
Herbivory (+,-)	Occurs when an <i>H. physophora</i> loses its associated <i>Allomerus</i> colony.	This review
Cleptobiosis (+,-)	Flying insect predators attracted by prey captured by <i>Allomerus</i> on their trap Large <i>Zelus</i> nymphs stealing prey from <i>Allomerus</i> .	This review
Parasitism (+,-)	Castration parasitism was noted when <i>Allomerus</i> workers destroyed <i>H. physophora</i> buds.	This review
Parasitoidism (+,-)	Solitary wasps <i>Telenomus</i> sp. (Scelionidae) that parasitize <i>Zelus</i> eggs.	This review; Ralston (1977)
Commensalism (+,0)	Social and non-social wasps nesting on <i>H. physophora</i> to be protected from other ants, mostly army ants.	This review
Semiochemicals (+,-)	Herbivore-induced plant volatiles were noted when leaf wounds triggered short- and long-range nestmate recruitment by <i>Allomerus</i> workers.	This review
Cases not noted in this review		
Amensalism (0,-)	Yet, a possible example was seen in <i>Allomerus</i> ants selecting Proteobacteria and Actinobacteria (other types of bacteria are likely eliminated)	Ruiz-González <i>et al.</i> (2019)

566

567 Availability of data and materials

568 Not applicable.

569

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780

Figure legends

781

782

783 **Figure 1:** (A) *Hirtella physophora* flowers; the gallery-shaped trap is visible along the stem

784 and at the bases of the leaf pouches. (B) A drupe fruit.

785

786 **Figure 2:** (A) Capture of a locust by *Allomerus decemarticulatus* next to a leaf pouch

787 (domatia). The gallery-shaped trap is clearly visible. (B) An *Agelaia pallipes* wasp robbing

788 pieces of the locust, whereas *Allomerus* ants continued their prey capture with numerous

789 workers mobilized. (C) A small cleptobiont social wasp was captured; at this stage, numerous

790 workers were recruited to stretch the prey against the gallery. (D) Triple benefit for *Allomerus*

791 ants that first captured a green grasshopper and then trapped two small cleptobiont social

792 wasps. (E) A locust was first caught by *Allomerus* ants and transported onto a leaf pouch

793 where it will be cut into pieces. A social wasp attracted by the scene tried to rob pieces of that

794 prey, but was captured in turn. Finally, a *Zelus annulosus*, free from attacks by the small

795 *Allomerus* ants thanks to a sticky substance situated at the base of the legs, is feeding on this

796 wasp (note that it attracted siblings whose legs are visible at the right of the picture; they also

797 stung the locust).

798

799 **Figure 3:** Nests of some social and one non-social wasp species sheltering under the leaves of

800 *Hirtella physophora* where they benefit from protection by *Allomerus* ants against other ants,

801 particularly army ants whose scouts can be captured. (A) *Myschocyttus oecothrix* (Polistinae:

802 *Mischocyttarini*). (B) *M. punctatus* that uses the central vein to build its nest. (C) *M. adolphi*

803 whose nest is built along the central vein. (D) *M. lecointei lecointei*; the petiole, bright,

804 installed on the central vein, was covered by ant-repellent secretions from the Van der

805 Vecht's, a gland. (E) Envelope-covered nest of *Angiopolybia pallens* (Polistinae: Epiponini).

806 (F) The solitary wasp *Nitela constructor* (Crabronidae: Mischophilini) whose females, to feed
807 their larvae, build their nests using spider silk collected from the surroundings and the hyphae
808 of an Ascomycota growing on the extrafloral nectaries of *H. physophora*.

809

810 **Figure 4:** Caterpillars of a Notodontidae eating a *Hirtella physophora* leaf (identification
811 Thierry Bouyer).

812

813 **Figure 5:** (A) A *Zelus annulosus* nymph captured a locust and fed on it by introducing its
814 rostrum into an inter-segmental zone. (B) Two nymphs sharing a prey fly. (C) Large prey can
815 be captured by third instar nymphs; here an adult female grasshopper is shared by four
816 siblings.

