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# The influence of biogeographical and evolutionary histories on morphological traitmatching and resource specialization in mutualistic hummingbirdplant networks

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8

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128 AMMG collected the data; BD, PKM, KH, TBZ, JVB and AMMG compiled and checked the  
129 database; JS (with help from BD, PKM, JDK, MSc) analysed the data; BD wrote the first draft of

130 the manuscript with inputs from PKM, JS, KH, BIS, MSc, AMMG. All authors contributed to the  
131 manuscript and gave final approval for publication.

132

133 **Data availability statement:** Upon the acceptance of the manuscript, all raw quantitative  
134 hummingbird-plant networks and complementary morphological trait data supporting the results  
135 will be archived in DRYAD and the data DOI will be included in the reference list.

136



137 **Abstract**

- 138 1. Functional traits can determine pairwise species interactions, such as those between plants and  
139 pollinators. However, the effects of biogeography and evolutionary history on trait-matching  
140 and trait-mediated resource specialization remain poorly understood.
- 141 2. We compiled a database of 93 mutualistic hummingbird-plant networks (including 181  
142 hummingbird and 1,256 plant species), complemented by morphological measures of  
143 hummingbird bill and floral corolla length. We divided the hummingbirds into their principal  
144 clades and used knowledge on hummingbird biogeography to divide the networks into four  
145 biogeographical regions: Lowland South America, Andes, North & Central America, and the  
146 Caribbean islands. We then tested: (i) whether hummingbird clades and biogeographical regions  
147 differ in hummingbird bill length, corolla length of visited flowers and resource specialization,  
148 and (ii) whether hummingbirds' bill length correlates with the corolla length of visited food  
149 plants and with their level of resource specialization.
- 150 3. Hummingbird clades dominated by long-billed species generally visited longer flowers and  
151 were the most exclusive in their resource use. Bill and corolla length and the degree of resource  
152 specialization were similar across mainland regions, but the Caribbean islands had shorter  
153 flowers and hummingbirds with more generalized interaction niches. Bill and corolla length  
154 correlated in all regions and most clades, i.e. trait-matching was a recurrent phenomenon in  
155 hummingbird-plant associations. In contrast, bill length did not generally mediate resource  
156 specialization, as bill length was only weakly correlated with resource specialization within one  
157 hummingbird clade (Brilliant) and in the regions of Lowland South America and the Andes in  
158 which plants and hummingbirds have a long co-evolutionary history.
- 159 4. These results demonstrate how biogeographical and evolutionary histories can modulate the  
160 effects of functional traits on species interactions, and that traits better predict functional groups

161 of interaction partners (i.e. trait-matching) than resource specialization. These findings reveal  
162 that functional traits have great potential, but also key limitations, as a tool for developing more  
163 mechanistic approaches in community ecology.

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168 **Keywords:** biogeography, island ecology, niche-partitioning, plant-animal interactions, resource  
169 specialization, species traits, specificity, trait-matching

## 170 **Introduction**

171 Species do not live and evolve in isolation, but are entangled within networks of interactions with  
172 other species (Bascompte & Jordano, 2007). As species' interactions play a key role in species  
173 coexistence and speciation (Phillips et al., 2020), it is important to understand when and why co-  
174 occurring species interact and specialize on each other. Recently, there is growing interest in the  
175 role of functional traits in determining pairwise interactions between species (McGill, 2006;  
176 Maruyama et al., 2018; Pigot et al., 2020; Schleuning et al., 2020; Sonne et al., 2020). If two co-  
177 occurring species have matching traits, the probability and efficiency of their interaction should  
178 increase (Eklöf et al., 2013; Maglianesi et al., 2014). Otherwise, mismatches in traits could render  
179 interactions inefficient or even impose barriers to interactions, resulting in so-called 'forbidden  
180 links' (Jordano et al., 2003). In other words, species' traits determine whether and how frequently  
181 co-occurring species interact and so species' traits should match and influence resource  
182 specialization (Maglianesi et al., 2014; Klumpers et al., 2019).

183           Mutualistic systems of plant-pollinator interactions contain classic examples of trait-  
184 matching that have been highlighted as textbook examples of coevolution (e.g. Thompson, 2005).  
185 For instance, the length of a moth's proboscis, or the length of a hummingbird's bill, often match  
186 the corolla length of their food plants (Nilsson, 1988; Temeles & Kress, 2003). Trait-matching and  
187 floral specificity may be energetically advantageous for the pollinators and increase pollination  
188 efficiency (Montgomerie et al., 1984). Accordingly, both trait-matching and trait-mediated resource  
189 specialization have been reported for local plant-pollinator interaction networks (Stang et al., 2009;  
190 Maglianesi et al., 2014; Vizentin-Bugoni et al., 2014; Weinstein & Graham, 2017; Klumpers et al.,  
191 2019). However, drawing general conclusions in community ecology requires comparative studies  
192 of local communities across biogeographic regions (Lessard et al., 2012), and biogeographical  
193 history has been suggested to influence the role traits play in mediating plant-pollinator interactions

194 (Dalsgaard et al., 2018). Notably, theory predicts that biogeographical regions where species have  
195 co-occurred for longer should contain species with more specialized associations and greater trait  
196 matching (Dalsgaard et al., 2011; Sonne et al., 2016, 2020). At the other extreme, pollinators on  
197 oceanic islands are predicted to have generalized feeding behaviours, probably because it is  
198 advantageous to be a generalist to colonize and establish on islands (Olesen et al., 2002). Moreover,  
199 as oceanic islands have an impoverished insect pollinator fauna (Olesen & Jordano, 2002),  
200 evolutionary processes may drive island pollinators, especially vertebrates, to evolve novel and  
201 generalized feeding niches (Olesen et al., 2002; Olesen & Valido, 2003; Traveset et al., 2015).  
202 Taken together, theory suggests that biogeographical history may influence trait-matching and how  
203 well traits predict resource specialization in plant-pollinator networks, but large-scale analyses  
204 across biogeographical regions are rare (Dalsgaard et al., 2018; Sonne et al., 2020).

