

**Article title:** The influence of biogeographical and evolutionary histories on morphological trait-matching and resource specialization in mutualistic hummingbird-plant networks

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## 1 **Abstract**

- 2 1. Functional traits can determine pairwise species interactions, such as those between plants and  
3 pollinators. However, the effects of biogeography and evolutionary history on trait-matching  
4 and trait-mediated resource specialization remain poorly understood.
- 5 2. We compiled a database of 93 mutualistic hummingbird-plant networks (including 181  
6 hummingbird and 1,256 plant species), complemented by morphological measures of  
7 hummingbird bill and floral corolla length. We divided the hummingbirds into their principal  
8 clades and used knowledge on hummingbird biogeography to divide the networks into four  
9 biogeographical regions: Lowland South America, Andes, North & Central America, and the  
10 Caribbean islands. We then tested: (i) whether hummingbird clades and biogeographical regions  
11 differ in hummingbird bill length, corolla length of visited flowers and resource specialization,  
12 and (ii) whether hummingbirds' bill length correlates with the corolla length of their food plants  
13 and with their level of resource specialization.
- 14 3. Hummingbird clades dominated by long-billed species generally visited longer flowers and  
15 were the most exclusive in their resource use. Bill and corolla length and the degree of resource  
16 specialization were similar across mainland regions, but the Caribbean islands had shorter  
17 flowers and hummingbirds with more generalized interaction niches. Bill and corolla length  
18 correlated in all regions and most clades, i.e. trait-matching was a recurrent phenomenon in  
19 hummingbird-plant associations. In contrast, bill length did not generally mediate resource  
20 specialization, as bill length was only weakly correlated with resource specialization within one  
21 hummingbird clade (Brilliant) and in the regions of Lowland South America and the Andes in  
22 which plants and hummingbirds have a long co-evolutionary history. Supplementary analyses  
23 including bill curvature confirmed that bill morphology (length and curvature) does not in  
24 general predict resource specialization.

25 4. These results demonstrate how biogeographical and evolutionary histories can modulate the  
26 effects of functional traits on species interactions, and that traits better predict functional groups  
27 of interaction partners (i.e. trait-matching) than resource specialization. These findings reveal  
28 that functional traits have great potential, but also key limitations, as a tool for developing more  
29 mechanistic approaches in community ecology.

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

## 36 **Introduction**

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

49           Mutualistic systems of plant-pollinator interactions contain classic examples of trait-  
50 matching that have been highlighted as textbook examples of coevolution (e.g. Thompson, 2005).  
51 For instance, the length of a moth's proboscis, or the length of a hummingbird's bill, often match  
52 the corolla length of their food plants (Nilsson, 1988; Temeles & Kress, 2003). Trait-matching and  
53 floral specificity may be energetically advantageous for the pollinators and increase pollination  
54 efficiency (Montgomerie et al., 1984). Accordingly, both trait-matching and trait-mediated resource  
55 specialization have been reported for local plant-pollinator interaction networks (Stang et al., 2009;  
56 Maglianesi et al., 2014; Vizentin-Bugoni et al., 2014; Weinstein & Graham, 2017; Klumpers et al.,  
57 2019). However, drawing general conclusions in community ecology requires comparative studies  
58 of local communities across biogeographic regions (Lessard et al., 2012), and biogeographical  
59 history has been suggested to influence the role traits play in mediating plant-pollinator interactions

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

71           In addition to biogeographical history, if trait-matching and trait-mediated resource  
72 specialization are invariant properties of pollination networks, they should manifest repeatedly  
73 among distinctly related groups within a pollination system. For example, the mutualistic  
74 association between hummingbirds and their nectar-food plants is the most specialized avian  
75 pollination system (Fleming & Muchhala, 2008; Zanata et al., 2017), largely manifested in the  
76 match between the length of hummingbird bills and the length of the flowers they feed on  
77 (Feinsinger & Colwell, 1978; Stiles, 1981; Cotton, 1998; Dalsgaard et al., 2009; Maruyama et al.,  
78 2014; Vizentin-Bugoni et al., 2014; Weinstein & Graham, 2017; Sonne et al., 2020). However,  
79 hummingbirds consist of nine evolutionary distinct clades (McGuire et al., 2014), which differ  
80 greatly in their bill morphology and floral preferences (Feinsinger & Colwell, 1978; Bleiweiss,  
81 1988). Thus, if trait-matching is universal for hummingbird-plant associations, bill length and  
82 corolla length of visited flowers should co-vary between hummingbird clades. In other words,  
83 hummingbird clades consisting of long-billed species should prefer flowers with long corollas and

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

91           To examine whether evolutionary and biogeographical histories influence the  
92 generality of trait-matching and trait-mediated resource specialization in assemblages of plants and  
93 pollinators, we compiled a database of 93 quantitative hummingbird-plant networks distributed  
94 widely across continental America and the Caribbean islands. Each network represents the  
95 mutualistic interactions occurring within local assemblages of hummingbirds and their food plants  
96 (Dalsgaard et al., 2011), for which we gathered data on hummingbird bill length and the effective  
97 floral corolla length (*sensu* Wolf et al., 1976). To test the generality of trait-matching and trait-  
98 mediated resource specialization across evolutionary and biogeographical histories, we divided the  
99 hummingbirds into their nine principal clades and used knowledge on hummingbird biogeography  
100 to divide the networks into four biogeographical regions: Lowland South America, Andes, North &  
101 Central America, and the Caribbean islands (McGuire et al., 2014). We used this unique set of  
102 hummingbird-plant networks and trait data to test: (i) whether hummingbird clades and  
103 biogeographical regions differ in hummingbird bill length, corolla length of visited flowers and  
104 resource specialization, and (ii) whether hummingbirds' bill length correlates with the corolla  
105 length of their food plants and with their level of resource specialization, which we examined both  
106 for the entire dataset and within each hummingbird clade and biogeographical region.

107

108 **Materials and methods**

109 *Datasets: hummingbird-plant networks and traits*

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

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

132 network, if data on floral corolla length was collected in several localities (~19% of the species), we  
133 calculated species averages across localities. In total, we obtained floral corolla length estimates for  
134 962 plant species (76% of the species; Appendix 3). As a supplement to bill and corolla length, we  
135 attempted to gather data on bill and corolla curvature, as these traits may also match and the  
136 combination of bill length and curvature may better predict hummingbird resource specialization  
137 than bill length alone (Maglianesi et al., 2014; Sonne et al., 2019). There was insufficient data  
138 available on floral curvature to be included in our analyses, but we were able to gather a  
139 comprehensive dataset for bill curvature (99% of the species; Appendix 2); this we used in  
140 supplementary analyses to validate our main focus on bill length. The bill length, bill curvature and  
141 corolla length trait data can be downloaded from DRYAD [Link to be inserted upon acceptance of  
142 the manuscript].

143

#### 144 *Measuring resource specialization*

145 For each hummingbird species within the 93 hummingbird-plant networks, we calculated resource  
146 specialization using two metrics, one reflecting niche overlap and one reflecting niche breadth. As a  
147 measure of niche overlap, we used the complementary specialization index  $d'$  (Blüthgen et al.,  
148 2006). We used this index as it is robust to variation in sampling effort, more than other measures of  
149 resource specialization (Fründ et al., 2016). The index derives from Shannon's entropy and  
150 quantifies the extent to which observed interaction frequencies differ from random encounter  
151 probabilities of species, as derived from species' total interaction frequencies (Blüthgen et al.,  
152 2006). This follows the assumption that if species specialize on specific interaction partners, these  
153 preferences should be captured as deviations from random encounters given by partner availability  
154 (Blüthgen et al., 2006). The index  $d'$  ranges between 0 and 1 for extreme generalization and extreme  
155 specialization, respectively (Blüthgen et al., 2006). As a measure of hummingbird niche breadth, we

156 used the proportional generality index; a quantitative version of proportional resource use  
157 (normalised degree in binary networks), making it suitable for comparisons between networks  
158 (Cusser et al., 2019). A proportional generality value at or close to zero indicates a narrow niche  
159 breadth (i.e. a specialized species), whereas higher values indicate a broader niche breadth. Note  
160 that the proportional generality index may be larger than one. For each hummingbird species in  
161 each network, we calculated species-level specialization  $d'$  and proportional generality using the  
162 *bipartite* package in R (Dormann et al., 2008).

