1 Is bigger always better? Neither body size nor aggressive behavior are good
2 predictors to measure the degree of specialization of hummingbird interaction
3 networks in rocky outcrops

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Abstract- Interspecific competition can strongly influence community structure and shape niche breadth and overlap. One of the main factors that determine the hummingbird community structure is competition for food. Hummingbirds functional attributes, such as beak length and body mass, influence nectar acquisition in the flowers, shaping foraging niches according to hummingbird dominance and foraging strategy. This study evaluates how the hummingbirds' functional and behavioral attributes are related to plants assemblage in rocky outcrops' habitats. We tested the following hypothesis: H1) Functional traits (beak length and body mass) are related to the richness and frequency of pollen grain morphotypes carried by hummingbirds; H2) Dominant and territorial hummingbirds carry a lower richness and frequency of pollen types when compared to subordinate hummingbirds, and H3) Hummingbird species carry different types of pollen grains. We conducted the study between September 2018 and March 2019 in a *Campo Rupestre* (rocky outcrops) in Southeastern Brazil. Hummingbirds were captured with a trap built based on trapdoors. We recorded their beak size and body masses, marked with commercial bird rings and ink on parts of the body, and then released. Behavioral responses to artificial feeders were collected regarding each visit's time and duration and the outcome of aggressive interactions. The pollen adhered to the body parts was collected and identified in the laboratory. Our results showed that neither body size nor aggressive behaviors influenced pollen richness and frequency in rocky outcrops. Beak length was the most important hummingbirds' attribute that influenced pollen richness, but not pollen frequency. Short-billed hummingbirds carried the greatest richness of pollen grains. Pollen grain richness and frequency were not related to hummingbird body mass or aggressive behavior. The hummingbird-pollen grain interaction network has shown to be generalized in the pollen grain transport. We conclude that hummingbirds' beak length is the central

49 morphological variable to measure pollen grain transport. It has direct implications for
50 the pollination of different plant species.

51 **Keywords:** interaction network, pollen transportation, territorial behavior, Trochilidae.

52

53 **Introduction**

54 Diet specialization, the use of a subset of available resources, is considered
55 one of the major mechanisms permitting species co-occurrence through reduced niche
56 overlap (Chesson 2000; Levine and HilleRisLambers 2009). Diet specialists often have
57 morphological adaptations that allow them to extract resources more efficiently than
58 species with generalist feeding morphologies (Forister et al. 2012).

59 Morphological attributes of hummingbirds, such as beak and tongue lengths,
60 and flowers, such as the corolla size, are directly related to the acquisition of nectar by
61 hummingbirds (Sick 1997, Fogden et al. 2014, Rico-Guevara et al. 2019). When
62 feeding, hummingbirds can carry a certain amount of pollen grains glued to different
63 parts of their bodies (Von Matter et al. 2010, Rico-Guevara et al. 2019). Flowers with a
64 large corolla opening are efficient in depositing pollen grains in the bodies of
65 hummingbirds (Buzato et al. 2000). Body mass is a morphological variable of great
66 importance in hummingbirds' interactions (Feinsinger 1976, Araya-Salas et al. 2018).
67 Short-billed hummingbirds are generally characterized as functionally generalized
68 pollinators, visiting many flowers with short corollas (Von Matter et al. 2010, Fogden et
69 al. 2014, Maruyama et al. 2016). On the other hand, hummingbirds with long and
70 curved beaks are adapted to the exploitation of flowers with long corollas (Collins &
71 Paton 1989, Buzato et al. 2000, Fogden et al. 2014).

72 Body size and body mass have been related to the dominance hierarchy of
73 hummingbirds in different assemblages. They can influence the composition of
74 hummingbird species exploring a given food source, explaining in part the niche
75 specialization observed in this group of birds (Wolf et al. 1976, Feinsinger & Colwell
76 1978, Lopez-Segoviano et al. 2018, Marquez-Luna et al. 2019). Overall, larger
77 hummingbird species tend to dominate, excluding smaller species from high-quality

energy resources (Araujo-Silva and Bessa 2010; Justino et al. 2012; Mendiola-Islas et al. 2016, Marquez-Luna et al. 2019). However, smaller species can also establish and defend foraging territories against larger contenders (Wolf et al. 1976; Antunes 2003). Hummingbirds of medium-large body masses are more efficient than... in expelling smaller species from food resources (Lanna et al. 2017, Lopez -Segoviano et al. 2018). Generally, medium-sized hummingbirds and small-medium straight beaks are territorial species, dominant in defense of the food resource (Feinsinger 1976). Thus, it is expected that medium-large body masses hummingbirds present less richness and less frequency of pollen adhered to their bodies when compared to small body masses species.

Dominance or subordination of hummingbirds can lead them to adopt two strategies for acquiring their food: 1) territorialism or, 2) trapline (Stiles 1975, Feinsinger & Colwell 1978). Hummingbirds that are subordinate and do not defend territories must forage for greater distances, visiting a more significant number of flower species, which can increase the richness and frequency of pollen grains adhered to their bodies. Since dominant hummingbirds defend and have exclusive access to more energetic food resources, they do not need to forage for greater distances, gaining more benefits than subordinate hummingbirds (Lanna et al. 2017). Dominant and more aggressive hummingbirds may have, then, less richness and less frequency of pollen grains adhered to their bodies (Lanna et al. 2017).