205           In addition to biogeographical history, if trait-matching and trait-mediated resource  
206 specialization are invariant properties of pollination networks, they should manifest repeatedly  
207 among distinctly related groups within a pollination system. For example, the mutualistic  
208 association between hummingbirds and their nectar-food plants is the most specialized avian  
209 pollination system (Fleming & Muchhala, 2008; Zanata et al., 2017), largely manifested in the  
210 match between the length of hummingbird bills and the length of the flowers they feed on  
211 (Feinsinger & Colwell, 1978; Stiles, 1981; Cotton, 1998; Dalsgaard et al., 2009; Maruyama et al.,  
212 2014; Vizentin-Bugoni et al., 2014; Weinstein & Graham, 2017; Sonne et al., 2020). However,  
213 hummingbirds consist of nine evolutionary distinct clades (McGuire et al., 2014), which differ  
214 greatly in their bill morphology and floral preferences (Feinsinger & Colwell, 1978; Bleiweiss,  
215 1988). Thus, if trait-matching is universal for hummingbird-plant associations, bill length and  
216 corolla length of visited flowers should co-vary between hummingbird clades. In other words,  
217 hummingbird clades consisting of long-billed species should prefer flowers with long corollas and

218 *vice versa* for clades with shorter bills. Moreover, bill length should correlate with corolla length  
219 both across all hummingbird species and across the species within each hummingbird clade.  
220 Likewise, if bill length predicts resource specialization (Maglianesi et al., 2014), then bill length  
221 should co-vary with resource specialization both between and within hummingbird clades.  
222 However, although evolutionary relatedness is known to structure plant-pollinator interaction  
223 networks (Rezende et al., 2007; Martín González et al., 2015), the role of evolutionary history in  
224 influencing trait-matching and trait-mediated resource specialization remains poorly understood.

225           To examine whether evolutionary and biogeographical histories influence the  
226 generality of trait-matching and trait-mediated resource specialization in assemblages of plants and  
227 pollinators, we compiled a database of 93 quantitative hummingbird-plant networks distributed  
228 widely across continental America and the Caribbean islands. Each network represents the  
229 mutualistic interactions occurring within local assemblages of hummingbirds and their food plants  
230 (Dalsgaard et al., 2011), for which we gathered data on hummingbird bill length and the effective  
231 floral corolla length (*sensu* Wolf et al., 1976). To test the generality of trait-matching and trait-  
232 mediated resource specialization across evolutionary and biogeographical histories, we divided the  
233 hummingbirds into their nine principal clades and used knowledge on hummingbird biogeography  
234 to divide the networks into four biogeographical regions: Lowland South America, Andes, North &  
235 Central America, and the Caribbean islands (McGuire et al., 2014). We used this unique set of  
236 hummingbird-plant networks and trait data to test: (i) whether hummingbird clades and  
237 biogeographical regions differ in hummingbird bill length, corolla length of visited flowers and  
238 resource specialization, and (ii) whether hummingbirds' bill length correlates with the corolla  
239 length of their food plants and with their level of resource specialization, which we examined both  
240 for the entire dataset and within each hummingbird clade and biogeographical region.

241

242 **Materials and methods**

243 *Datasets: hummingbird-plant networks and traits*

244 We compiled a dataset consisting of 93 quantitative hummingbird-plant interaction networks  
245 distributed widely across the Americas. The networks were sampled to represent all hummingbird  
246 clades and hummingbird-visited plant families without any taxonomic bias. Each of the 93 networks  
247 describes interaction frequencies within assemblages of hummingbirds and their food plants in a  
248 specific location. We only included mutualistic interactions in which a given hummingbird was  
249 observed drinking nectar and touching the stigma / anthers of the given flower, thereby potentially  
250 acting as a pollinator (see Appendix 1). Species names of the hummingbirds follow the International  
251 Ornithological Committee World List (IOC version 9.2; [www.worldbirdnames.org](http://www.worldbirdnames.org); Appendix 2).  
252 Hummingbirds were divided into nine clades following McGuire et al. (2014). Species names and  
253 families of the plants follow ‘The Plant List’ (TPL version 1.1; [www.theplantlist.org](http://www.theplantlist.org)), with a few  
254 exceptions where species names of recorded plants were not found in TPL (specified in Appendix 3).  
255 The networks can be downloaded from DRYAD [Link to be inserted upon acceptance of the  
256 manuscript].

257 We compiled two trait datasets: one for all hummingbird species and one for all plant  
258 species observed in the 93 networks (Appendix 2 and 3). In total, these contained 181 hummingbird  
259 species and 1,256 plant species for which we gathered data on hummingbird bill length and the  
260 effective floral corolla length (*sensu* Wolf et al., 1976). Data on hummingbird bill length was based  
261 on museum specimens, most averaged across five males and five females (specified in Appendix 2).  
262 In total, we obtained bill length estimates for 180 of the 181 hummingbird species (99% of the  
263 species; Appendix 2). Data on floral corolla length was based on measurements in the field. Apart  
264 from a few cases (~1% of the species), data on floral corolla length was collected at the same  
265 locality as the network. For plant species present in more than one network, if data on floral corolla

266 length was collected in several localities (~19% of the species), we calculated species averages  
267 across localities. In total, we obtained floral corolla length estimates for 962 plant species (76% of  
268 the species; Appendix 3). As a supplement to bill and corolla length, we attempted to gather data on  
269 bill and corolla curvature, as these traits may also match and the combination of bill length and  
270 curvature may better predict resource specialization than bill length alone (Maglianesi et al., 2014;  
271 Sonne et al., 2019). We were only able to gather a comprehensive dataset for bill curvature (99% of  
272 the species; Appendix 2), which we used in supplementary analyses to validate our main focus on  
273 bill length. The trait data can be downloaded from DRYAD [Link to be inserted upon acceptance of  
274 the manuscript].

275

#### 276 *Measuring resource specialization*

277 For each hummingbird species within the 93 hummingbird-plant networks, we calculated resource  
278 specialization using two metrics, one reflecting niche overlap and one reflecting niche breadth. As a  
279 measure of niche overlap, we used the complementary specialization index  $d'$  (Blüthgen et al.,  
280 2006). We used this index as it is robust to variation in sampling effort, more than other measures of  
281 resource specialization (Fründ et al., 2016). The index derives from Shannon's entropy and  
282 quantifies the extent to which observed interaction frequencies differ from random encounter  
283 probabilities of species, as derived from species' total interaction frequencies (Blüthgen et al.,  
284 2006). This follows the assumption that if species specialize on specific interaction partners, these  
285 preferences should be captured as deviations from random encounters given by partner availability  
286 (Blüthgen et al., 2006). The index  $d'$  ranges between 0 and 1 for extreme generalization and extreme  
287 specialization, respectively (Blüthgen et al., 2006). As a measure of hummingbird niche breadth, we  
288 used the proportional generality index. The proportional generality index is a quantitative version of  
289 proportional resource use (normalised degree in binary networks), making it suitable for

290 comparisons between networks (Cusser et al., 2019). A proportional generality value at or close to  
291 zero indicates a narrow niche breadth (i.e. a specialized species), whereas higher values indicate a  
292 broader niche breadth. Note that the proportional generality index may be larger than one. For each  
293 hummingbird species in each network, we calculated species-level specialization  $d'$  and  
294 proportional generality using the *bipartite* package in R (Dormann et al., 2008).