163

#### 164 *Biogeographical regions*

165 The datasets were separated into four major biogeographical regions: Lowland South America,  
166 Andes, North & Central America, and the Caribbean (Fig. 1). This separation is based on the  
167 division of hummingbirds into biogeographical regions, as extant hummingbirds supposedly  
168 originated in lowland South America ~ 22 million years ago, then expanded into the Andes (~ 16  
169 million years ago) and north of the Isthmus of Panama (~ 12 million years ago), arriving in the  
170 Caribbean region more recently ~ 5 million years ago (McGuire et al., 2014). Our ‘Lowland South  
171 America’ region includes all networks south of the Isthmus of Panama, excluding networks located  
172 in the Andean mountains. The ‘Andes’ region includes all networks within the Andean mountains  
173 as defined by Rahbek et al. (2019). The ‘North & Central America’ region includes all networks  
174 located on the mainland north of the Isthmus of Panama. Finally, the ‘Caribbean’ region includes all  
175 networks located on oceanic islands in the Caribbean Basin, excluding the continental island of  
176 Trinidad located south of Bond’s line, which for biogeographical reasons was included in the  
177 ‘Lowland South America’ region (Carstensen et al., 2013). The 93 hummingbird-plant interaction  
178 networks were distributed as follows: 41 in Lowland South America, 22 in North & Central  
179 America, 21 in the Andes, and nine in the Caribbean (Fig. 1). We observed most hummingbird

180 species in the Andean networks (76 species), followed by Lowland South America (55 species),  
181 North & Central America (53 species) and the Caribbean island networks (12 species). With respect  
182 to hummingbird-visited plant species, we recorded 641 species in Lowland South America, 367  
183 species in the Andes, 233 species in North & Central America, and 65 species in the Caribbean.

184

#### 185 *Statistical analyses*

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

195 Second, we used one-way ANOVA tests followed by FDR adjusted multiple comparisons to  
196 examine whether functional traits (hummingbird bill length, floral corolla length) and hummingbird  
197 resource specialization (complementary specialization  $d'$ , proportional generality) varied between  
198 (a) biogeographical regions and (b) hummingbird clades. When performing the one-way ANOVAs  
199 between hummingbird clades, the comparison was based on all observed species within each clade,  
200 irrespective of regional affinities. We only used the seven most species-rich clades in our dataset,  
201 excluding the species-poor clades *Topazes* (three species) and *Patagona* (one species). When  
202 performing the one-way ANOVAs between biogeographical regions, the comparison was based on  
203 all species observed within each region, i.e. we allowed species to be affiliated with multiple

204 biogeographical regions (only 13 hummingbird species, i.e. ~7%, occurred in two regions; four  
205 species, i.e. ~2%, in three regions, and no species occurred in four regions). For all analyses, we  
206 log-transformed bill and corolla length. Supplementary one-way ANOVAs showed that bill  
207 curvature varied significantly between hummingbird clades and regions (for details, see Fig. S3 in  
208 Supporting Information), but there was no indication that this caused clade and regional differences  
209 in resource specialization (compare Figs. 2-3 with Fig. S3). The ANOVA tests were run using the  
210 ‘anova’ function in R. We also constructed linear-mixed effect models with the aim to control for  
211 clades as a random intercept (when examining how regions differ in traits and specialization level)  
212 and regions as a random intercept (when examining how clades differ in traits and specialization  
213 level). However, these models did not converge due to singularities (see Bates et al., 2015),  
214 probably caused by hummingbird clades being non-randomly distributed across regions (see the  
215 Results of Fisher's Exact Test). Third, we investigated whether the length of hummingbird  
216 bills was associated positively with: (a) the mean corolla length of the flowers they visit, and (b)  
217 their degree of resource specialization (complementary specialization  $d'$ , proportional generality)  
218 averaged across networks. We tested these potential associations with linear mixed-effects models  
219 (LMMs) using the *nlme* package in R (Pinheiro et al., 2019). Models simultaneously including  
220 hummingbird clade and biogeographical region as random effects did not converge, so for each  
221 response variable we built two sets of models both with bill length as a fixed effect: one considering  
222 hummingbird clades and another considering biogeographical regions as random intercepts. When  
223 using clades as a random factor, mean corolla length and resource specialization were estimated for  
224 each species irrespective of regional affinity. When using regions as random factor, mean corolla  
225 length and resource specialization were estimated for each species within each region. For each of  
226 these analyses, we constructed both unweighted and weighted models, the latter weighted with the  
227 square root number of plants visited (when predicting mean corolla length) and the square root

228 number of networks (when predicting mean resource specialization; Maglianesi et al., 2014). The  
229 weighting procedure gave higher weight to hummingbird species that visited more plants and  
230 occurred in more networks, respectively. We constructed both weighted and unweighted LMMs  
231 because estimates of mean corolla length and mean resource specialization may be more reliable  
232 when based on larger sample sizes, however, it may also bias the results towards frequent and  
233 geographically widespread species. To estimate the significance of bill length in the LMMs, we  
234 used the function ‘anova’ from the *car* package in R (Fox & Weisberg, 2019). We estimated the  
235 proportion of variance explained by bill length in the LMMs as marginal  $R^2$ , and the proportion of  
236 variance explained by both fixed and random factors as conditional  $R^2$  with the function  
237 ‘r.squaredGLMM’ in the MuMIn package (Nakagawa et al., 2017; Barton, 2020).

238 In addition to the LMMs, we estimated Pagel’s lambda  $\lambda$  to assess how well evolutionary  
239 relatedness explains the distribution of bill length, mean corolla length and resource specialization  
240 among hummingbird species (Pagel, 1999). Subsequently, to account for the non-independence of  
241 the species-level data in our regression analyses, we repeated these using phylogenetic least squares  
242 regression (PGLS) with the function ‘pgls’ in the R package *caper* (Orme et al., 2018). In these  
243 analyses, we derived the expected co-variances among all species using the maximum clade  
244 credibility tree of McGuire et al. (2014). To reconcile our trait dataset with the McGuire et al.  
245 (2014) phylogeny we: (1) excluded species included in our networks but not sampled in the tree, (2)  
246 dropped species that McGuire et al. (2014) showed evidence as being either paraphyletic or  
247 polyphyletic with respect to other taxa included in our networks, and (3) used the mean bill / corolla  
248 length and specialization value for species pairs that represent recent taxonomic splits only  
249 represented by a single tip in the McGuire et al. (2014) phylogeny. These amendments reduced our  
250 dataset to 155 species (bill vs. corolla length) and 158 species (bill vs. specialization) in the PGLS  
251 regressions. As additional analyses, we repeated the unweighted / weighted and PGLS regressions

252 separately for each of the seven hummingbird clades (not for Topazes and *Patagona* with only three  
253 and one species, respectively) and for each of the four biogeographical regions. Finally, we  
254 constructed supplementary LMM and PGLS regressions to examine whether bill curvature provides  
255 additional explanatory power in predicting resource specialization. These analyses showed  
256 negligible effects of bill curvature (for details, see Table S1).

## 257 **Results**

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

259 There was a clear difference in the distribution of hummingbird clades between the four regions ( $P$   
260  $< 0.05$ ; Fig. 1). Notably, the Brilliants and Coquettes dominated the Andean networks and appeared  
261 in very low numbers outside of the Andes. In the other three regions, Emeralds were the most  
262 species-rich group, along with Hermits (Lowland South America), Mountain Gems and Bees (North  
263 & Central America) and Mangoes (Caribbean). Likewise, there were clear differences in plant  
264 family distribution between the four regions ( $P < 0.05$ ; Fig. 1). The Ericaceae dominated the  
265 Andean networks, and were well represented in North & Central America, but had few species in  
266 the Lowland South American and the Caribbean networks. The Bromeliaceae dominated in the  
267 Lowland South American networks, and were well represented in the Andean and North & Central  
268 American networks, but were poorly represented in the Caribbean. The Caribbean networks were  
269 dominated by the Rubiaceae, which were also well represented in the other regions (Fig. 1).

270

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

272 The hummingbird clades differed significantly in bill length and the length of the flowers visited  
273 (bill length:  $F_{6, 165} = 25.29$ ,  $P < 0.001$ ; corolla length:  $F_{6, 165} = 12.64$ ,  $P < 0.001$ ; Fig. 2a). The  
274 Hermits and Mountain Gems had the longest bills, although the bill length of Mountain Gems was

275 not significantly longer than those of Mangoes and Brilliants. The shortest bills were those of the  
276 Coquettes and Bees, whereas Emeralds had bills of intermediate length, only overlapping with those  
277 of Mangoes. The Hermits and Mountain Gems visited the longest flowers, although the flowers  
278 visited by Mountain Gems were not significantly longer than those visited by Brilliants and  
279 Mangoes. The Coquettes and Bees visited the shortest flowers, although the Coquettes' flowers  
280 were not significantly shorter than those visited by Emeralds and Mangoes (Fig. 2a). Hummingbird  
281 resource specialization  $d'$  largely differed among clades in accordance with differences in bill /  
282 corolla length: the Hermits and Mountain Gems were the most specialized clades (i.e., species in  
283 these clades were most exclusive in their resource use), significantly more specialized than the Bees  
284 and Emeralds, with intermediate levels of specialization shown by Mangoes, Brilliants and  
285 Coquettes ( $F_{6, 169} = 4.29, P < 0.001$ ; Fig. 2b). The hummingbird clades showed similar level of  
286 proportional generality, i.e. niche breadth did not differ significantly between clades ( $F_{6, 169} = 1.19,$   
287  $P = 0.32$ ; Fig. 2c).

288 Hummingbird bill length was similar across regions ( $F_{3, 191} = 1.15, P = 0.33$ ), but there  
289 were regional differences in the corolla length of hummingbird-visited flowers ( $F_{3, 575} = 10.15, P <$   
290  $0.001$ ): North & Central American flowers were the longest, whereas the Caribbean flowers were  
291 significantly shorter than flowers in all mainland regions (Fig. 3a). There were also regional  
292 differences in hummingbird resource specialization, both when measured as  $d'$  ( $F_{3, 192} = 2.82, P <$   
293  $0.05$ ) and proportional generality ( $F_{3, 192} = 13.35, P < 0.05$ ). Notably, the Caribbean hummingbirds  
294 were more generalized than mainland hummingbirds, both in terms of niche overlap ( $d'$ ) and niche  
295 breadth (proportional generality) (Fig. 3b-c).