Thus, hummingbirds' and plants' morphological attributes can facilitate or hinder the relationship between them when referring to pollination (Sick 1997), which may influence the transport and transfer of pollen grain from one flower to another (Sick 1997, Von Matter et al. 2010).

102 The interaction between plants that provide food to birds (in the form of nectar
103 or fruits) and birds that provide positive services to the plants (in the way of pollen
104 transfer or seed dispersal) has attracted the attention of biologists since Darwin's time
105 (Bascompte and Jordano, 2007, Maruyama et al. 2016, Vizentin-Bugoni et al. 2016).
106 Studies are now using complex interaction network metrics to describe bird-plant
107 mutualistic interactions and are contributing to the development of the conceptual
108 framework of ecological networks (Bascompte and Jordano, 2007; Ings et al., 2009;
109 Heleno et al., 2014; Gu et al., 2015; Rodríguez -Flores et al. 2019, Simmons et al.
110 2019). A pattern is emerging from these studies: the interspecific interactions between
111 plant-hummingbird present an uneven distribution between species (i.e., the degree of
112 dependence of one species to another), with some hummingbirds interacting with
113 several plant species and others interacting with few plant species (Rezende et al. 2007).

114 In the context presented above, in our study, we investigate whether
115 morphological traits (beak length and body mass) and behavioral traits (dominance
116 hierarchy) influence the richness and frequency of pollen grains transported in the
117 hummingbirds in outcrops in southeastern Brazil. We also investigate the influence of
118 these traits in metrics of the plants-hummingbird ecological network. We tested the
119 following hypotheses: H1) The richness and frequency of pollen grains transported in
120 the hummingbird body are influenced by beak length and body mass. We expected
121 short-billed hummingbirds to carry more richness and pollen frequency because they
122 visit more flowers than long-billed hummingbirds. H2) Dominance behavior will
123 influence the richness and frequency of pollen grains in the hummingbird body. We
124 expect that dominant hummingbirds will carry a lower richness and frequency of pollen
125 grain than subordinate hummingbirds because they spend more time feeding and
126 defending a single spot of a preferred resource. H3) The hummingbird-plant interactions

will present specializations with a lower overlap in the composition of morphotypes. We expect hummingbirds' morphological and behavioral characteristics to influence the richness and frequency of pollen carried by the birds with a lower overlap in the pollen types composition.

Methods

Study area

The study was conducted between September 2018 and March 2019 in a *Campo Rupestre* (rocky outcrops) site located in Ouro Preto city, Minas Gerais, Southeastern Brazil (20°22'16.62" S, 43°30'23.43" W). The altitude is 1,397m, and the climate is humid-mesothermal (humid temperate, with dry winters and hot and rainy summers; Álvares et al. 2013).

Hummingbird capture

The study was divided into two stages: 1) capture of hummingbirds, held in September and November 2018, and 2) sampling of hummingbirds' dominance behaviors, which were carried out in January and February 2019, in two periods (morning and afternoon), totaling 195 hours in 30 non-consecutive days. The study summed a sampling effort of 438 hours.

Hummingbirds were captured with a trap built based on trapdoors used in hunting wild birds. It consists of a crate 50-60cm wide and 50cm high. All sides of the box were closed with a fine screen (green net). On one side of 60cm, a door was kept open with a 30cm bamboo rod. A string was attached to the door's support rod, which, when pulled, caused the door to close, trapping the hummingbirds inside.

Hummingbirds were attracted to the trap's interior with artificial drinking fountains filled with a 25% sugar concentration solution (Lanna et al. 2017). The drinkers were offered from 6:00 am to 6:00 pm, two days before data collection. In the

152 following three days, the hummingbirds were captured from 2:00 pm to 6:00 pm to
153 ensure that these individuals had visited natural flowers during the morning. The
154 sampled individuals were marked with commercial bird rings and ink on three parts of
155 the body (chest, tail, and back). This method was preferred to minimize possible loss of
156 pollen due to the manipulation of hummingbirds in ornithological nets traditionally used
157 in this type of work (Borgella et al. 2001, Avalos et al. 2011). The capture of
158 hummingbirds was authorized by the Brazilian Agency of Environment and Renewable
159 Sources (SISBIO license number 51082-1). We measure each bird's beak length using a
160 digital caliper (model ZAAS 1.004®) and body mass, using a Pesola precision scale
161 (LogNature®).

162 The pollen adhered to the body, forehead, throat, and beak of the individuals
163 was removed using small portions of glycerin gelatin stained with fuchsin (Beattie
164 1971) and stored in Eppendorf for subsequent preparation of the slides for
165 identification. Identification of morphotypes was performed considering the
166 ornamentation, shape, and size of the pollen grains (Murcia & Feinsinger 1996, Fonseca
167 et al. 2016).

168

169 **Hummingbird behavior**

170 We documented the interactions among hummingbirds by conducting
171 behavioral observations at a distance using an artificial drinking fountain to attract
172 hummingbirds (following Lanna et al., 2017). The artificial drinker contained three 200
173 ml (Mr. Pet®) artificial flowers and was filled with a 25% sugar solution. The fountain
174 was remained available throughout the day, and the mixture was always replaced every
175 morning (Lanna et al. 2016, Lanna et al. 2017).

