

2021-03-21

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

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<http://hdl.handle.net/10026.1/17028>

10.1111/1365-2435.13784

Functional Ecology

Wiley

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1 **This is a pre-submission version of:**

2 **Dalsgaard B, et al. (2021) The influence of biogeographical and evolutionary histories on**
3 **morphological trait-matching and resource specialization in mutualistic hummingbird–plant**
4 **networks. *Funct Ecol.* 2021;00:1–14. <https://doi.org/10.1111/1365-2435.13784>**

5

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

8

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99 **Acknowledgements**

100 BD, KH and BIS thank the Independent Research Fund Denmark for its support (grant nr. 0135-
101 00333B). BD, JS, KH, CR, and AMMG thank the Danish National Research Foundation for its
102 support of the Center for Macroecology, Evolution and Climate (grant no. DNRF96). This study
103 was supported by CAPES (PDSE scholarship proc. 8105/2014-6 to TBZ; Finance Code 001 to TBZ
104 and TSM; CNPq grant 445405/2014-7 and PQ scholarship 313801/2017-7 to IGV). ACA and EF
105 thanks CAPES/FUNDECT PAPOS for the grant 23/200.638/2014, ACA thanks CNPq for a PQ

106 scholarship (310999/2018-9) and EF thanks to CNPq researcher grant (306345/2019-6). AGC,
107 MMDF and ENN also thanks the CAPES for funding. CL received financial support from
108 ESDEPED-UATx. CGM was funded by FAPESB and CNPq. MAM thank the Consejo Nacional
109 para Investigaciones Científicas y Tecnológicas (CONICIT) and the research-funding program
110 “LOEWE-Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz” of
111 Hesse’s Ministry of Higher Education, Research, and the Arts. OHM-G was supported by the
112 National Council of Science and Technology (CONACYT 417094) and the Doctoral Program of
113 the Instituto de Ecología, A.C. (INECOL). RO-P thanks CONACyt (project grant # 258364). MBR-
114 B thanks COLCIENCIAS for a Ph.D. scholarship (617-2013), IDEA WILD for equipment
115 donation, and Fundación ProAves and American Bird Conservancy for support to conduct
116 fieldwork. ARR and SNPQ thanks CAPES (Financial code 001) for funding. IS and MS thank the
117 National Council of Scientific and Technological Development - CNPq grants 300992/79-ZO and
118 302781/2016-1, respectively. BIS was supported by Royal Commission for the Exhibition of 1851
119 Research Fellowship. JV-B thank the US Army and CERL-ERDC for funding, and SW thanks The
120 British Ecological Society, The Biodiversity Trust and The Anglo Peruvian Society for grants.
121 AMMG was supported through a Marie Skłodowska-Curie Individual Fellowship (H2020-MSCA-
122 IF-2015-704409).