295

### 296 *Biogeographical regions*

297 The datasets were separated into four major biogeographical regions: Lowland South America,  
298 Andes, North & Central America, and the Caribbean (Fig. 1). This separation is based on the  
299 division of hummingbirds into biogeographical regions, as extant hummingbirds supposedly  
300 originated in lowland South America ~ 22 million years ago, then expanded into the Andes (~ 16  
301 million years ago) and north of the Isthmus of Panama (~ 12 million years ago), arriving in the  
302 Caribbean region more recently ~ 5 million years ago (McGuire et al., 2014). Our 'Lowland South  
303 America' region includes all networks south of the Isthmus of Panama, excluding networks located  
304 in the Andean mountains. The 'Andes' region includes all networks within the Andean mountains  
305 as defined by Rahbek et al. (2019). The 'North & Central America' region includes all networks  
306 located on the mainland north of the Isthmus of Panama. Finally, the 'Caribbean' region includes all  
307 networks located on oceanic islands in the Caribbean Basin, excluding the continental island of  
308 Trinidad located south of Bond's line, which for biogeographical reasons was included in the  
309 'Lowland South America' region (Carstensen et al., 2013). The 93 hummingbird-plant interaction  
310 networks were distributed as follows: 41 in Lowland South America, 22 in North & Central  
311 America, 21 in the Andes, and nine in the Caribbean (Fig. 1). We observed most hummingbird  
312 species in the Andean networks (76 species), followed by Lowland South America (55 species),  
313 North & Central America (53 species) and the Caribbean island networks (12 species). With respect



314 to hummingbird-visited plant species, we recorded 641 species in Lowland South America, 367  
315 species in the Andes, 233 species in North & Central America, and 65 species in the Caribbean.

316

### 317 *Statistical analyses*

318 First, we explored whether the hummingbird clades and plant families were distributed randomly  
319 across the four biogeographical regions. To examine whether differences in hummingbird clade and  
320 plant family distribution were statistically different between the four biogeographical regions, we  
321 used Fisher's Exact Test followed by False Discovery Rate (FDR) adjusted pairwise comparisons  
322 between all regions for both hummingbird clades and plant families. Significance levels were  
323 calculated with the function 'fisher.test' in R (R Development Core Team, 2016) using Monte Carlo  
324 simulations with 10,000 replicates. For plants, to focus on the main families, only the five most  
325 frequent families in each region were chosen, which across the four regions gave a total of 11  
326 families (plus the category 'others' including the rest of the plant families).

327 Second, we used one-way ANOVA tests followed by FDR adjusted multiple comparisons to  
328 examine whether functional traits (hummingbird bill length, floral corolla length) and hummingbird  
329 resource specialization (complementary specialization  $d'$ , proportional generality) varied between  
330 biogeographical regions and hummingbird clades. When performing the one-way ANOVAs  
331 between hummingbird clades, the comparison was based on all observed species within each clade,  
332 irrespective of regional affinities. We only used the seven most species-rich clades in our dataset,  
333 excluding the species-poor clades Topazes (three species) and *Patagona* (one species). When  
334 performing the one-way ANOVAs between biogeographical regions, the comparison was based on  
335 all species observed within each region, i.e. we allowed species to be affiliated with multiple  
336 biogeographical regions (only 13 hummingbird species, i.e. ~7%, occurred in two regions; four  
337 species, i.e. ~2%, in three regions, and no species occurred in four regions). For all analyses, we

338 log-transformed bill and corolla length. Supplementary one-way ANOVAs showed that bill  
339 curvature varied significantly between hummingbird clades and regions (for details, see Fig. S3 in  
340 Supporting Information), but there was no indication that this caused clade and regional differences  
341 in resource specialization (compare Figs. 2-3 with Fig. S3). The ANOVA tests were run using the  
342 ‘anova’ function in R. We also constructed linear-mixed effect models (LMMs) using the *nlme*  
343 package in R (Pinheiro et al., 2019), with the aim to control for clades as a random intercept (when  
344 examining how regions differ in traits and specialization level) and regions as a random intercept  
345 (when examining how clades differ in traits and specialization level). However, these models did  
346 not converge due to singularities (see Bates et al., 2015), likely caused by hummingbird clades  
347 being non-randomly distributed across regions (see the Results of Fisher's Exact Test).

348           Third, we investigated whether the length of hummingbird bills was associated  
349 positively with: (a) the mean corolla length of the flowers they visit, and (b) their degree of resource  
350 specialization (complementary specialization  $d'$ , proportional generality) averaged across networks.  
351 We tested these potential associations with linear mixed-effects models. For each response variable,  
352 we built two sets of models both with bill length as a fixed effect: one considering hummingbird  
353 clades and another considering biogeographical regions as random intercepts because models  
354 including both random effects simultaneously did not converge. When using clades as a random  
355 factor, mean corolla length and resource specialization were estimated for each species irrespective  
356 of regional affinity. When using regions as random factor, mean corolla length and resource  
357 specialization were estimated for each species within each region. For each of these analyses, we  
358 constructed both unweighted and weighted models, the latter weighted with the square root number  
359 of plants visited (when predicting mean corolla length) and the square root number of networks  
360 (when predicting mean resource specialization; Maglianesi et al., 2014). The weighting procedure  
361 gave higher weight to hummingbird species that visited more plants and occurred in more networks,

362 respectively. We used this weighting procedure as estimates of mean corolla length and mean  
363 resource specialization may be more reliable when based on larger sample sizes, however, it may  
364 also bias the results towards frequent and geographically widespread species. Thus both weighted  
365 and unweighted LMMs were constructed. To estimate the significance of bill length in the LMMs,  
366 we used the function ‘anova’ from the *car* package in R (Fox & Weisberg, 2019). We estimated the  
367 proportion of variance explained by bill length in the LMMs as marginal  $R^2$ , and the proportion of  
368 variance explained by both fixed and random factors as conditional  $R^2$  with the function  
369 ‘r.squaredGLMM’ in the MuMIn package (Nakagawa et al., 2017; Barton, 2020).