296

297 *Associations between traits and between traits and resource specialization*

298 In LMMs across all hummingbird species, when including region as a random factor, bill length  
299 was strongly and positively associated with mean corolla length (Table 1; Fig. 4b), weakly related  
300 to mean resource specialization when measured as  $d'$  (Table 1; Fig. 4d), but unrelated to mean  
301 proportional generality (Table 1; Fig. 4f). When including clade as a random factor, hummingbird  
302 bill length was also strongly positively associated with mean corolla length (Table 1; Fig. 4a), but  
303 bill length was not associated with neither measure of resource specialization (Table 1; Fig. 4c, Fig.  
304 4e). The species-level variation in bill length, mean corolla length of visited flowers, and both  
305 measures of resource specialization displayed a phylogenetic signal that was significantly greater  
306 than zero ( $P < 0.001$  in all cases). In particular, closely related hummingbird species are likely to be  
307 similar in bill length ( $\lambda = 0.97$ ), more so than the corolla length of visited flowers ( $\lambda = 0.59$ ), with  
308 weaker phylogenetic signals for resource specialization: proportional generality ( $\lambda = 0.51$ ) and  $d'$  ( $\lambda$   
309  $= 0.23$ ). When accounting for this phylogenetic non-independence using PGLS, we continued to  
310 detect a strong positive association between bill length and mean floral corolla length ( $\beta = 0.83$ ,  $R^2$   
311  $= 0.32$ ,  $P < 0.001$ ), but there was no association between bill length and mean resource  
312 specialization, both measured as  $d'$  ( $\beta = 0.05$ ,  $R^2 = 0.00$ ,  $P = 0.70$ ) and proportional generality ( $\beta =$   
313  $0.01$ ,  $R^2 = 0.00$ ,  $P = 0.94$ ).

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

320           For all four regions analysed separately, there was a strong positive association  
321 between hummingbird bill length and the mean corolla length of their flowers (Fig. S2a). The

322 association between bill length and resource specialization  $d'$  was non-significant for all four  
323 regions when using unweighted regressions and PGLS, but weakly positive for Andes and Lowland  
324 South America when using weighted regressions (Fig. S2b). There was no association between bill  
325 length and proportional generality, i.e. niche breadth, in any of the regions (all fits had  $P > 0.05$ ).

326

## 327 **Discussion**

328 We demonstrate that morphological trait-matching is a recurrent phenomenon in hummingbird-  
329 plant networks throughout the Americas, i.e. the length of a hummingbird's bill correlates with the  
330 corolla length of the flowers they visit (Fig. 4a-b). This result was repeated within all  
331 biogeographical regions and within most hummingbird clades (Fig. S1-S2). Trait-matching was also  
332 evident when comparing between clades, as clades dominated by long-billed hummingbirds (e.g.  
333 Hermits) generally visited longer flowers and *vice versa* for clades with shorter bills (Fig. 2a).  
334 These recurrent patterns of trait-matching may have been even stronger if we had local trait  
335 measurements for all hummingbird-visited flowers and local measurements of hummingbird traits  
336 (rather than from museum specimens). When comparing between clades, traits also largely co-  
337 varied with resource specialization (niche overlap  $d'$ ), e.g. the long-billed Hermits were also the  
338 most exclusive in their resource use (Fig. 2b). However, there was no difference in niche breadth  
339 between clades (Fig. 2c) and morphological traits did not generally mediate resource specialization,  
340 as bill length was only a weak predictor of both measures of resource specialization (Fig. 4c-f; bill  
341 curvature had negligible effects on resource specialization, see Table S1). Notably, bill length was  
342 associated with resource specialization only within the Brilliants and within the regions of Lowland  
343 South America and the Andes (Fig. S1-S2). Taken together, these results demonstrate the strong  
344 influence of biogeographical and evolutionary histories on recurrent patterns of morphological trait-  
345 matching, and the weak relationships between hummingbird bill length and resource specialization.

346           The recurrent patterns of trait-matching show that hummingbirds feed on flowers  
347 approximately similar in length to their bills, even though long-billed hummingbirds are able to  
348 access nectar from flowers with shorter corollas. This may be because long-billed hummingbirds  
349 minimize competition with short-billed hummingbirds by primarily feeding on flowers inaccessible  
350 to short-billed hummingbirds (Maglianesi et al., 2015; Sonne et al., 2020). It may also be related to  
351 nectar production, as longer flowers often offer more nectar than shorter flowers (Stiles, 1981;  
352 Buzato et al., 2000; Ornelas et al., 2007; Dalsgaard et al., 2009), making it energetically optimal for  
353 longer-billed hummingbirds to feed on flowers with longer corollas, up to a given corolla length  
354 threshold (Montgomerie et al., 1984). From the plant's perspective, it may also be an advantage to  
355 attract hummingbirds with matching traits, thereby increasing pollination efficiency (Montgomerie  
356 et al., 1984). Therefore, there is a clear association between functional traits of hummingbirds and  
357 plants. Similar patterns have been reported for other plant-pollinator systems, such as hawkmoths  
358 and other insect pollinators visiting flowers approximately similar in length to their proboscises  
359 (Stang et al., 2009; Sazatornil et al., 2016; Klumpers et al., 2019). Interestingly, on average, species  
360 in all hummingbird clades visited flowers with slightly longer corollas than their bills (Fig. 2a),  
361 reflecting that hummingbirds extend their tongues while drinking nectar (Hainsworth, 1973;  
362 Montgomerie et al., 1984). Notably, the Bees and the Coquettes – the two clades with the shortest  
363 bills – visited flowers considerably longer than their bills (Fig. 2a). This may be because short-  
364 billed hummingbirds have evolved the ability to extend their tongues proportionally longer than  
365 long-billed hummingbirds (Hainsworth, 1973), allowing short-billed species to exploit flowers with  
366 longer corolla and more nectar than short-corolla flowers fitting their bills.

367           Floral corolla length was on average also longer than hummingbird bill length across  
368 all mainland regions, but not in the Caribbean islands where floral corolla length was shorter than  
369 on the mainland (Fig. 3a). This result is consistent with reports that the Caribbean hummingbirds

370 often feed on shorter insect-syndrome flowers (Dalsgaard et al., 2009; Lehmann et al., 2019) and  
371 have a more generalized feeding behaviour than mainland hummingbirds, both in terms of floral  
372 niche overlap and niche breadth (Fig. 3b-c). Although the majority of the plants visited by  
373 Caribbean hummingbirds are either endemic or native to the region (~33% endemic and ~55%  
374 native in our dataset), their opportunism also makes Caribbean hummingbirds more likely to  
375 incorporate introduced plants into their feeding niche (~12% in our dataset; Maruyama et al., 2016).  
376 As all except one of the Caribbean hummingbirds are endemic to the region (Dalsgaard et al.,  
377 2018), these Caribbean *vs.* mainland patterns are in agreement with the idea that many plants and  
378 pollinators on oceanic islands have evolved towards generalism (Olesen et al., 2002; Olesen &  
379 Valido, 2003; Traveset et al., 2015), but may also reflect the more recent colonization history and  
380 limited trait evolution among Caribbean hummingbirds (Dalsgaard et al., 2018). Taken together,  
381 despite the distribution of plant families and hummingbird clades differ significantly across regions  
382 (Fig. 1), functional traits and the degree of resource specialization were similar across mainland  
383 regions, but the Caribbean islands had both shorter flowers and hummingbirds with more  
384 generalized niches (Fig. 3). Traits and resource specialization (niche overlap  $d'$ ) also largely co-  
385 varied between hummingbird clades (Fig. 2), illustrating the influence of biogeographical and  
386 evolutionary histories in both functional traits and floral specificity.