176 Dominance behaviors were recorded from 6 am to 10 am and from 2 pm to 4
177 pm on five consecutive days. Birds were observed at a distance of 8m, using a 10x50
178 binocular (Nikon TX Extreme), and the hummingbirds were identified according to
179 Sigrist (2009). For each observation, we recorded the hummingbird species, the time
180 and duration of each visit, and the outcome of aggressive interactions. The aggressive
181 interactions were characterized by a hummingbird chasing (without contact) and/or
182 attacking other hummingbirds (Cotton 1998, Camfield 2006). The winner was identified
183 as the hummingbird that returned to feed or perch nearby after it had successfully
184 defended and/or chased off another hummingbird from the feeder. For behavioral data
185 collection, the focal sampling was used to record all agonistic behavior occurrences
186 (Altmann 1974).

187 **Statistical analysis**

188 We determined pollen grain frequency in the hummingbirds' bodies by
189 summarizing the abundance of pollen morphotypes in each hummingbird species
190 divided by the number of individuals of each hummingbird species. Morphotypes with a
191 frequency lower than 3% were removed because these types could represent possible
192 contamination of pollen grains from a plant species deposited on the flower by any
193 other floral visitor (Talavera et al. 2001).

194 Pearson's correlation analysis was performed to verify whether the
195 hummingbird's beak length was correlated with its body mass. Subsequently,
196 Generalized Linear Models were built, and, in the case of non-significant variables, the
197 models were reduced to the minimum ideal model (Zuur et al. 2009). We created four
198 models, two related to the hummingbird's morphology, and the other two related to the
199 hummingbird behavior. To analyze if the hummingbird's morphology affected the
200 pollen richness and the frequency of pollen morphotypes (as response variables) and the

201 beak length and body mass as explanatory variables (the error distributions were
202 Poisson and Gaussian, respectively). And, to analyze if the hummingbird behavior
203 affected the pollen richness and the frequency of pollen morphotypes (the response
204 variables), the models had the number of victories in agonistic combats and the time the
205 species spent feeding as explanatory variables (the error distribution were negative
206 binomial and Gaussian, respectively).

207 A mutualistic network was built to evaluate the relationship between the
208 frequency of each pollen grain morphotype transported by the hummingbird. We used a
209 quantitative matrix with hummingbirds in the columns and pollen morphotypes in the
210 lines (Blüthgen et al. 2006). The indexes used were: complementary specialization (H2')
211 and modularity (Q). The H2' index is derived from Shannon entropy, which describes
212 the diversity of interactions (distribution of the interactions' weight in the interaction
213 network). It is suggested to be robust for differences in the sampling effort and the size
214 of the analyzed network (Fründ et al. 2016). Values close to 0 indicate high
215 specialization, and values relative to 1 indicate high generalization (Newman 2006,
216 Blüthgen et al. 2007). The Q index shows the most related species within the network,
217 ranging from 0 to 1. Values close to 0 indicate no connection between individuals, and
218 close to 1 indicates individuals high connected in the network (Dormann and Strauss
219 2014). We used the bipartite package to calculate and analyze the interaction network
220 (Dormann et al. 2009) of the software R version 3.1.0 (R Development Core Team
221 2017).

222 **Results**

223 Eight species of hummingbirds were recorded (Table 1) in the study. Of these,
224 only *Phaethornis eurynome* was not observed during behavioral sampling. Pearson's
225 correlation analysis showed that the length of the beak (mm) and body mass (g) of

hummingbirds were not correlated variables. In our study, the highest richness of pollen grain morphotypes was recorded in *Amazilia lactea* ($s = 8$), and the lowest richness was recorded in *Phaethornis pretrei* ($s = 1$) (Table 1). The highest frequency of pollen grain morphotypes was found in *Phaethornis pretrei* ($n = 96.02\%$), and the lowest frequency in *Heliodoxa rubricauda* ($n = 27.02\%$) (Table 1). The hummingbird species with the highest body mass was *Eupetomena macroura* (8.5g), and the lowest was *Chlorostilbon lucidus* (4.0g) (Table 1). The species with the longest beak was *Phaethornis pretrei* (33.5mm), and the shortest beak was *Amazilia lactea* (14.3mm) (Table 1).

Insert Table 1

Forty-seven morphotypes of pollen-grains adhered to the hummingbirds' bodies were identified. With the removal of the morphotypes that had a frequency below 3%, the final richness was 15 pollen grains morphotypes. In this study, the species that obtained the highest number of victories in interspecific and intraspecific agonistic combats was *Heliodoxa rubricauda* ($n = 840$), and the least amount was *Chlorostilbon lucidus* ($n = 1$) (Table 1). The species that spent the longest time feeding was *Chlorostilbon lucidus* (35 sec), and the shortest time was *Eupetomena macroura* (1.3 sec) (Table 1).

The richness of pollen grain morphotypes was inversely related to the length of the hummingbird beak ($F_{1, 6.3218} = 25.755$, $p < 0.004$) (Figure 1; Table 2) and did not vary with hummingbirds' body mass ($F_{1, 0.008} = 0.0287$, $p < 0.873$). The frequency of pollen grain morphotypes did not vary significantly depending on the length of the beak ($F_{1, 828.07} = 0.8126$, $p < 0.409$) and on the body mass of hummingbirds ($F_{1, 521.35} = 0.4560$, $p < 0.536$) (Table 2).