370 In addition to the LMMs, we estimated Pagel’s lambda  $\lambda$  to assess how well evolutionary  
371 relatedness explains the distribution of bill length, mean corolla length and resource specialization  
372 among hummingbird species (Pagel, 1999). Subsequently, to account for the non-independence of  
373 the species-level data in our regression analyses, we repeated these using phylogenetic least squares  
374 regression (PGLS) with the function ‘pgls’ in the package *caper* (Orme et al., 2018). In these  
375 analyses, we derived the expected co-variances among all species using the maximum clade  
376 credibility tree of McGuire et al. (2014). To reconcile our trait dataset with the McGuire et al.  
377 (2014) phylogeny we: (1) excluded species included in our networks but not sampled in the tree, (2)  
378 dropped species that McGuire et al. (2014) demonstrated to be paraphyletic or polyphyletic with  
379 respect to other taxa included in our networks, and (3) used the mean bill / corolla length and  
380 specialization value for species pairs that represent recent taxonomic splits only represented by a  
381 single tip in the McGuire et al. (2014) phylogeny. These amendments reduced our dataset to 155  
382 species (bill vs. corolla length) and 158 species (bill vs. specialization) in the PGLS regressions. As  
383 additional analyses, we repeated the unweighted / weighted and PGLS regressions separately for  
384 each of the seven hummingbird clades (excluding *Topazes* and *Patagona* with only three and one  
385 species, respectively) and for each of the four biogeographical regions. Finally, we constructed

386 supplementary LMM and PGLS regressions to examine whether bill curvature provides additional  
387 explanatory power in predicting resource specialization. These analyses showed negligible effects  
388 of bill curvature (for details, see Table S1).

## 389 **Results**

### 390 *Hummingbird clade and plant family distribution across regions*

391 There was a clear difference in the distribution of hummingbird clades between the four regions ( $P$   
392  $< 0.05$ ; Fig. 1). Notably, the Brilliants and Coquettes dominated the Andean networks and appeared  
393 in very low numbers outside of the Andes. In the other three regions, Emeralds were the most  
394 species-rich group, along with Hermits (Lowland South America), Mountain Gems and Bees (North  
395 & Central America) and Mangoes (Caribbean). Likewise, there were clear differences in plant  
396 family distribution between the four regions ( $P < 0.05$ ; Fig. 1). The Ericaceae dominated the  
397 Andean networks, and were well represented in North & Central America, but had few species in  
398 the Lowland South American and the Caribbean networks. The Bromeliaceae dominated in the  
399 Lowland South American networks, and were well represented in the Andean and North & Central  
400 American networks, but were poorly represented in the Caribbean. The Caribbean networks were  
401 dominated by the Rubiaceae, which were also well represented in the other regions (Fig. 1).

402

### 403 *Comparing traits and resource specialization between clades and between regions*

404 The hummingbird clades differed significantly in bill length and the length of the flowers visited  
405 (bill length:  $F_{6, 165} = 25.29$ ,  $P < 0.001$ ; corolla length:  $F_{6, 165} = 12.64$ ,  $P < 0.001$ ; Fig. 2a). The  
406 Hermits and Mountain Gems had the longest bills, although the bill length of Mountain Gems was  
407 not significantly longer than those of Mangoes and Brilliants. The shortest bills were those of the  
408 Coquettes and Bees, whereas Emeralds had bills of intermediate length, only overlapping with those

409 of Mangoes. The Hermits and Mountain Gems visited the longest flowers, although the flowers  
410 visited by Mountain Gems were not significantly longer than those visited by Brilliants and  
411 Mangoes. The Coquettes and Bees visited the shortest flowers, although the Coquettes' flowers  
412 were not significantly shorter than those visited by Emeralds and Mangoes (Fig. 2a). Hummingbird  
413 resource specialization  $d'$  largely differed among clades in accordance with differences in bill /  
414 corolla length: the Hermits and Mountain Gems were the most specialized clades (i.e., species in  
415 these clades were most exclusive in their resource use), significantly more specialized than the Bees  
416 and Emeralds, with intermediate levels of specialization shown by Mangoes, Brilliants and  
417 Coquettes ( $F_{6, 169} = 4.29$ ,  $P < 0.001$ ; Fig. 2b). The hummingbird clades showed similar level of  
418 proportional generality, i.e. niche breadth did not differ significantly between clades ( $F_{6, 169} = 1.19$ ,  
419  $P = 0.32$ ; Fig. 2c).

420 Hummingbird bill length was similar across regions ( $F_{3, 191} = 1.15$ ,  $P = 0.33$ ), but there  
421 were regional differences in the corolla length of hummingbird-visited flowers ( $F_{3, 575} = 10.15$ ,  $P <$   
422  $0.001$ ): North & Central American flowers were the longest, whereas the Caribbean flowers were  
423 significantly shorter than flowers in all mainland regions (Fig. 3a). There were also regional  
424 differences in hummingbird resource specialization, both when measured as  $d'$  ( $F_{3, 192} = 2.82$ ,  $P <$   
425  $0.05$ ) and proportional generality ( $F_{3, 192} = 13.35$ ,  $P < 0.05$ ). Notably, the Caribbean hummingbirds  
426 were more generalized than mainland hummingbirds, both in terms of niche overlap  $d'$  and  
427 proportional generality (Fig. 3b-c).

428

#### 429 *Associations between traits and between traits and resource specialization*

430 In LMMs across all hummingbird species, when including region as a random factor, bill length  
431 was strongly and positively associated with mean corolla length (Table 1; Fig. 4b), weakly related

432 to mean resource specialization when measured as  $d'$  (Table 1; Fig. 4d), but unrelated to mean  
433 proportional generality (Table 1; Fig. 4f). When including clade as a random factor, hummingbird  
434 bill length was also strongly positively associated with mean corolla length (Table 1; Fig. 4a), but  
435 bill length was not associated with neither measure of resource specialization (Table 1; Fig. 4c, Fig.  
436 4e). The species-level variation in bill length, mean corolla length of visited flowers, and both  
437 measures of resource specialization displayed a phylogenetic signal that was significantly greater  
438 than zero ( $P < 0.001$  in all cases). In particular, closely related hummingbird species are likely to be  
439 similar in bill length ( $\lambda = 0.97$ ), more so than the corolla length of visited flowers ( $\lambda = 0.59$ ), with  
440 weaker phylogenetic signals for resource specialization: proportional generality ( $\lambda = 0.51$ ) and  $d'$  ( $\lambda$   
441  $= 0.23$ ). When accounting for this phylogenetic non-independence using PGLS, we continued to  
442 detect a strong positive association between bill length and mean floral corolla length ( $\beta = 0.83$ ,  $R^2$   
443  $= 0.32$ ,  $P < 0.001$ ), but there was no association between bill length and mean resource  
444 specialization, both measured as  $d'$  ( $\beta = 0.05$ ,  $R^2 = 0.00$ ,  $P = 0.70$ ) and proportional generality ( $\beta =$   
445  $0.01$ ,  $R^2 = 0.00$ ,  $P = 0.94$ ).