387           Despite the generally consistent trait-matching and associated level of resource  
388 specialization observed between clades, and between mainland *vs.* islands (Figs. 2-3), trait-  
389 matching did not generally translate into trait-mediated resource specialization for individual  
390 species, as bill length and bill curvature were only weak predictors of resource specialization (Fig.  
391 4c-f; Table 1, Table S1). While the diversity of hummingbird traits within a community influences  
392 overall community-level specialization and partitioning of interactions (Maruyama et al., 2018),  
393 additional mechanisms appear to operate at the species-level (Tinoco et al., 2017; Simmons et al.,

394 2019). Notably, while functional traits may constrain species into their fundamental niche of  
395 possible pairwise interactions (Junker et al., 2013), a hierarchy of multiple mechanisms determines  
396 the realized niche, i.e. which of the possible pairwise interactions are realized (Junker et al., 2013).  
397 In hummingbird-plant communities, trait-matching determines which interactions are possible  
398 (Sonne et al., 2020), but other mechanisms – such as hummingbird abundance and local conditions  
399 related to resource availability and competition with other floral visitors – determine how often  
400 these interactions are realized (Tinoco et al., 2017; Dalsgaard et al., 2018; Simmons et al., 2019).  
401 Thus, there is no general relationship between bill morphology and level of resource specialisation  
402 (Fig. 4c-f; Table 1, Table S1), illustrating that morphological and ecological specialization can be  
403 disassociated (Ollerton et al., 2007; Armbruster, 2017). The only exceptions to this trend are the  
404 weak associations we found between bill length and resource specialization within the Brilliants and  
405 within Lowland South America and the Andes. Here, hummingbirds and plants have had the longest  
406 co-evolutionary history (McGuire et al., 2014) and have experienced more benign conditions during  
407 the Quaternary to evolve more specialized associations (Dalsgaard et al., 2011; Sonne et al., 2016).  
408 A prime example of this is the association between the Andean species of *Passiflora* and the Sword-  
409 billed Hummingbird (*Ensifera ensifera*), an iconic long-billed species within the Brilliants  
410 (Abrahamczyk et al., 2014). The Sword-billed Hummingbird has by far the longest bill of any  
411 hummingbird species, uses very long-corolla flowers, and tends to be ecologically specialized (Figs.  
412 4, S1-S2). In line with that, long-tubed *Passiflora*, and other plants specialized on hummingbird-  
413 pollination, tend to have evolved longer corolla flowers when compared to related plants pollinated  
414 by most other groups of pollinators (Abrahamczyk et al., 2014; Pauw, 2019). Nevertheless, even  
415 these specialised systems with tightly matching traits may have been dynamic over evolutionary  
416 time (Abrahamczyk et al., 2017) and generally long-billed and short-billed hummingbirds show  
417 similar degrees of resource specialization (Fig. 4c-f).

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

429

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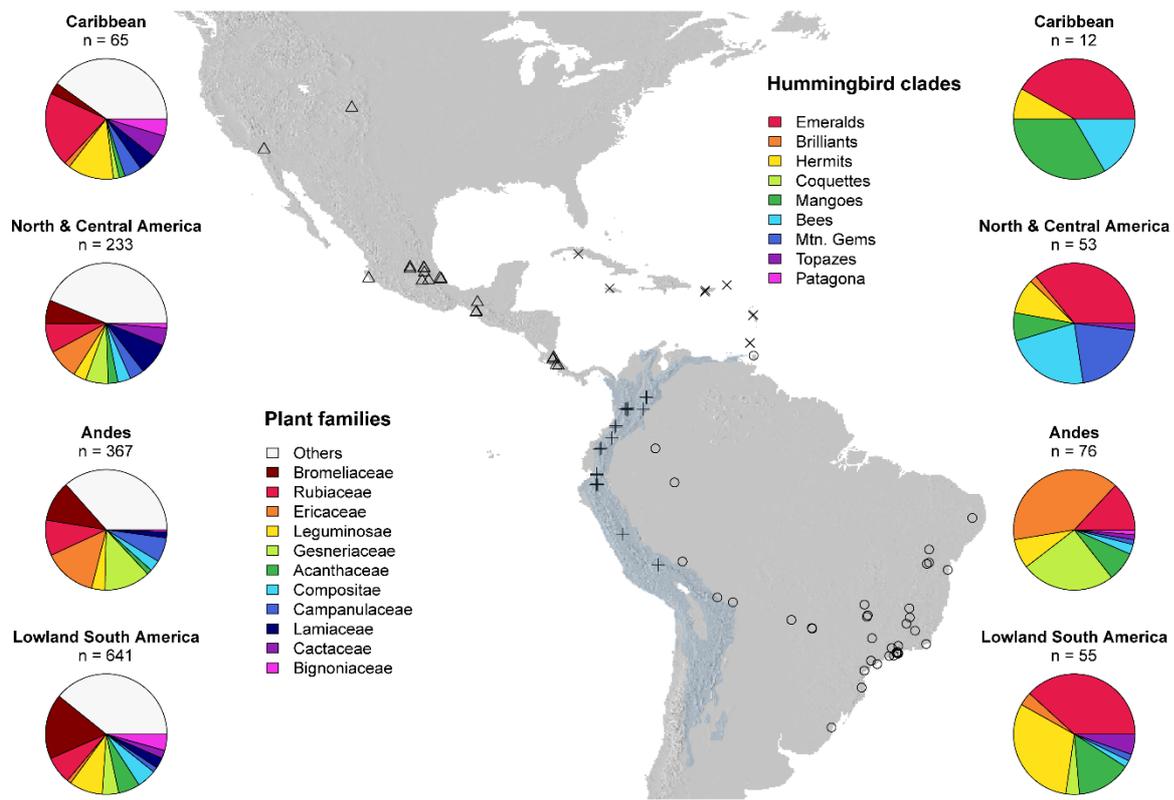
628 **Table 1.** Linear mixed-effects models (LMMs), analysing the association between hummingbird bill  
629 length and (a) mean corolla length of visited flowers, and mean resource specialization, measured  
630 both as (b) complementary specialization  $d'$  and (c) proportional generality. For each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

<b>Model</b>	<b>Random factor</b>	<b><math>R^2</math> marginal</b>	<b><math>R^2</math> conditional</b>	<b>coefficient</b>	<b>Std Error</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 generality	<b>clade</b>	<b>0.00</b>	<b>0.02</b>	<b>0.07<sup>NS</sup></b>	<b>0.12</b>
	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

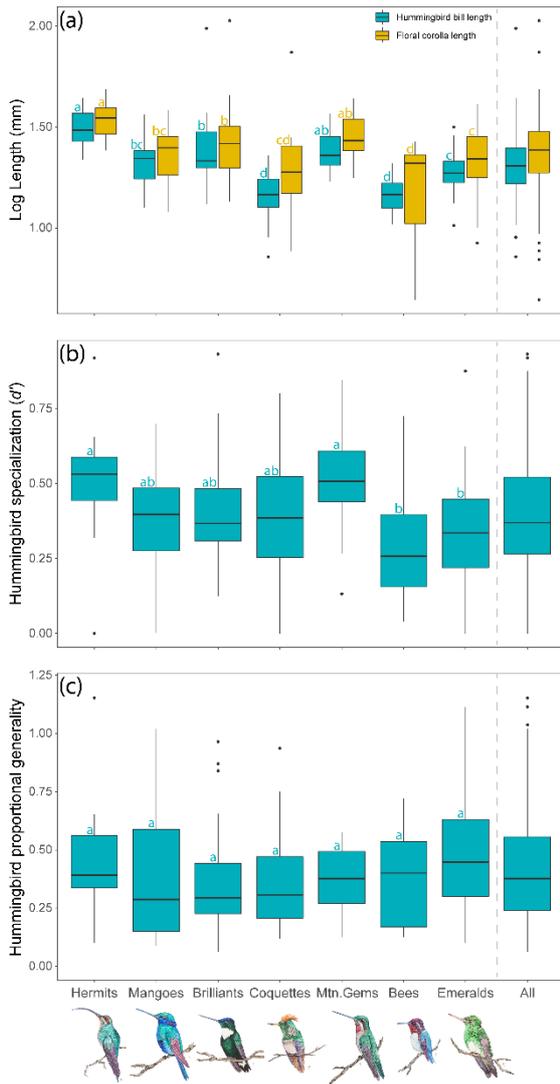
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640 \*\*P<0.001, \*p < 0.05, <sup>NS</sup> P > 0.05