Insert Figure 1 and Table 2

250 Dominance behaviors in the artificial feeder did not vary with the richness and
251 frequency of pollen grain morphotypes found in hummingbirds (Table 3).
252 Hummingbirds' body mass also did not change concerning the number of victories in
253 agonistic interactions on artificial feeders (Table 3).

254 _____Insert Table 3_____

255 The interaction network between hummingbirds and pollen grain morphotypes
256 showed median values of modularity ($Q = 0.533$) (Figure 2) and the complementary
257 specialization index $H_2'(0.675)$ (Figure 3), indicating medium connectivity between
258 hummingbirds and plants and a tendency for generalization of these relations.

259 _____Insert Figures 2 and 3_____

260

261 Discussion

262 Our results showed that neither body size nor aggressive behaviors influenced
263 pollen richness and frequency in rocky outcrops. Beak length was the most crucial
264 hummingbirds' attribute that influenced pollen richness, but not pollen frequency. We
265 expected short-billed hummingbirds to carry a higher richness of pollen morphotypes
266 with higher frequency because they need to visit more flowers than long-billed
267 hummingbirds. We found that the length of the beak of hummingbird species proved to
268 be the most important morphological trait related to the richness of plants visited
269 (measured by the pollen grains richness) and less important to explain the frequency of
270 pollen grains transported. This greater richness of pollen grains is probably the result of
271 an adjustment of the hummingbirds' beak with the flowers they feed on (Sick 1997,
272 Fogden et al. 2014, Rico-Guevara et al. 2019). Due to that adjustment, the interaction
273 network between hummingbirds and pollen grains was moderately specialized,
274 reflecting the importance of hummingbirds in different plants' pollination process.

275 The short-billed hummingbird species in our study (*A. lactea*, *T. glaucopis*,
276 and *C. lucidus*) carried a higher pollen grain richness. These species are considered
277 functional generalists because they can visit a great diversity of native and exotic plants
278 (Maruyama et al. 2016), mainly plants with short corollas (Maruyama et al. 2016).). At
279 the other extreme, the longest, curved-billed hummingbird species (*P. eurynome* and *P.*
280 *petrei*) carried a lower richness of pollen grains when compared to the intermediate-
281 billed species (*A. cirrochloris*, *H. rubricauda*, and *E. macroura*) and to the short-billed
282 (*A. lactea*, *T. glaucopis*, and *C. lucidus*). These two *Phaethornis* hummingbirds are
283 considered specialized in feeding nectar from long tube bromeliad flowers found in the
284 Atlantic forest, restricting access to nectar for short-billed species hummingbirds
285 (Buzato et al. 2000, Maruyama et al. 2016, Sonne et al. 2019). The morphological
286 match and phenological overlap are important factors predicting plant–hummingbird
287 interactions, showing the role of these characteristics in organizing plant–hummingbird
288 communities (Brown & Bowers 1985, Vizentin-Bugoni et al. 2014). Thus, the lowest
289 richness of pollen grains found in *Phaethornis* species may reflect the lack of long,
290 tubular flowers in the study area, remembering that our study was conducted in the
291 *Campo Rupestre* area where more plant species have open and narrow corollas (Giulietti
292 et al. 1997).

293 On the other hand, the frequency of pollen grains was not significantly related
294 to the beak length. Our results indicate that the potential pollinators' effectiveness may
295 be mediated more closely by the abundance of pollinators (hummingbirds) than the
296 trait-matching compatibility. The potential pollinators are the pollinator's total
297 contribution to the plant's fitness, measure, for example, by the number of pollen grains
298 deposited (Schupp et al. 2017, Missagia and Alves 2018). Although hummingbirds'
299 beak was not a good predictor to explain a higher frequency of pollen grains in our

study, the result points to a tendency for long, curved-billed species to carry a higher frequency of pollen grains than short-billed species. However, it is crucial to consider the environment where the present study was carried out. A rocky outcrop is a less complex and more unpredictable habitat; although deemed a megadiverse, it presents less flower diversity than forest habitats (Silveira et al. 2016). Maybe, more complex, flower-diverse habitats, like forests and urban environments, could increase the frequency of pollen carried by short-billed hummingbirds, due mainly to differences in plant (flower) composition (Maglianesi et al. 2014).

The richness and frequency of pollen grain morphotypes were not influenced by the hummingbirds' body masses, as expected. Lighter hummingbirds, such as the Phaethornithinae (*P. eurynome* and *P. pretrei*) and the Trochilinae (*C. lucidus*, *A. lactea*, and *T. glaucopis*), were expected to carry more morphotypes and more pollen grains. Species that are subordinate avoid combats with more dominant species. Therefore, they need to visit more plant species, which would increase the chances of having more pollen of different plant species in their bodies. Thus, hummingbirds' body mass and dominance behavior were not variables that explained the differences in the number of pollen grains transported. In contrast, some authors cite that the behavior and the body mass are the most important variables for structuring the hummingbird assembly in a resource spot (Sick 1997, Forgden et al. 2014, Lanna et al. 2017). More aggressive and dominant species can share the territory with more peaceful and less hostile species (Stiles 2008, Lanna et al. 2017, Lopez-Segoviano et al. 2018). Marques-Luna et al. (2019) found that the dominant species of hummingbirds in the assemblage (*L. clemenciae* and *C. thalassinus*) weigh more than 6 g, representing the largest hummingbird species in the community. The higher rank within the dominance hierarchy was associated with large body size species, which coincides with that

325 reported by different authors (Dearborn 1998; Justino et al. 2012). However, the bigger
326 species are not always the most aggressive and dominant, observed by Martin and
327 Ghalambor (2014) and Márquez-Luna et al. (2019), where smaller species of
328 hummingbirds dominated bigger ones in some of the interactions.