446           Analysing the individual hummingbird clades separately showed that most clades had  
447 a moderate to strong positive association between bill length and mean corolla length, although  
448 relationships for some clades were weak and non-significant (Fig. S1a). Only Brilliants showed a  
449 weakly positive association between bill length and mean resource specialization  $d'$  (Fig. S1b). For  
450 all other clades, there were no significant associations between bill length and both measures of  
451 resource specialization (Fig. S1b).

452           For all four regions analysed separately, there was a strong positive association  
453 between hummingbird bill length and the mean corolla length of their flowers (Fig. S2a). The  
454 association between bill length and resource specialization  $d'$  was non-significant for all four  
455 regions when using unweighted regressions and PGLS, but weakly positive for Andes and Lowland

456 South America when using weighted regressions (Fig. S2b). For all regions, there was no  
457 association between bill length and proportional generality, i.e. niche breadth (all fits had  $P > 0.05$ ).

458

## 459 **Discussion**

460 We demonstrate that morphological trait-matching is a recurrent phenomenon in hummingbird-  
461 plant networks throughout the Americas, i.e. the length of a hummingbird's bill correlates with the  
462 corolla length of the flowers they visit (Fig. 4a-b). This result was repeated within all  
463 biogeographical regions and within most hummingbird clades (Fig. S1-S2). Trait-matching was also  
464 evident when comparing between clades, as clades dominated by long-billed hummingbirds (e.g.  
465 Hermits) generally visited longer flowers and *vice versa* for clades with shorter bills (Fig. 2a). If we  
466 had local floral trait measurements for all hummingbird-visited plants and local measurements of  
467 hummingbird traits (rather than from museum specimens), these recurrent patterns of trait-matching  
468 may have been even stronger. When comparing between clades, traits also largely co-varied with  
469 resource specialization  $d'$ , e.g. the long-billed Hermits were also the most exclusive in their resource  
470 use (Fig. 2b). However, there was no difference in niche breadth between clades (Fig. 2c) and  
471 morphological traits did not generally mediate resource specialization, as bill length was only a  
472 weak predictor of both measures of resource specialization (Fig. 4c-f; bill curvature also had  
473 negligible effects on resource specialization, see Table S1). Notably, bill length was associated with  
474 resource specialization only within the Brilliants and within the regions of Lowland South America  
475 and the Andes (Fig. S1-S2). Taken together, these results demonstrate the strong influence of  
476 biogeographical and evolutionary histories on recurrent patterns of morphological trait-matching,  
477 and the weak relationships between hummingbird bill length and resource specialization.

478           The recurrent patterns of trait-matching show that hummingbirds feed on flowers  
479 approximately similar in length to their bills, even though long-billed hummingbirds are able to

480 access nectar from flowers with shorter corollas. This may be because long-billed hummingbirds  
481 minimize competition with short-billed hummingbirds by primarily feeding on flowers inaccessible  
482 to short-billed hummingbirds (Maglianesi et al., 2015; Sonne et al., 2020). It may also be related to  
483 nectar production, as longer flowers often offer more nectar than shorter flowers (Stiles, 1981;  
484 Buzato et al., 2000; Ornelas et al., 2007; Dalsgaard et al., 2009), making it energetically optimal for  
485 longer-billed hummingbirds to feed on flowers with longer corollas, up to a given threshold  
486 (Montgomerie et al., 1984). From the plant's perspective, it may also be an advantage to attract  
487 hummingbirds with matching traits, thereby increasing pollination efficiency (Montgomerie et al.,  
488 1984). Therefore, there is a clear association between these functional traits of hummingbirds and  
489 plants. Similar patterns have been reported for other plant-pollinator systems, such as hawkmoths  
490 and other insect pollinators visiting flowers approximately similar in length to their proboscises  
491 (Stang et al., 2009; Sazatornil et al., 2016; Klumpers et al., 2019). Interestingly, on average, species  
492 in all hummingbird clades visited flowers with slightly longer corollas than their bills (Fig. 2a),  
493 reflecting that hummingbirds extend their tongues while drinking nectar (Hainsworth, 1973;  
494 Montgomerie et al., 1984). The Bees and the Coquettes, the two clades with the shortest bills,  
495 visited flowers considerably longer than their bills (Fig. 2a). This may be because short-billed  
496 hummingbirds benefit from exploring flowers with longer corolla, as these offer more nectar than  
497 short-corolla flowers fitting their bills. Therefore, short-billed hummingbirds may have evolved the  
498 ability to extend their tongues proportionally longer than long-billed hummingbirds (Hainsworth,  
499 1973).

500 Floral corolla length was on average longer than hummingbird bill length across all  
501 mainland regions, but not in the Caribbean islands where floral corolla length was shorter than on  
502 the mainland (Fig. 3a). This result is consistent with reports that the Caribbean hummingbirds often  
503 feed on shorter insect-syndrome flowers (Dalsgaard et al., 2009; Lehmann et al., 2019) and have a



504 more generalized feeding behaviour than mainland hummingbirds, both in terms of floral niche  
505 overlap and niche breadth (Fig. 3b-c). Although the majority of the plants visited by Caribbean  
506 hummingbirds are either endemic or native to the region (~33% endemic and ~55% native in our  
507 dataset), their opportunism also makes Caribbean hummingbirds more likely to incorporate  
508 introduced plants into their feeding niche (~12% in our dataset; Maruyama et al., 2016). As all  
509 except one of the Caribbean hummingbirds are endemic to the region (Dalsgaard et al., 2018), these  
510 Caribbean vs. mainland patterns are in agreement with the idea that many plants and pollinators on  
511 oceanic islands have evolved towards generalism (Olesen et al., 2002; Olesen & Valido, 2003;  
512 Traveset et al., 2015), but may also reflect the more recent colonization history and limited trait  
513 evolution among Caribbean hummingbirds (Dalsgaard et al., 2018). Despite the distribution of plant  
514 families and hummingbird clades differing significantly across regions (Fig. 1), functional traits and  
515 the degree of resource specialization were similar across mainland regions, but the Caribbean  
516 islands had both shorter flowers and hummingbirds with more generalized niches (Fig. 3). Traits  
517 and resource specialization (niche overlap  $d'$ ) also largely co-varied between hummingbird clades  
518 (Fig. 2), illustrating the influence of biogeographical and evolutionary histories in both functional  
519 traits and floral specificity.