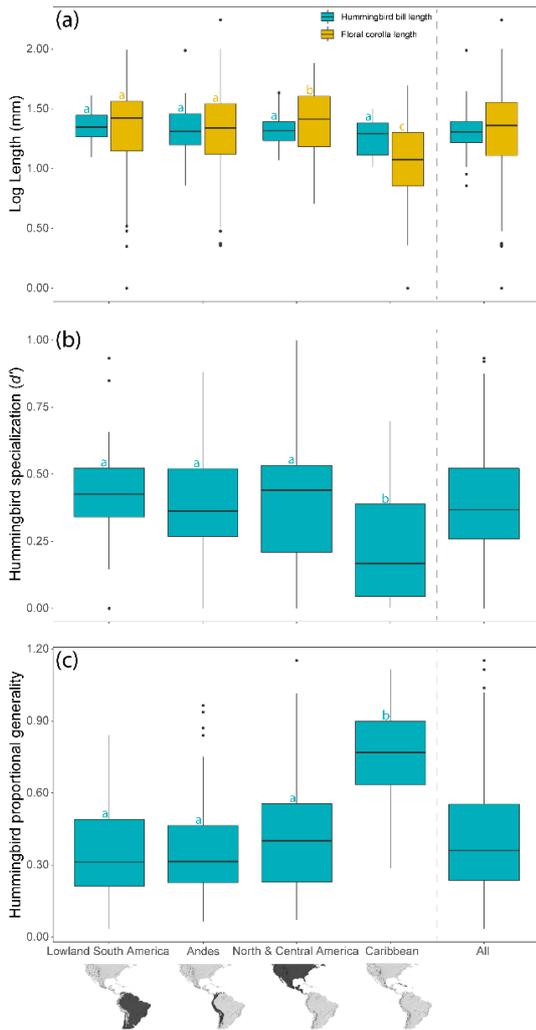
641

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



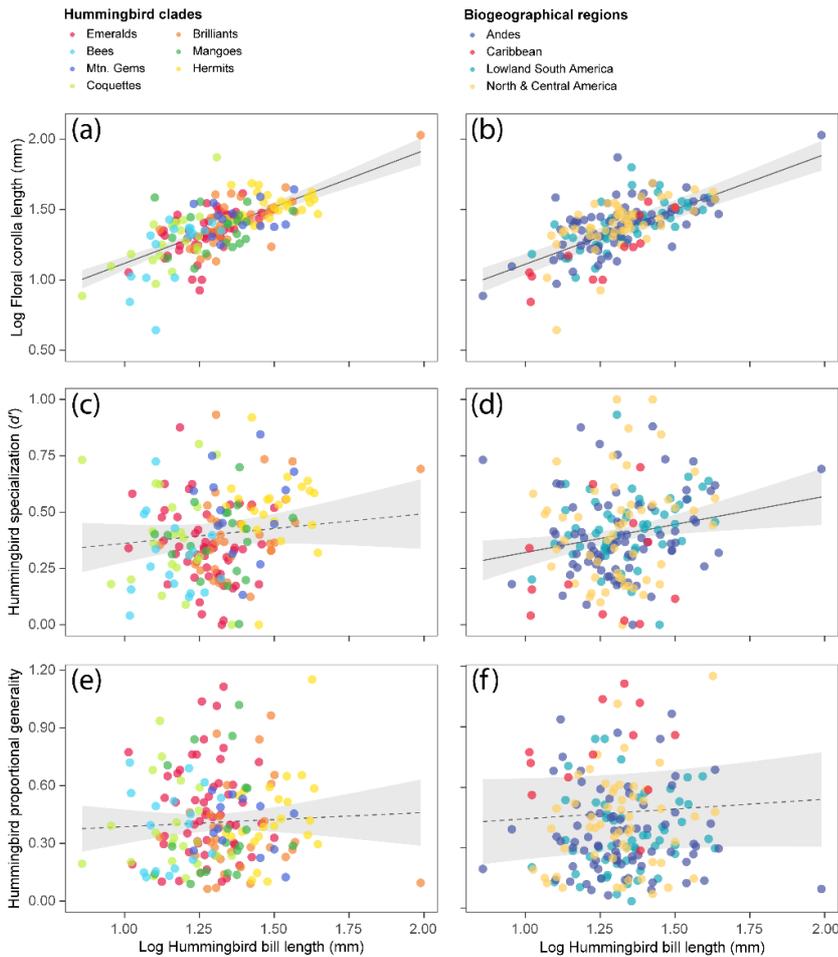
653

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



663

664 **Figure 3.** Differences in (a) hummingbird bill length (turquoise) and mean floral corolla length  
 665 (yellow), (b) mean hummingbird specialization  $d'$ , and (c) mean hummingbird specialization  
 666 measured as proportional generality between biogeographical regions: Lowland South America,  
 667 Andes, Central & North America, and the Caribbean. The comparison was based on the species  
 668 pool for each region, as extracted in the networks within each region. Boxes indicate the first and  
 669 third quartiles (Q1 and Q3), horizontal lines inside boxes are medians, vertical lines indicate Q1/Q3  
 670 + 1.5 x interquartile ranges (IQR), and circles are outliers. Different letters represent statistical  
 671 difference ( $P < 0.05$ ) according to one-way ANOVAs with multiple post-hoc comparisons using the  
 672 False Discovery Rate (FDR). For comparison, on the right in both figures we show boxplots across  
 673 all species.



674

675 **Figure 4.** Associations between hummingbird bill length and (a, b) mean floral corolla length, (c, d)

676 mean hummingbird specialization  $d'$  and (e, f) mean hummingbird specialization measured as

677 proportional generality, when using hummingbird clades as a random factor (clades in different

678 colours: a, c and e;  $n = 172$  and  $n = 177$ ) and when using biogeographical regions as a random

679 factor (regions in different colours: b, d and f;  $n = 191$  and  $n = 195$ ). The black lines (with grey 95%

680 confidence intervals) represent the overall fits of unweighted linear-mixed effects models; the

681 dotted line represents a non-significant fit. Note that a few hummingbird species were recorded in

682 more than one region and, thus, appear more than once in the analyses including regions as a

683 random factor (b, d and f). See supplementary Figure S1 for individual plots for each hummingbird

684 clade and Figure S2 for individual plots for each biogeographical regions.

## Supporting Information

**Article title:** The influence of biogeographical and evolutionary histories on morphological trait-matching and resource specialization in mutualistic hummingbird-plant networks

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## Appendix 1. Mutualistic hummingbird-plant interaction networks

The dataset consists of 93 quantitative hummingbird-plant interaction networks distributed widely across the Americas. Each of the 93 networks describes interaction frequencies between assemblages of hummingbirds and their food plants in a specific location. We only included mutualistic interactions in which a given hummingbird was observed drinking nectar and touching the stigma / anthers of the given flower, thereby potentially acting as a pollinator, i.e. non-mutualistic interactions such as nectar robbing were not included. Below we provide information on sampling locations, their assigned biogeographical region (Lowland South America, Andes, North & Central America, or the Caribbean) and their original sources. The networks can be downloaded at Dryad Digital Repository

(<https://doi.org/10.5061/dryad.rr4xgxd7n>, Dalsgaard et al., 2021).

Country	Latitude	Longitude	Biogeographical region	Source
USA	38.98	-106.97	North & Central America	Ollerton et al. (2009) <i>Ann. Bot.</i> , 103, 1471–1480
USA	34.22	-116.95	North & Central America	Alarcón et al. (2008) <i>Oikos</i> , 117, 1796–1807
Cuba	22.28	-81.20	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754
Mexico	20.76	-100.35	North & Central America	Own unpublished data
Mexico	20.70	-98.77	North & Central America	Martínez-García & Ortiz-Pulido (2014) <i>Ornit Neotrop</i> , 25, 273–289
Mexico	20.68	-98.76	North & Central America	Martínez-García & Ortiz-Pulido (2014) <i>Ornit Neotrop</i> , 25, 273–289
Mexico	20.61	-98.75	North & Central America	Martínez-García & Ortiz-Pulido (2014) <i>Ornit Neotrop</i> , 25, 273–289
Mexico	20.52	-100.35	North & Central America	Own unpublished data
Mexico	20.13	-98.71	North & Central America	Own unpublished data
Mexico	19.50	-105.05	North & Central America	Arizmendi & Ornelas (1990) <i>Biotropica</i> , 22, 172–180
Mexico	19.50	-96.95	North & Central America	Own unpublished data
Mexico	19.40	-96.80	North & Central America	Own unpublished data
Mexico	19.28	-98.23	North & Central America	Own unpublished data
Mexico	19.23	-98.97	North & Central America	Own unpublished data
British Virgin Islands	18.73	-64.32	Caribbean	Own unpublished data
Jamaica	18.35	-77.65	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754

Puerto Rico	18.13	-66.76	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754
Puerto Rico	17.95	-66.82	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754
Mexico	16.75	-92.68	North & Central America	Partida-Lara et al. (2012) <i>Rev. Biol. Trop.</i> , 60, 1621-1630
Mexico	15.66	-92.80	North & Central America	Partida-Lara et al. (2018) <i>J. Trop. Ecol.</i> , 34, 293-307
Mexico	15.63	-92.81	North & Central America	Partida-Lara et al. (2018) <i>J. Trop. Ecol.</i> , 34, 293-307
Mexico	15.59	-92.85	North & Central America	Partida-Lara et al. (2018) <i>J. Trop. Ecol.</i> , 34, 293-307
Dominica	15.35	-61.30	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754
Dominica	15.25	-61.37	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754
Grenada	12.10	-61.70	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754
Grenada	12.10	-61.68	Caribbean	Dalsgaard et al. (2018) <i>Proc. R. Soc. B.</i> , 285, 20172754
Trinidad & Tobago	10.67	-61.28	Lowland South America	Snow & Snow (1972) <i>J. Anim. Ecol.</i> , 41, 471-485
Costa Rica	10.44	-84.01	North & Central America	Maglianesi et al. (2014) <i>Ecology</i> 95, 3325-3334
Costa Rica	10.27	-84.08	North & Central America	Maglianesi et al. (2014) <i>Ecology</i> 95, 3325-3334
Costa Rica	10.18	-84.11	North & Central America	Maglianesi et al. (2014) <i>Ecology</i> 95, 3325-3334
Costa Rica	9.57	-83.73	North & Central America	Wolf et al. (1976) <i>J. Anim. Ecol.</i> , 45, 349-379
Costa Rica	9.48	-83.48	North & Central America	Wolf et al. (1976) <i>J. Anim. Ecol.</i> , 45, 349-379
Colombia	5.92	-73.53	Andes	Snow & Snow (1980) <i>Bull. Br. Mus. Nat. Hist. (Zool.)</i> , 38, 105-139
Colombia	5.90	-73.42	Andes	Snow & Snow (1980) <i>Bull. Br. Mus. Nat. Hist. (Zool.)</i> , 38, 105-139
Colombia	4.67	-75.57	Andes	Own unpublished data
Colombia	4.58	-75.86	Andes	Own unpublished data
Colombia	4.54	-75.77	Andes	Own unpublished data
Colombia	4.53	-73.85	Andes	Snow & Snow (1980) <i>Bull. Br. Mus. Nat. Hist. (Zool.)</i> , 38, 105-139
Colombia	4.50	-75.60	Andes	Own unpublished data
Colombia	2.67	-76.95	Andes	Ramírez-Burbano et al. (2017) <i>Biotropica</i> , 49, 555-564
Colombia	2.52	-76.98	Andes	Ramírez-Burbano et al. (2017) <i>Biotropica</i> , 49, 555-564
Colombia	1.25	-77.43	Andes	Gutierrez Aquiles EZ, Rojas-Nossa SV (2001) Dinámica anual de la interacción de colibrí-flor en ecosistemas altoandinos del volcán Galeras, sur de Colombia: Título de biología, Universidad Nacional de Colombia.
Ecuador	0.12	-78.63	Andes	Graham & Weinstein (2018) <i>Ecol. Lett.</i> , 21, 1299-1310
Ecuador	0.12	-78.60	Andes	Graham & Weinstein (2018) <i>Ecol. Lett.</i> , 21, 1299-1310
Colombia	0.07	-72.45	Lowland South America	Lasprilla & Sazima M (2004) <i>Ornit. Neotrop.</i> , 15, 183-190
Ecuador	-0.02	-78.77	Andes	Walther & Brieschke (2001) <i>Internat. J. Ornithol.</i> , 4, 115-135
Ecuador	-2.83	-79.13	Andes	Tinoco et al. (2017) <i>Oikos</i> , 126, 52-60.