329 The interaction network between hummingbirds and pollen grain morphotypes
330 tended to be generalized, with an overlap of pollen grain morphotypes carried by each
331 hummingbird species. Most of the hummingbirds recorded in the present study
332 belonged to the Emerald clade (*Chlorostilbon*, *Eupetomena*, *Amazilia*, *Thalurania*, and
333 *Aphantochroa*), formed by evolutionarily recent and more generalist hummingbirds.
334 The *Phaetornis* genus belongs to the Hermit clade, formed by evolutionarily older and
335 more specialized hummingbirds (Rodrigues-Flores et al. 2019). Because of the
336 hummingbird species involved, we expected and observed in this study a heterogeneous
337 connectivity distribution, with a tendency to generalize the species interaction in the
338 network, because most hummingbirds visited more plant species and few hummingbirds
339 visited few of the plants.

340 Hummingbirds present a preference in selecting their food resource (Stiles
341 1975, Feinsinger et al. 1979, Maruyama et al. 2014, Lanna et al. 2017). This
342 specialization can be related to the flower morphology (Maglianesi et al. 2014, Fonseca
343 et al. 2016) and to hummingbird morphology (beak length) (Maglianesi et al. 2014).
344 However, it was shown that hummingbirds relaxed their specialized relationships and
345 became less specific in their interactions in non-forested areas (Morrison and
346 Mendenhall, 2020). Our research was conducted in a *Campo Rupestre* area, where trees
347 are rare, reinforcing our result of a lower specialization in our interaction network.
348 Thus, most hummingbirds explored many flowers, ornithophilous or not, while the
349 hermits remained to explore few plant species.

350 We conclude that the hummingbirds' beak's length is the most important
351 variable to explain the richness, specialization, and segregation in the transport of pollen
352 grains by hummingbirds in rocky outcrops. As suggested by Maglianesi et al. (2014),
353 hummingbird species' morphological traits influence ecological specialization patterns.
354 The beak's morphology seems to be more important than body mass in determining the
355 niche partition within the community. In this way, the beak of hummingbirds is of great
356 importance for the pollination process of many species of plants.

357

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363

364 **References**

- 365 Altmann, J. (1974). Observational study of behavior: sampling methods. **Behavior** 49:
366 227–267.
- 367 Álvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M., & Sparovek, G. (2013).
368 Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift** 22:
369 711-728.
- 370 Antunes, A.Z. (2003). Partilha de néctar de *Eucalyptus* spp., territorialidade e hierarquia
371 de dominância em beija-flores (Aves: Trochilidae) no sudeste do Brasil. **Ararajuba**
372 11: 39-44.

- 373 Araujo-Silva, L.E., & Bessa, L. (2010). Territorial behavior and dominance hierarchy of
374 *Anthracothonax nigricollis* Vieillot 1817 (Aves: Trochilidae) on food resources.
375 **Revista Brasileira de Ornitologia** 18: 89-96.
- 376 Araya-Salas, M., Gonzalez-Gomez, P., Wojczulanis-Jakubas, K., Lopez, V., & Wright
377 T.F. (2018). Spatial memory is as important as weapon and body size for territorial
378 ownership in a lekking hummingbird. **Scientific Reports** 8:1–11.
- 379 Avalos, G., Soto, A., & Alfaro, W. (2011). Effect of artificial feeders on pollen loads of
380 the hummingbirds of Cerro de La Muerte, Costa Rica. **J. Trop. Biol.** 60: 65-73.
- 381 Bascompte, J., & Jordano, P. (2007). Plant-animal mutualistic networks: the
382 architecture of biodiversity. **Annual Reviews of Ecology and Systematics** 38: 567-
383 593.
- 384 Beattie, A.J. (1971). A technique for the study of insect-borne pollen. **Pan Pacific**
385 **Entomologist** 47-82.
- 386 Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species
387 interaction networks. *Ecology* 6(1), 9. doi:10.1186/1472-6785-6-9
- 388 Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B., & Blüthgen, N.
389 (2007). Specialization, Constraints, and Conflicting Interests in Mutualistic
390 Networks. **Current Biology** 17: 341–346.
- 391 Borgella Jr, R., Snow, A.A., & Gavin, T.A. (2001). Species richness and pollen loads of
392 hummingbirds using forest fragments in southern Costa Rica. **Biotropica** 33: 90-109.
- 393 Brown, J.H. & Bowers, M.A. (1985) Community organization in hummingbirds:
394 relationship between morphology and ecology. **Auk** 102: 251–669.
- 395 Buzato, S., Sazima, M., & Sazima, I. (2000). Hummingbird-pollinated floras at three
396 Atlantic forest sites. **Biotropica** 32: 824-841.