520           Despite the generally consistent trait-matching and associated level of resource  
521 specialization observed between clades, and between mainland vs. islands (Figs. 2-3), trait-  
522 matching did not generally translate into trait-mediated resource specialization for individual  
523 species, as bill length and bill curvature were only weak predictors of resource specialization (Fig.  
524 4c-f; Table 1, Table S1). While the diversity of hummingbird traits within a community influences  
525 overall community-level specialization and partitioning of interactions (Maruyama et al., 2018),  
526 additional mechanisms appear to operate at the species-level (Tinoco et al., 2017; Simmons et al.,  
527 2019). Notably, while functional traits may constrain species into their fundamental niche of

528 possible pairwise interactions (Junker et al., 2013), a hierarchy of multiple mechanisms determines  
529 the realized niche, i.e. which of the possible pairwise interactions are realized (Junker et al., 2013).  
530 In hummingbird-plant communities, trait-matching determines which interactions are possible  
531 (Sonne et al., 2020), but other mechanisms – such as hummingbird abundance and local conditions  
532 related to resource availability and competition with other floral visitors – determine how often  
533 these interactions are realized (Tinoco et al., 2017; Dalsgaard et al., 2018; Simmons et al., 2019).  
534 Thus, there is no general relationship between bill morphology and level of resource specialisation  
535 (Fig. 4c-f; Table 1, Table S1), illustrating that morphological and ecological specialization can be  
536 disassociated (Ollerton et al., 2007; Armbruster, 2017). The only exceptions to this trend are the  
537 weak associations we found between bill length and resource specialization within the Brilliants and  
538 within Lowland South America and the Andes. Here, hummingbirds and plants have had the longest  
539 co-evolutionary history (McGuire et al., 2014) and have experienced more benign conditions during  
540 the Quaternary to evolve more specialized associations (Dalsgaard et al., 2011; Sonne et al., 2016).  
541 A prime example of this is the association between the Andean species of *Passiflora* and the Sword-  
542 billed Hummingbird (*Ensifera ensifera*), an iconic long-billed species within the Brilliants  
543 (Abrahamczyk et al., 2014). The Sword-billed Hummingbird has by far the longest bill of any  
544 hummingbird species, uses very long-corolla flowers, and tends to be ecologically specialized (Figs.  
545 4, S1-S2). In line with that, long-tubed *Passiflora*, and other plants specialized on hummingbird-  
546 pollination, tend to have evolved longer corolla flowers when compared to related plants pollinated  
547 by most other groups of pollinators (Abrahamczyk et al., 2014; Pauw, 2019). Nevertheless, even  
548 these specialised systems with tightly matching traits may have been dynamic over evolutionary  
549 time (Abrahamczyk et al., 2017) and generally long-billed and short-billed hummingbirds show  
550 similar degrees of resource specialization (Fig. 4c-f).

551 In conclusion, we demonstrate the influence of biogeographical and evolutionary  
552 histories on recurrent patterns of trait-matching in hummingbird-plant associations, and weak  
553 effects of functional traits on resource specialization. These findings indicate that morphological  
554 traits can be used to predict resource utilization, not only at the level of resource type (e.g.,  
555 nectarivore, frugivore, granivore, and others; Pigot et al., 2020), but even at the level of specific  
556 species or functional groups of resources. Thus, our macroecological study reveals that trait-  
557 matching rules are generally good predictors of interaction partners across trophic levels, whereas  
558 the degree of resource specialisation is less predictable by morphological traits but is highly  
559 dependent on the biogeographical, ecological and evolutionary context. These findings reveal that  
560 functional traits have great potential, but also key limitations, as a toolkit for understanding trophic  
561 interactions in ecological communities.

562

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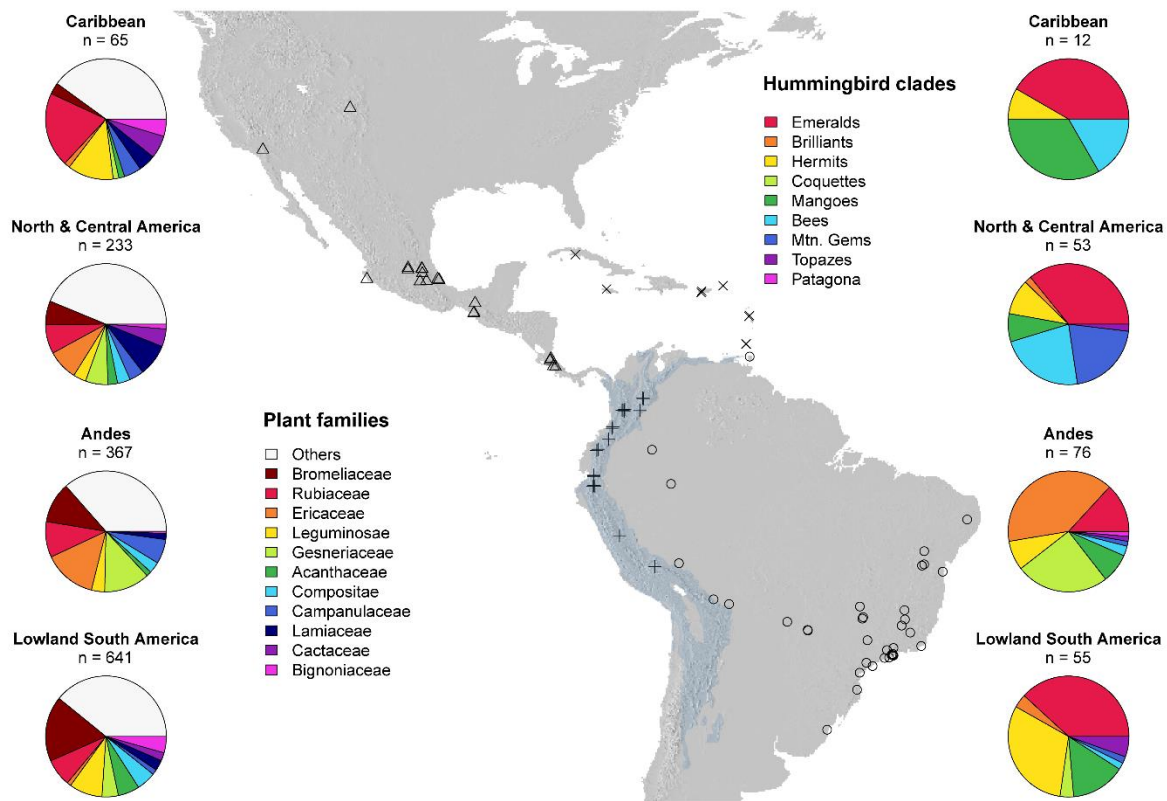
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761 **Table 1.** Linear mixed-effects models (LMMs), analysing the association between hummingbird bill  
762 length and (a) mean corolla length of visited flowers, and mean resource specialization, measured  
763 both as (b) complementary specialization  $d'$  and (c) proportional generality. For each response  
764 variable, we built two sets of models: one considering hummingbird clades and another considering  
765 biogeographical regions as random intercepts, with bill length as a fixed effect. For each of these  
766 analyses, we constructed both unweighted and weighted models, the latter weighted with the square  
767 root number of plants visited (when predicting mean corolla length) and the square root number of  
768 networks (when predicting mean specialization  $d'$  and mean proportional generality). Unweighted  
769 models are in bold. We estimated the proportion of variance explained by bill length in the LMMs as  
770 marginal  $R^2$ , and the proportion of variance explained by both bill length and random factors as  
771 conditional  $R^2$ . We also report standardized coefficient estimates as well as corresponding  $P$ -values  
772 and standard errors.