817



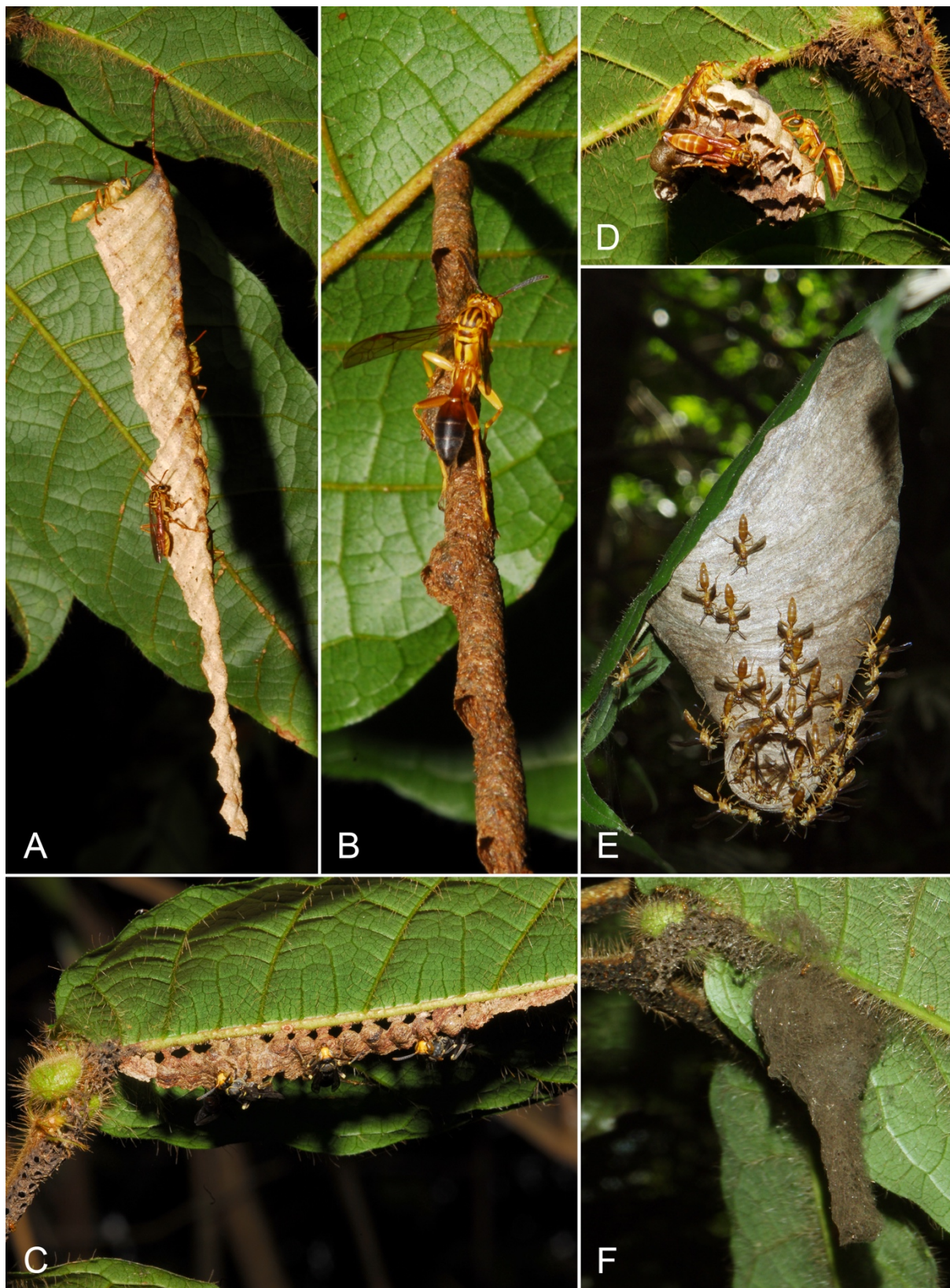
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