397 Camfield, A.F. (2006). Resource value affects territorial defense by Broad-tailed and
398 Rufous hummingbirds. **Journal of Field Ornithology** 77: 120-125.

399 Chesson, P. (2000). Mechanisms of maintenance of species diversity. **Annual Review**
400 **of Ecology and Systematics** 31: 343-366.

401 Collins B.G., & Paton D.C. (1989). Consequences of differences in body size, wing
402 length and leg morphology for nectar-feeding birds. **Australian Journal of Ecology**
403 14: 269-289.

404 Cotton, P.A. (1998). Temporal partitioning of a floral resource by territorial
405 hummingbirds. **Ibis** 140: 647-653.

406 Dormann, C.F., & Strauss, R. (2014). A method for detecting modules in quantitative
407 bipartite networks. **Methods in Ecology and Evolution** 5: 90–98.

408 Dormann, C.F., Fruend, J., Bluethgen, N & Gruber, B. (2009). Indices, graphs and null
409 models: analyzing bipartite ecological networks. **The Open Ecology Journal** 2: 7-
410 24.

411 Feinsinger, P. (1976). Organization of a tropical guild of nectarivorous birds.
412 **Ecological Monographs** 46: 257-291.

413 Feinsinger, P., & Colwell, R.K. (1978). Community organization among neotropical
414 nectar-feeding birds. **American Zoologist** 18:779-795.

415 Feinsinger, P., Linhart, Y.B., Swarm, L.A., & Wolfe, J.A. (1979). Aspects of the
416 pollination biology of three *Erythrina* species on Trinidad and Tobago. **Annals of**
417 **the Missouri Botanical Garden** 66: 451-471.

418 Fogden, M. Taylor, M and Sheri L. (2014). Hummingbirds: A life-size guide to every
419 species. Williamson. Harper Collins, New York, New York, USA.: pg. 400. ISBN:
420 978-0-06-228064-0.

421 Fonseca, L.C.N., Rech, A.R. Bergamo, P.J., Esteves, V.G., & Sazima, M. (2016).
422 Heterospecific pollen deposition among plants sharing hummingbird pollinators in
423 the Brazilian Atlantic Forest. **Rodriguésia** 67(2): 335-345.

424 Forister, M.L., Dyer, L.A., Singer, M.S., Stireman III, J.O., & Lill, J.T. (2012).
425 Revisiting the evolution of ecological specialization, with emphasis on insect-plant
426 interactions. **Ecology** 93: 981-991.

427 Fründ, J., McCann, K.S., & Williams, N.M. (2016). Sampling bias is a challenge for
428 quantifying specialization and network structure: lessons from a quantitative niche
429 model. **Oikos** 125: 502–513.

430 Giulietti, A.M., Pirani, J.R., & Harley, R.M. (1997). Espinhaço Range region, eastern
431 Brazil. In: Davis, S.D., Heywood, V.H., Herrera-MacBryde, O., Villa-Lobos, J., &
432 Hamilton, A.C. (Eds.), **Centres of plant diversity: a guide and strategy for their**
433 **conservation**. Information Press, Oxford, v.3, p.397-404.

434 Gu, H., Goodale, E., & Chen, J. (2015). Emerging directions in the study of the ecology
435 and evolution of plant-animal mutualistic networks: a review. **Zoological Research**
436 36: 65-71.

437 Ings, T.C., Montoya, J.M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C.F.,
438 Edwards, F., Figueroa, D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E.,
439 Lewis, H.M., Olesen, J.M., Frank van Veen, F.J., Warren, P.H., & Woodward, G.
440 (2009). Ecological networks: beyond food webs. **Journal of Animal Ecology** 78:
441 253-269.

442 Heleno, R., Garcia, C., Jordano, P., Travaset, A., Gómez, J.M., Blüthgen, N., Memmott,
443 J., Moora, M., Cerdeira, J., Rodríguez-Echeverria, S., Freitas, H., & Olesen, J.M.
444 (2014). Ecological networks: delving into the architecture of biodiversity. **Biology**
445 **Letters** 10: 20131000.

- 446 Justino, D.G., Maruyama, P.K., & Oliveira, P.E. (2012). Floral resource availability and
447 hummingbird territorial behaviour on a Neotropical savanna shrub. **Journal of**
448 **Ornithology** 153: 189-197.
- 449 Lanna, L.L., Azevedo, C.S., Claudino, R.M., Oliveira, R., & Antonini, Y. (2016). Perch
450 Usage by Hummingbirds in a Fragment of Atlantic Forest in Brazil. **The Wilson**
451 **Journal of Ornithology** 128: 453-45.
- 452 Lanna, L.L., Azevedo, C.S., Claudino, R.M., Oliveira, R., & Antonini, Y. (2017).
453 Feeding behavior by hummingbirds (Aves: Trochilidae) in artificial food patches in
454 an Atlantic Forest remnant in southeastern Brazil. **Zoologia** 34. e13228
- 455 Levine, J.M., & HilleRisLambers, J. (2009). The importance of niches for the
456 maintenance of species diversity. **Nature** 461: 254-257.
- 457 Lopez-Segoviano, G., Bribiesca, R., & Arizmendi, M.C. (2018). The role of size and
458 dominance in the feeding behaviour of coexisting hummingbirds. **Ibis** 160: 283–292.
- 459 Marquez -Luna, U., Lura, Corcuera, P., & Valverde, P.L. (2019). Factors affecting the
460 dominance hierarchy dynamics in a hummingbird assemblage. **Current Zoology** 65:
461 261–268.
- 462 Martin, P.R., & Ghalambor, C.K. (2014). When David beats Goliath: the advantage of
463 large size in interspecific aggressive contests declines over evolutionary time. **Plos**
464 **One** 9(9): e108741.
- 465 Maruyama, P.K., Vizentin-Bugoni, J., Oliveira, G.M., Oliveira, P.E., & Dalsgaard, B.
466 (2014). Morphological and Spatio-Temporal Mismatches Shape a Neotropical
467 Savanna Plant-Hummingbird Network. **Biotropica** 46: 740–747.
- 468 Maruyama, P.K., Vizentin-Bugoni, J., Sonne, J., Martín González, A.M., Schleuning,
469 M., Araujo, A. C., & Dalsgaard, B. (2016). The integration of alien plants in