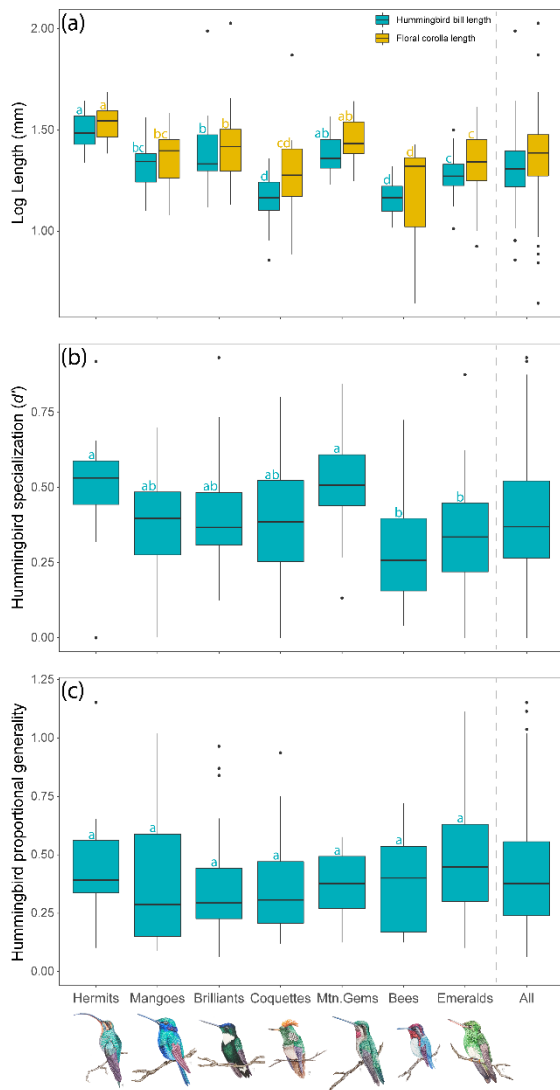
<b>Model</b>	<b>Random</b>	$R^2$	$R^2$	<b>coefficient</b>	<b>Std Error</b>
	<b>factor</b>	<b>marginal</b>	<b>conditional</b>		
(a) Corolla length	<b>clade</b>	<b>0.45</b>	<b>0.45</b>	<b>0.80**</b>	<b>0.07</b>
	clade	0.11	0.11	0.87**	0.08
	<b>region</b>	<b>0.41</b>	<b>0.50</b>	<b>0.78**</b>	<b>0.06</b>
	region	0.11	0.13	0.86**	0.07
(b) Specialization $d'$	<b>clade</b>	<b>0.01</b>	<b>0.10</b>	<b>0.13<sup>NS</sup></b>	<b>0.11</b>
	clade	0.00	0.01	0.09 <sup>NS</sup>	0.12
	<b>region</b>	<b>0.04</b>	<b>0.04</b>	<b>0.25*</b>	<b>0.09</b>
	region	0.00	0.02	0.18 <sup>NS</sup>	0.10
(c) Proportional	<b>clade</b>	<b>0.00</b>	<b>0.02</b>	<b>0.07<sup>NS</sup></b>	<b>0.12</b>
generality	clade	0.00	0.01	0.14 <sup>NS</sup>	0.14
	<b>region</b>	<b>0.00</b>	<b>0.43</b>	<b>0.10<sup>NS</sup></b>	<b>0.10</b>
	region	0.00	0.10	0.10 <sup>NS</sup>	0.11