Ecuador	-2.87	-79.12	Andes	Tinoco et al. (2017) <i>Oikos</i> , 126, 52–60.
Ecuador	-2.96	-79.10	Andes	Tinoco et al. (2017) <i>Oikos</i> , 126, 52–60.
Colombia	-3.82	-70.27	Lowland South America	Cotton (1998) <i>Ibis</i> 140, 639–646.
Ecuador	-3.97	-79.07	Andes	Sonne et al. (2019) <i>Biotropica</i> 51, 205–218
Ecuador	-4.11	-79.17	Andes	Sonne et al. (2019) <i>Biotropica</i> 51, 205–218
Ecuador	-4.11	-78.97	Andes	Sonne et al. (2019) <i>Biotropica</i> 51, 205–218
Brazil	-7.87	-36.40	Lowland South America	Las Casas et al. (2012) <i>Braz. J. Biol.</i> , 72, 51–58.
Peru	-9.71	-76.16	Andes	Gonzalez & Loiselle (2016) <i>PeerJ</i> , 4, e2789.
Brazil	-11.48	-41.32	Lowland South America	Own unpublished data
Peru	-12.85	-69.37	Lowland South America	Watts et al. (2016). <i>Ann. Bot.</i> 118, 415–429.
Brazil	-12.98	-41.33	Lowland South America	Machado et al. (2007) <i>Rev. Bras. Ornitol.</i> , 15, 215–227
Brazil	-13.12	-41.58	Lowland South America	Machado (2009) <i>Zoologia</i> , 26, 55–65
Brazil	-13.12	-41.57	Lowland South America	Machado (2014) <i>Biosci. J.</i> , 30, 1578–1587.
Peru	-13.22	-72.12	Andes	Watts et al. (2016). <i>Ann. Bot.</i> 118, 415–429.
Brazil	-13.81	-39.20	Lowland South America	Own unpublished data
Bolivia	-16.96	-65.41	Lowland South America	Abrahamczyk & Kessler (2010) <i>J. Ornithol.</i> , 151, 615–625.
Bolivia	-17.51	-63.63	Lowland South America	Abrahamczyk & Kessler (2010) <i>J. Ornithol.</i> , 151, 615–625.
Brazil	-17.78	-48.68	Lowland South America	Machado & Oliveira (2015) <i>Rodriguésia</i> , 66, 1–19.
Brazil	-18.20	-43.57	Lowland South America	Own unpublished data
Brazil	-18.99	-48.30	Lowland South America	Araújo et al. (2011). <i>Flora</i> , 206, 827–835 / Maruyama et al. (2013) <i>Naturwissenschaften</i> , 100, 1061–1068.
Brazil	-19.16	-48.39	Lowland South America	Araújo et al. (2013) <i>Plant Syst. Evol.</i> , 299, 1119–1133/ Maruyama et al. (2014) <i>Biotropica</i> , 46, 740–747.
Brazil	-19.25	-43.52	Lowland South America	Rodrigues & Rodrigues (2014) <i>Braz. J. Biol.</i> , 74, 659–676.
Brazil	-19.52	-56.98	Lowland South America	Araujo & Sazima M (2003) <i>Flora</i> , 198, 427–435
Brazil	-19.95	-43.90	Lowland South America	Vasconcelos & Lombardi (1999) <i>Ararajuba</i> , 7, 71–79.
Brazil	-20.44	-54.65	Lowland South America	Rodrigues & Araujo (2011) <i>Braz. J. Biol.</i> , 71, 611–622.
Brazil	-20.51	-54.62	Lowland South America	Barbosa-Filho & Araujo (2013). <i>Biota Neotrop.</i> , 13, 21–27
Brazil	-20.75	-42.92	Lowland South America	Abreu & Vieira (2004) <i>Lundiana</i> , 5, 129–134
Brazil	-21.62	-47.81	Lowland South America	Own unpublished data
Brazil	-22.28	-41.66	Lowland South America	Fonseca et al. (2015) <i>An. Acad. Bras. Ciên.</i> , 87, 2163–2175
Brazil	-22.50	-44.83	Lowland South America	Canela (2006) Ph.D thesis. University of Campinas, Brazil.
Brazil	-22.73	-45.58	Lowland South America	Sazima et al. (1996) <i>Bot. Acta</i> , 109, 149–160
Brazil	-23.17	-44.92	Lowland South America	Buzato et al. (2000) <i>Biotropica</i> , 32, 824–841

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Brazil	-23.28	-45.05	Lowland South America	Vizentin-Bugoni et al. (2016) <i>J. Anim. Ecol.</i> , 85, 262–272
Brazil	-23.32	-44.94	Lowland South America	Maruyama et al. (2015) <i>Oecologia</i> , 178,783–793
Brazil	-23.33	-44.83	Lowland South America	Maruyama et al. (2015) <i>Oecologia</i> , 178,783–793
Brazil	-23.35	-44.83	Lowland South America	Own unpublished data
Brazil	-23.36	-44.85	Lowland South America	Maruyama et al. (2015) <i>Oecologia</i> , 178,783–793
Brazil	-23.58	-45.33	Lowland South America	Buzato et al. (2000) <i>Biotropica</i> , 32, 824–841
Brazil	-23.63	-45.85	Lowland South America	Snow & Snow (1986) <i>Hornero</i> , 12, 286–296
Brazil	-24.18	-47.93	Lowland South America	Own unpublished data
Brazil	-24.56	-47.23	Lowland South America	Own unpublished data
Brazil	-25.32	-48.70	Lowland South America	Own unpublished data
Brazil	-27.25	-49.00	Lowland South America	Own unpublished data
Brazil	-27.25	-49.00	Lowland South America	Own unpublished data
Brazil	-27.25	-49.00	Lowland South America	Own unpublished data
Brazil	-31.80	-52.42	Lowland South America	Own unpublished data

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## Appendix 2. Hummingbird taxonomy, and bill length and curvature estimates

Species names of the hummingbirds follow the International Ornithological Committee World List (IOC version 9.2; [www.worldbirdnames.org](http://www.worldbirdnames.org)). We measured museum specimens to obtain bill lengths and curvatures for the 181 hummingbird species present in the plant-hummingbird networks (Appendix S1). To measure bill length/curvature, we took lateral photographs of the bill placed close to a ruler as a scale reference. The camera was positioned perpendicular to the right-sagittal plane of the bill, keeping a distance of 15 cm from the specimens to avoid image distortion. Bill length represents the chord of the exposed culmen, measured from the tip to the anterior extension of the feathers on the bill (Zanata et al., 2019). Bill curvature was measured as the angle of deflection of the exposed culmen, measured by trigonometry rules. First, we placed a scaled grid above the photograph, ensuring that the straight part of the bill was parallel to the horizontal lines of the grid. We then measured bill length and deflection length, using the scaled grid. Bill length was measured as a line from the bill tip to the anterior extension of feathers, following the horizontal line from the grid, while deflection length was measured as a line from the bill tip to the bill length's line, creating a 90° angle and, thereby, a right-angle triangle. To detect the angle of deflection, we used the tangent rule. Finally, we converted radians to degree. For each species, we measured 10 adult individuals, except for *Phaethornis subochraceus* (n=7), *Amazilia brevirostris* (n=5) and *Eriocnemis mirabilis* (n=2). When possible, we measured five females and five males (we were unable to do this for 38 species, i.e. ~21% of the species). For five species that were split taxonomically after our bill length measurements (*Colibri cyanotus*, *Eugenes spectabilis*, *Heliangelus clarisse*, *Schistes albogularis*, *Stephanoxis loddigesii*), we gave the derived species the same bill length estimates as their previous conspecifics (*Colibri.thalassinus*, *Eugenes.fulgens*, *Heliangelus amethysticollis*, *Schistes.geoffroyi*, *Stephanoxis.lalandi*). We obtained bill length and curvature estimates for 180 of the 181 hummingbird species (~99% of the species). The trait data

can be downloaded at Dryad Digital Repository (<https://doi.org/10.5061/dryad.rr4xgxd7n>, Dalsgaard et al., 2021).