470 mutualistic plant-hummingbird networks across the Americas: the importance of
471 species traits and insularity. **Diversity and Distributions** 22: 672–681.

472 Maglianesi, M.A., Blüthgen, N., Böhning-Gaese, K., & Schleuning, M.
473 (2014). Morphological traits determine specialization and resource use in plant–
474 hummingbird networks in the neotropics. **Ecology** 95: 3325–3334.

475 Mendiola-Islas, V., Lara, C., Corcuera, P., & Valverde, P.L. (2016). Residency in
476 White-eared hummingbirds (*Hylocharis leucotis*) and its effect in territorial contest
477 resolution. **PeerJ** 4: e2588.

478 Missagia, C.C.C., & Alves, M.A.S. (2018). Does beak size predict the pollination
479 performance of hummingbirds at long and tubular flowers? A case study of a
480 Neotropical spiral ginger. **Journal of Zoology** 305: 1-7.

481 Morrison, B.M.L., & Mendenhall, C.D. (2020). Hummingbird-plant interactions are
482 more specialized in forest compared to coffee plantations. **Diversity** 12: 126.

483 Murcia, C & Feinsinger, P. (1996). Interspecific pollen loss by hummingbirds visiting
484 flower mixtures: effects of floral architecture. **Ecology** 77: 550-560.

485 Newman, M.E.J. (2006). Modularity and community structure in networks.
486 **Proceedings of the National Academy of Sciences** 103: 8577–8582.

487 R Core Team. (2017). R: A language and environment for statistical computing. R
488 Foundation for Statistical Computing, Vienna, Austria. URL [https://www.R-](https://www.R-project.org/)
489 [project.org/](https://www.R-project.org/).

490 Rezende, E. L., Jordano, P., & Bascompte, J. (2007). Effects of phenotypic
491 complementarity and phylogeny on the nested structure of mutualistic networks.
492 **Oikos** 116: 1919–1929.

493 Rico-Guevara, A., Rubega, M.A., Hurme, K.J., & Dudley, R. (2019). Shifting
494 paradigms in the mechanics of nectar extraction and hummingbird bill morphology.
495 **Integrative Organismal Biology** 1: 1–15.

496 Rodríguez-Flores, C.I., Ornelas, J.F., Wethington, S., & Arizmendi, M.d.C. (2019). Are
497 hummingbirds generalists or specialists? Using network analysis to explore the
498 mechanisms influencing their interaction with nectar resources. **PLoS ONE** 14:
499 e0211855.

500 Schupp, E.W., Jordano, P., Gómez, J.M. (2017). A general framework for effectiveness
501 concepts in mutualisms. **Ecology Letters** 20: 577-590.

502 Sick, H. (1997). **Ornitologia Brasileira**. 2^a ed. Rio de Janeiro: Ed. Nova Fronteira.

503 Sigrist, T. (2009). **Avifauna Brasileira**, vol. 2. Rio de Janeiro: Ed. Avis Brasilis.

504 Silveira, F.A.O., Negreiros, D., Barbosa, N.P.U., Buisson, E., Carmos, F.F., Carstensen,
505 D.W., Conceição, A.A., Cornelissen, T.G., Echternacht, L., Fernandes, G.W., Garcia,
506 Q.S., Guerra, T.J., Jacobi, C.M., Lemos-Filho, J.P., Le Stradic, S., Morellato, L.P.C.,
507 Neves, F.S., Oliveira, R.S., Schaefer, C.E., Viana, P.L., & Lambers, H. (2016).
508 Ecology and evolution of plant diversity in the endangered campo rupestre: a
509 neglected conservation priority. **Plant Soil** 403: 129-152.

510 Simmons, B.I., Vizentin-Bugoni, J., Maruyama, P.K., Cotton, P.A., Marín-Gómez,
511 O.H., Lara, C., Rosero-Lasprilla, L., Maglianesi, M.A., Ortiz-Pulido, R., Rocca,
512 M.A., Rodrigues, L.C., Tinoco, B.A., Vasconcelos, M.F., Sazima, M., González,
513 A.M.M., Sonne, J., Rahbek, C., Dicks, L.V., Dalsgaard, B., & Sutherland, W.J.
514 (2019). Abundance drives broad patterns of generalization in plant–hummingbird
515 pollination networks. **Oikos** 128: 1287–1295.