773 \*\* $P < 0.001$ , \* $p < 0.05$ , <sup>NS</sup>  $P > 0.05$



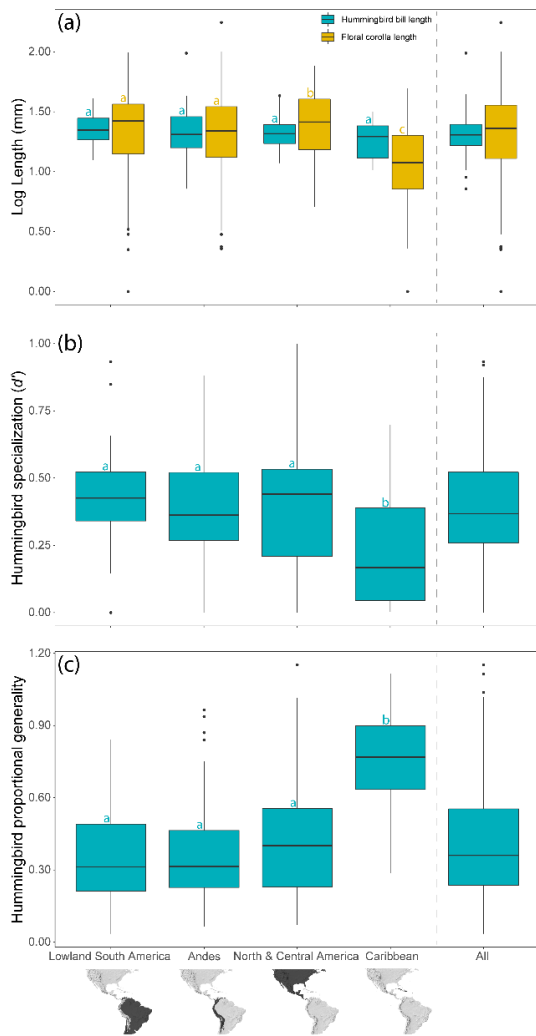
774

775 **Figure 1.** The distribution of 93 hummingbird-plant interaction networks across four  
 776 biogeographical regions (O = Lowland South America, + = Andes, Δ = Central & North America,  
 777 × = Caribbean). The pie charts visualize the distribution of all nine hummingbird clades (right) and  
 778 the 11 most frequently visited hummingbird-plant families (left) within each biogeographical  
 779 region. The category ‘others’ includes the rest of the plant families visited by hummingbirds.  
 780 Within a given pie chart, the size of a clade/family reflects the number of species observed in the  
 781 networks within a given region. Fisher’s exact test showed that all regions differed in respect to  
 782 plant family distribution ( $P < 0.05$  for all pairwise comparisons). For hummingbirds, all mainland  
 783 regions differed significantly in clade distribution ( $P < 0.05$ ), but the Caribbean was not  
 784 significantly different from North & Central America and Lowland South America ( $P > 0.05$ ), the  
 785 two regions from where hummingbirds colonized the Caribbean (Dalsgaard et al., 2018).



786

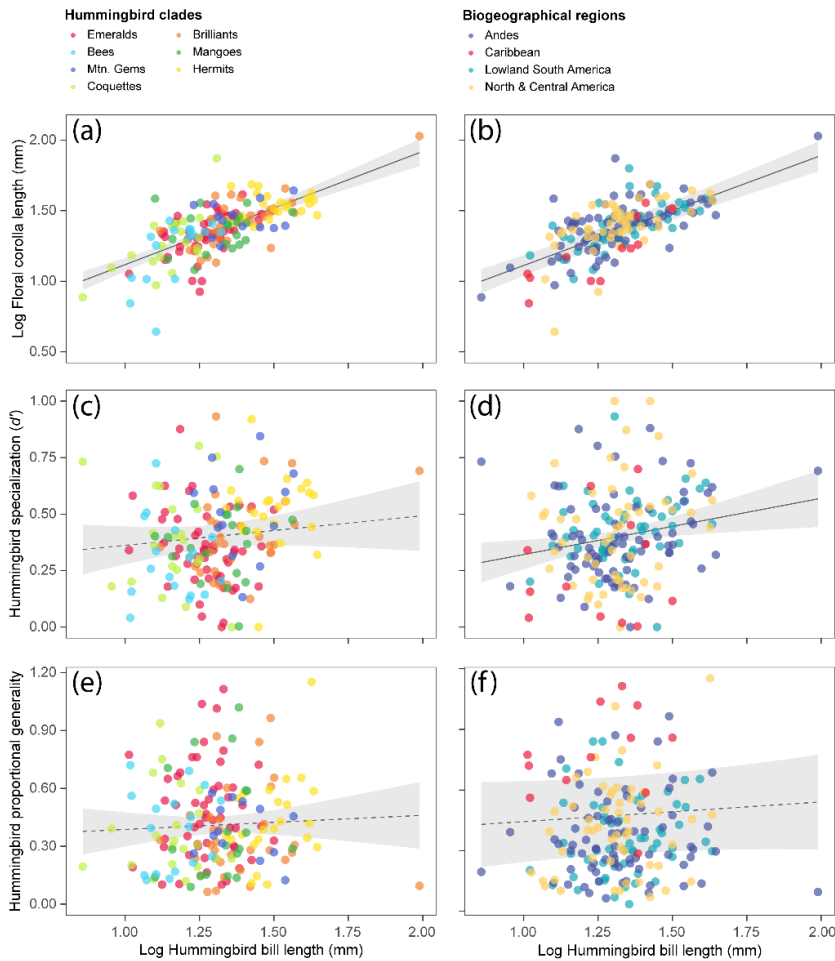
787 **Figure 2.** Hummingbird clade specific differences in (a) hummingbird bill length (turquoise) and  
 788 mean corolla length of visited flowers (yellow), and (b) mean hummingbird specialization  $d'$ , and  
 789 (c) mean hummingbird specialization measured as proportional generality. This comparison was  
 790 based on all species within each clade, irrespectively of regional affinities. Boxes indicate the first  
 791 and third quartiles (Q1 and Q3), horizontal lines inside boxes are medians, vertical lines indicate  
 792 Q1/Q3 + 1.5 x interquartile ranges (IQR), and circles are outliers. Different letters represent  
 793 statistical difference ( $P < 0.05$ ) according to one-way ANOVAs with multiple post-hoc  
 794 comparisons using the fFalse Discovery Rate (FDR). For comparison, on the right in both figures  
 795 we show boxplots across all species. Hummingbird paintings by Katrine Hansen.



796

797 **Figure 3.** Differences in (a) hummingbird bill length (turquoise) and mean floral corolla length  
 798 (yellow), (b) mean hummingbird specialization  $d'$ , and (c) mean hummingbird specialization  
 799 measured as proportional generality between biogeographical regions: Lowland South America,  
 800 Andes, Central & North America, and the Caribbean. The comparison was based on the species  
 801 pool for each region, as extracted in the networks within each region. Boxes indicate the first and  
 802 third quartiles (Q1 and Q3), horizontal lines inside boxes are medians, vertical lines indicate Q1/Q3  
 803 + 1.5 x interquartile ranges (IQR), and circles are outliers. Different letters represent statistical  
 804 difference ( $P < 0.05$ ) according to one-way ANOVAs with multiple post-hoc comparisons using the  
 805 False Discovery Rate (FDR). For comparison, on the right in both figures we show boxplots across  
 806 all species.





807

808 **Figure 4.** Associations between hummingbird bill length and (a, b) mean floral corolla length, (c, d)  
 809 mean hummingbird specialization  $d'$  and (e, f) mean hummingbird specialization measured as  
 810 proportional generality, when using hummingbird clades as a random factor (clades in different  
 811 colours: a, c and e;  $n = 172$  and  $n = 177$ ) and when using biogeographical regions as a random  
 812 factor (regions in different colours: b, d and f;  $n = 191$  and  $n = 195$ ). The black lines (with grey 95%  
 813 confidence intervals) represent the overall fits of unweighted linear-mixed effects models; the  
 814 dotted line represents a non-significant fit. Note that a few hummingbird species were recorded in  
 815 more than one region and, thus, appear more than once in the analyses including regions as a  
 816 random factor (b, d and f). See supplementary Figure S1 for individual plots for each hummingbird  
 817 clade and Figure S2 for individual plots for each biogeographical regions.