Museums from where specimens were measured: AMNH – American Museum of Natural History (New York, USA); FMNH – Field Museum of Natural History (Chicago, USA); MBML – Museu de Biologia Mello Leitão (Santa Teresa, Brasil); MNRJ – Museu Nacional (Rio de Janeiro, Brasil); MPEG – Museu Paraense Emílio Goeldi (Belém, Brasil); NHMT – Natural History Museum of Tring (Tring, UK); SMF – Senckenberg Naturmuseum Frankfurt (Frankfurt, Germany); USNM – Smithsonian National Museum of Natural History (Washington, USA); ZFMK – Zoologisches Forschungsmuseum Alexander Koenig (Bonn, Germany); ZMSNM – Zoologisk Museum, Statens Naturhistoriske Museum (Copenhagen, Denmark).

#### Reference:

Zanata, T.B., Dalsgaard, B., Rahbek, C. & Varassin, I.G. (2019). Bill measurements of hummingbirds in the ecological network database. figshare. Dataset.

<https://doi.org/10.6084/m9.figshare.6151196.v4>

### Appendix 3. Plant taxonomy and corolla length estimates

Species names and families of the plants follow ‘The Plant List’ (TPL version 1.1; [www.theplantlist.org](http://www.theplantlist.org)), with a few exceptions where species names of registered plants were not found in TPL (three species, i.e. ~0.2% of the species in our dataset). For the 1,256 plant species observed in the plant-hummingbird networks (Appendix S1), most species had been identified to the species level (1060 species, i.e. ~84%). The remaining 196 species (~16%) had been identified to at least the family level. For all species, we aimed to gather data on the effective floral corolla length, i.e. the length from the base of the nectar chamber to the distal part of the flower that limited how far the head of a feeding hummingbird could fit into a given flower (*sensu* Wolf et al., 1976). Apart from a few cases (~1% of the species), data on floral corolla length was collected at the same locality as the interaction network. For plant species where data on floral corolla length was collected in several localities (~19% of the species), we averaged the corolla length across localities. In total, we obtained floral corolla length estimates for 962 plant species (~76% of the species). The trait data can be downloaded at Dryad Digital Repository (<https://doi.org/10.5061/dryad.rr4xgxd7n>, Dalsgaard et al., 2021).

#### Reference:

Wolf, L.L., Stiles, F.G. & Hainsworth, F.R. (1976). Ecological organization of a tropical, highland hummingbird community. *J. Anim. Ecol.*, 45, 349–379.

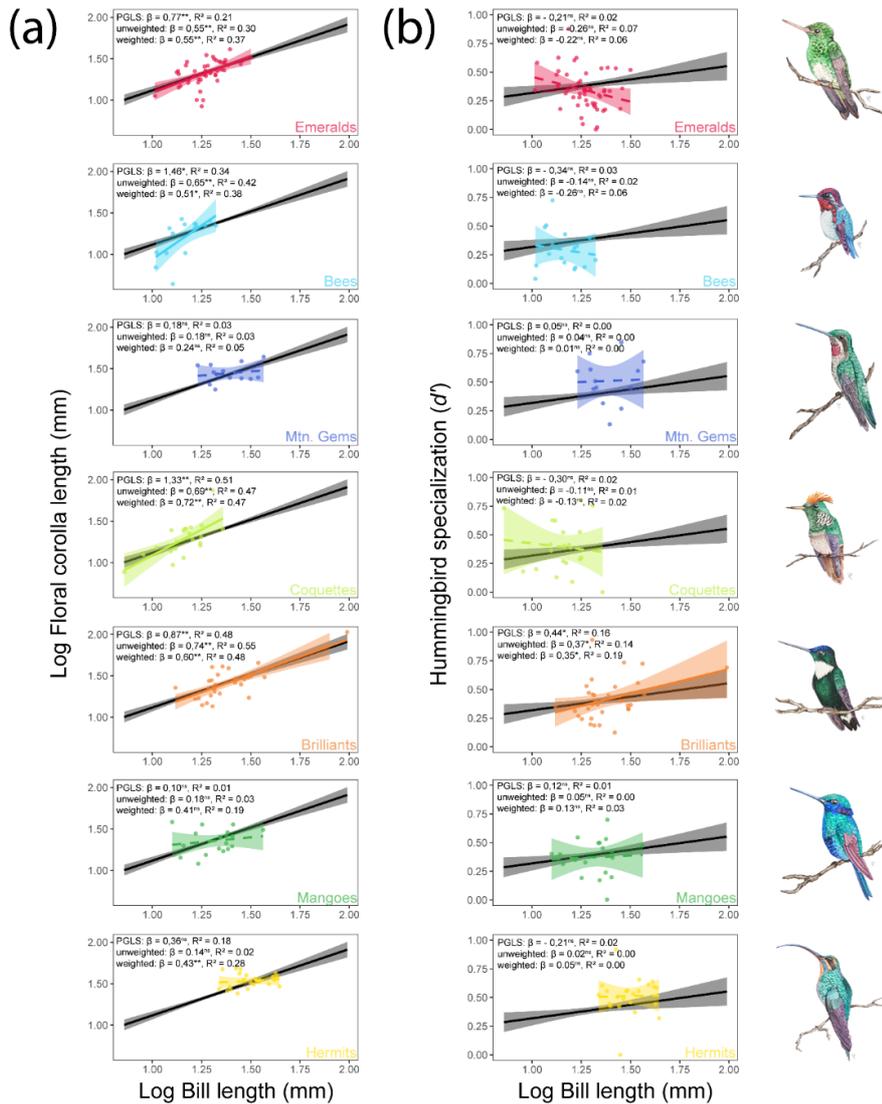
**Table S1.** The association between hummingbird bill length and bill curvature and mean resource specialization, measured both as (a) complementary specialization  $d'$  and (b) proportional generality. For each response variable, we built two sets of Linear Mixed-effects Models (LMMs): one considering hummingbird clades and another considering biogeographical regions as random intercepts, with bill length, bill curvature and their interaction term as fixed effects. For each of these analyses, we constructed both unweighted and weighted models, the latter weighted with square root number of networks. Unweighted models are in bold. We estimated the proportion of variance explained by the fixed effects (bill length, bill curvature, and their interaction term) in the LMMs as marginal  $R^2$ , and the proportion of variance explained by both fixed effects and random factors as conditional  $R^2$ . We also report standardized coefficient estimates as well as corresponding  $P$ -values and standard errors. These LMM analyses showed that hummingbird bill curvature added only marginally to bill length in predicting resource specialization, both for  $d'$  and proportional generality. Moreover, hummingbird bill curvature displayed a strong phylogenetic signal ( $\lambda = 0.92$ ,  $P < 0.001$ ), and when accounting for this phylogenetic non-independence using PGLS, there was no association between bill morphology (length, curvature and their interaction term) and mean resource specialization, both for  $d'$  ( $R^2 = 0.02$ ,  $P > 0.05$ ) and proportional generality ( $R^2 = 0.02$ ,  $P > 0.05$ ).

<b>Model</b>	<b>Random factor</b>	<b><math>R^2</math> marginal</b>	<b><math>R^2</math> conditional</b>	<b>Fixed effect</b>	<b>coefficient</b>	<b>Std Error</b>
(a) Specialization $d'$	<b>clade</b>	<b>0.02</b>	<b>0.09</b>	<b>bill length</b>	<b>0.13<sup>NS</sup></b>	<b>0.09</b>
				<b>bill curvature</b>	<b>-0.05<sup>NS</sup></b>	<b>0.10</b>
				<b>length x curv.</b>	<b>0.10<sup>NS</sup></b>	<b>0.09</b>
	clade	0.02	0.15	bill length	0.09 <sup>NS</sup>	0.11
				bill curvature	-0.06 <sup>NS</sup>	0.12
				length x curv.	0.11 <sup>NS</sup>	0.10