516 Sonne, J., Zanata, T.B., González, A.M.M., Torres, N.L.C., Fjeldså, J., Colwell, R.K.,
517 Tinoco, B.A., Rahbek, C., & Dalsgaard, B. (2019). The distributions of

518 morphologically specialized hummingbirds coincide with floral trait matching across
 519 an Andean elevational gradient. **Biotropica** 1–14 DOI: 10.1111/btp.12637

520 Stiles, F.G. (1975). Ecology, flowering phenology, and hummingbird pollination of
 521 some Costa Rican Heliconia species. **Ecology** 56: 285–301.

522 Stiles, F.G. (2008). Ecomorphology and phylogeny of hummingbirds: Divergence and
 523 convergence in adaptations to high elevations. **Ornitologia Neotropical**. 19: 511–
 524 519.

525 Talavera, S., Bastida, F., Ortiz, P.L., Arista, M. (2001). Pollinator attendance and
 526 reproductive success in *Cistus libanotis* L. (Cistacea). **International Journal of**
 527 **Plant Science** 162: 343-352.

528 Vizentin-Bugoni, J., Maruyama, P. K., & Sazima, M. (2014). Processes entangling
 529 interactions in communities: forbidden links are more important than abundance in a
 530 hummingbird–plant network. **Proceedings of the Royal Society B: Biological**
 531 **Sciences**, 281(1780), 20132397.

532 Vizentin-Bugoni, J., Maruyama, P.K., Debastiani, V.J., Duarte, L.S., Dalsgaard, B., &
 533 Sazima, M. (2016). Influences of sampling effort on detected patterns and structuring
 534 processes of a Neotropical plant–hummingbird network. **Journal of Animal**
 535 **Ecology** 85: 262–272.

536 Von Matter, S., Straube, F.C., Accordi, I., Piacentini., & Cândido-Jr, J. (2010).
 537 Ornitologia e Conservação. Ciência Aplicada, Técnicas de Pesquisa e Levantamento.
 538 1ª ed. Rio de Janeiro: Ed. Technical book. 516pg

539 Wolf, L.L., Stiles, F.G., & Hainsworth, F.R. (1976). Ecological organization of a
 540 tropical, highland hummingbird community. **Journal of Animal Ecology** 45:349–
 541 379.

542 Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., & Smith, G.M. (2009). Mixed
 543 Effects Models and Extensions in Ecology with R. Springer, New York.
 544
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Table 1: Physical attributes of the recorded hummingbirds, average time spent feeding, and the number of victories in the agonistic interactions while serving in the drinking fountain. Richness and frequency of pollen grains morphotypes transported by each hummingbird species.

Hummingbird species	Number of individuals	Pollen morphotype richness	Pollen morphotype frequency (%)	Victories in agonistic interactions	Time spent feeding (sec)	Beak length (mm)	Body mass (g)
<i>Amazilia lactea</i>	11	8	41.68	73	9.4	14.3	4.3
<i>Aphantochroa cirrochloris</i>	3	4	95.00	453	7.3	17.0	6.5
<i>Chlorostilbon lucidus</i>	2	5	38.89	1	35.0	15.3	4.0
<i>Eupetomena macroura</i>	1	4	40.06	37	1.2	21.0	8.5
<i>Heliodoxa rubricauda</i>	8	4	27.02	840	6.7	17.4	7.0
<i>Phaethornis eurynome</i>	2	2	95.97	-	-	33.5	5.3
<i>Phaethornis pretrei</i>	8	1	96.02	14	3.7	28.8	5.7
<i>Thalurania glaucopis</i>	14	7	94.01	225	8.5	15.7	4.8

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553 **Table 2:** Results of the GLM models for richness and abundance of pollen grains
 554 morphotypes collected in hummingbirds according to the functional attributes body
 555 mass plus beak length.

Response variable	Explanatory variable	DF	Devianc e	F	Estimativ e	P
Pollen richness	Beak length	1	6.322	25.75	-0.126	0.004*
				5		*
Pollen richness	Body mass	1	0.009	0.029	0.014	0.873
Pollen frequency	Beak length	1	828.07	0.813	2.338	0.408
Pollen frequency	Body mass	1	521.35	0.456	-6.208	0.536

556 ** Statistical significance; DF = degrees of freedom

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Table 3: Richness and frequency of pollen grain morphotypes as a function of the total time spent in feeding and the number of victories in agonistic interactions.

Response variable	Explanatory variable	DF	Deviance	F	Estimative	P
Pollen frequency	Time spent feeding	1	446.17	0.407	2.410	0.551
Pollen frequency	Victories	1	131.83	0.106	-0.033	0.760
Pollen richness	Time spent feeding	1	8.696	1.913	0.336	0.225
Pollen richness	Victories	1	0.105	0.021	-0.002	0.890

DF = degrees of freedom

Figure 1: Relationship between richness of pollen grains morphotypes carried by hummingbirds and the length of the beak.

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577 **Figure 2:** Interaction network (modules) formed by the hummingbird species and the
578 pollen grains morphotypes concerning their beak lengths. The most vibrant colors
579 represent the highest frequency of pollen carried by them.

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582 **Figure 3:** Interaction network representing the overlapping trophic niche of
583 hummingbirds based on the frequency of pollen grain transported by them.

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