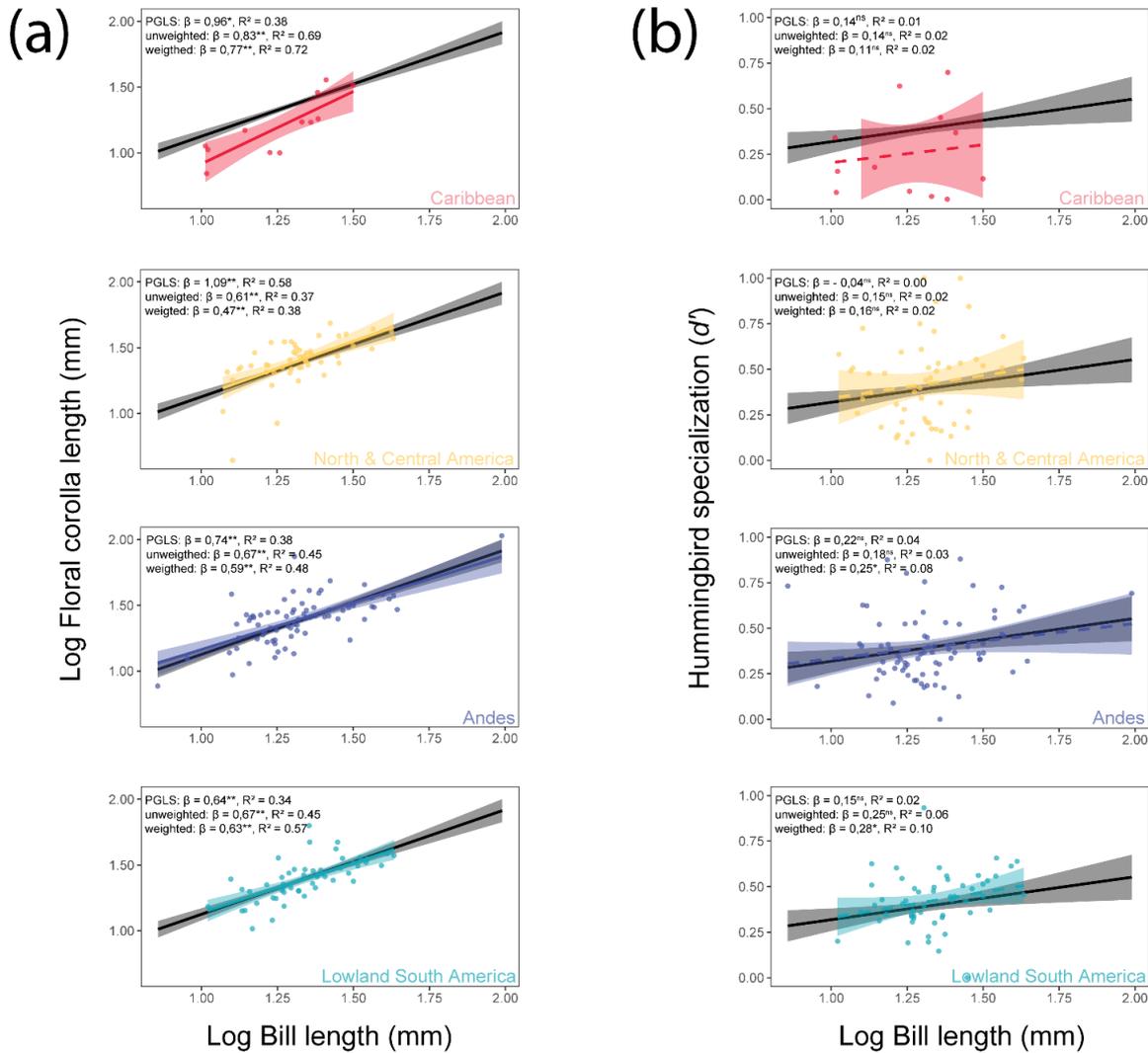
	<b>region</b>	<b>0.07</b>	<b>0.07</b>	<b>bill length</b>	<b>0.24**</b>	<b>0.05</b>
				<b>bill curvature</b>	<b>-0.02<sup>NS</sup></b>	<b>0.06</b>
				<b>length x curv.</b>	<b>0.12*</b>	<b>0.06</b>
	region	0.08	0.08	bill length	0.20**	0.07
				bill curvature	-0.10 <sup>NS</sup>	0.07
				length x curv.	0.15*	0.07
(b) Proportional	<b>clade</b>	<b>0.03</b>	<b>0.06</b>	<b>bill length</b>	<b>0.09<sup>NS</sup></b>	<b>0.09</b>
generality				<b>bill curvature</b>	<b>-0.18<sup>NS</sup></b>	<b>0.10</b>
				<b>length x curv.</b>	<b>0.17<sup>NS</sup></b>	<b>0.09</b>
	clade	0.09	0.17	bill length	0.17 <sup>NS</sup>	0.11
				bill curvature	-0.17 <sup>NS</sup>	0.11
				length x curv.	<b>0.25**</b>	0.10
	<b>region</b>	<b>0.08</b>	<b>0.57</b>	<b>bill length</b>	<b>0.04<sup>NS</sup></b>	<b>0.05</b>
				<b>bill curvature</b>	<b>-0.19**</b>	<b>0.06</b>
				<b>length x curv.</b>	<b>0.23**</b>	<b>0.06</b>
	region	0.08	0.57	bill length	0.10 <sup>NS</sup>	0.07
				bill curvature	-0.22**	0.07
				length x curv.	0.35**	0.06

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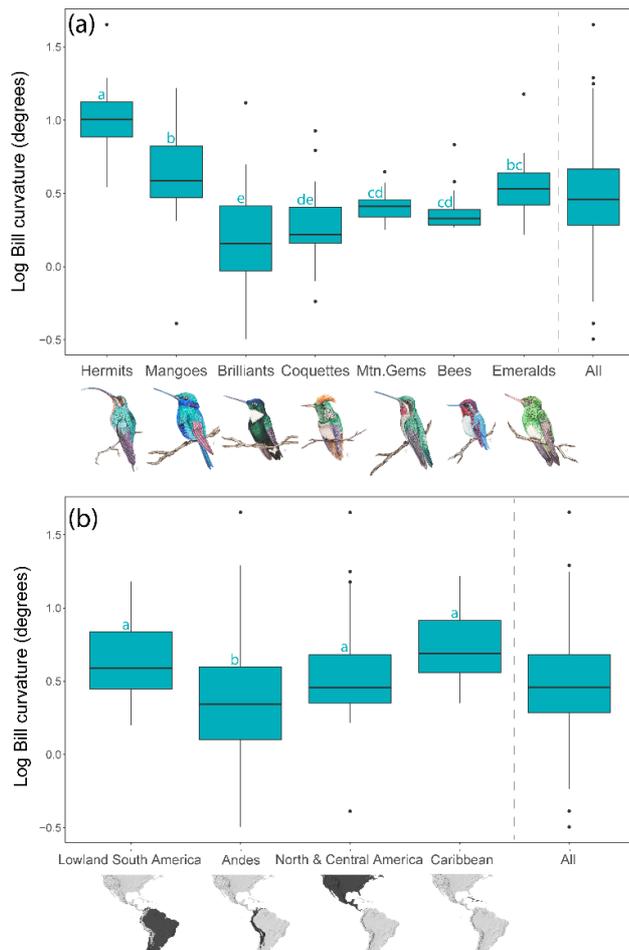
\*\*P<0.001, \*p < 0.05, <sup>NS</sup> P > 0.05



**Figure S1.** Associations between hummingbird bill length and (a) mean floral corolla length and (b) mean resource specialization index  $d'$ , plotted in colour (with grey 95% confidence intervals) for each of seven hummingbird clades based on unweighted regression; full lines represent significant fits and dotted lines represent non-significant fits. The black line (with grey 95% confidence intervals) represents the overall association between bill length and (a) mean corolla length and (b) mean resource specialization  $d'$ , respectively, based on unweighted regression and all hummingbird species. For each clade, we give the statistics for PGLS, unweighted and weighted regressions (see Materials and methods). We also modelled how bill length related to proportional generality (all fits had  $P > 0.05$ ). \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; ns  $P > 0.05$ . Hummingbird paintings by Katrine Hansen.



**Fig. S2.** Associations between hummingbird bill length and (a) mean floral corolla length and (b) mean resource specialization  $d'$ , plotted in colour (with grey 95% confidence intervals) for each of the four biogeographical regions based on unweighted regression; full lines represent significant fits and dotted lines represent non-significant fits. The black line (with grey 95% confidence intervals) represents the overall association between bill length and (a) mean corolla length and (b) mean resource specialization  $d'$ , respectively, based on unweighted regression and all hummingbird species. For each region, we give the statistics for PGLS, unweighted and weighted regressions (see Materials and methods). We also modelled how bill length related to proportional generality (all fits had  $P > 0.05$ ). \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; <sup>ns</sup>  $P > 0.05$ .



**Fig. S3.** Differences in hummingbird bill curvature in relation to (a) hummingbird clades, and (b) biogeographical regions: Lowland South America, Andes, Central & North America, and the Caribbean. In the clade specific comparison, it was based on all species within each clade, irrespectively of regional affinities. In the regional comparison, it was based on the species pool for each region, as extracted in the networks within each region. Boxes indicate the first and third quartiles (Q1 and Q3), horizontal lines inside boxes are medians, vertical lines indicate  $Q1/Q3 + 1.5 \times$  interquartile ranges (IQR), and circles are outliers. ANOVA analyses showed that bill curvature varied significantly between clades ( $F_{6, 165} = 28.82, P < 0.001$ ) and regions ( $F_{3, 191} = 9.22, P < 0.001$ ). Different letters represent statistical difference ( $P < 0.05$ ) according to one-way ANOVAs with multiple post-hoc comparisons using the FDR. For comparison, on the right in both figures we show boxplots across all species. Hummingbird paintings by Katrine Hansen.