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124 **Author contributions:** BD, PKM, JS, KH, CR, MSc and AMMG conceived the study; BD, PKM,
125 JS, TBZ, SA, RA, ACA, FPA, SB, EC-G, AGC, PAC, RDV, MFD, PLE, MMD-F, EF, GK, CL,
126 FMGL-C, LRL, AOM, CGM, MAM, TSM, OHM-G, VM-G, SMA-Jr, ENN, PEO, JFO, RO-P,
127 RPL, BIP-G, SNPQ, MBR-B, ARR, MAR, LCR, AMR, IS, MS, BT, IGV, MFV, JV-B, SW and
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; 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), 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 λ 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' (λ
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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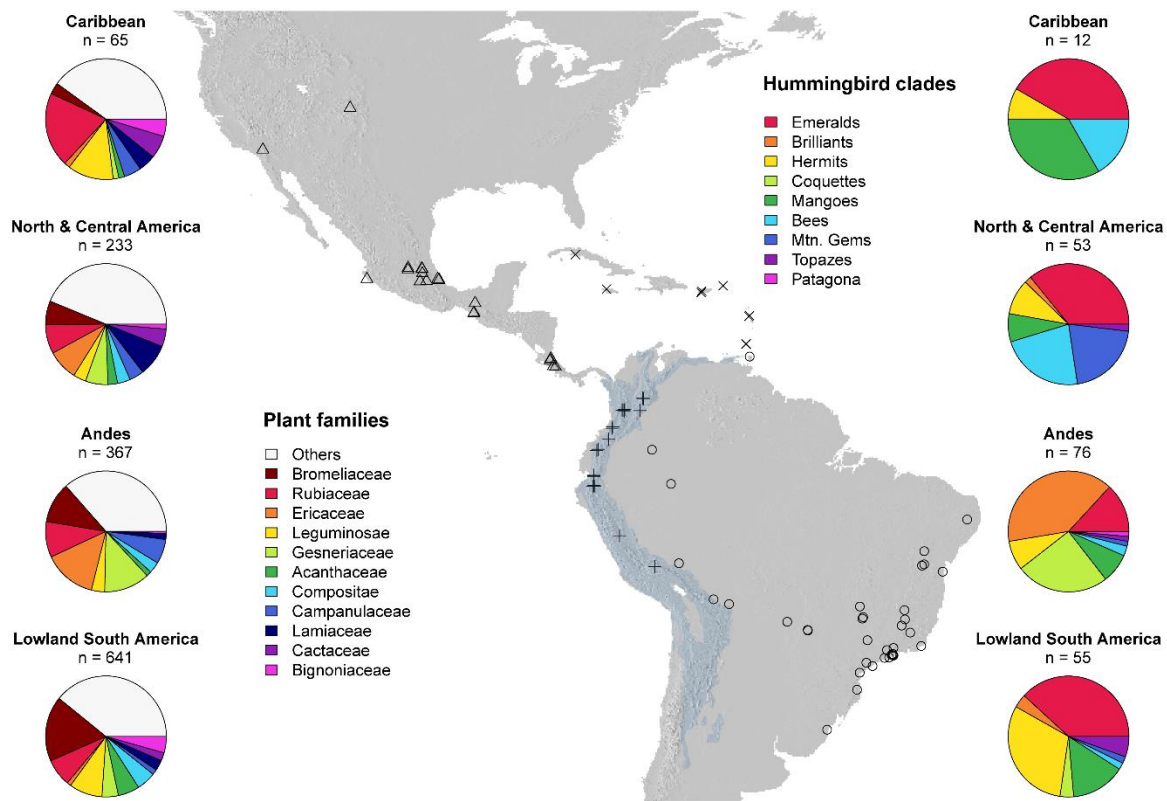
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761 **Table 1.** Linear mixed-effects models (LMMs), analysing the association between hummingbird bill
762 length and (a) mean corolla length of visited flowers, and mean resource specialization, measured
763 both as (b) complementary specialization 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.

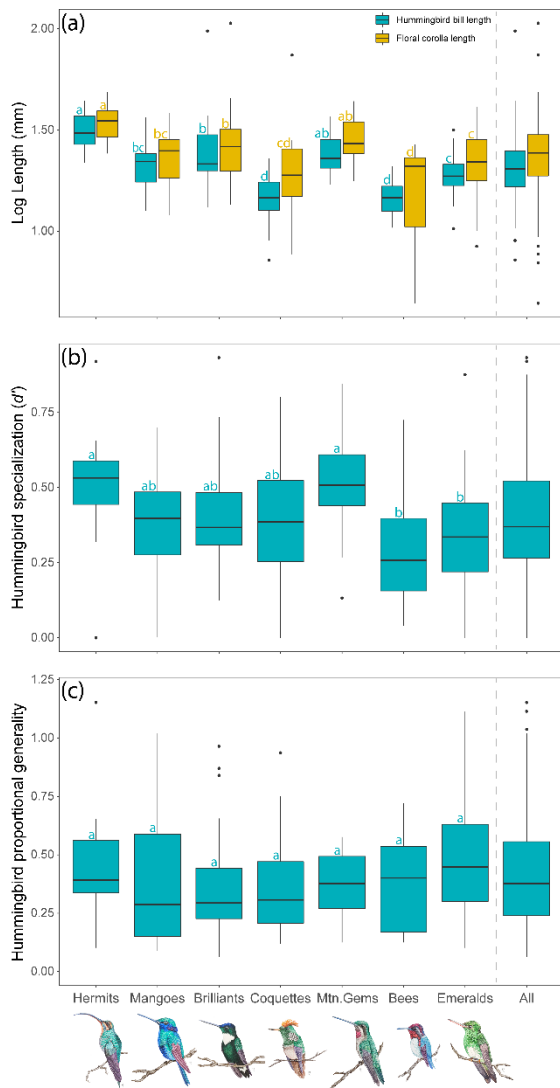
Model	Random factor	R^2 marginal	R^2 conditional	coefficient	Std Error
(a) Corolla length	clade	0.45	0.45	0.80**	0.07
	clade	0.11	0.11	0.87**	0.08
	region	0.41	0.50	0.78**	0.06
	region	0.11	0.13	0.86**	0.07
(b) Specialization d'	clade	0.01	0.10	0.13^{NS}	0.11
	clade	0.00	0.01	0.09 ^{NS}	0.12
	region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

773 ** $P < 0.001$, * $p < 0.05$, ^{NS} $P > 0.05$



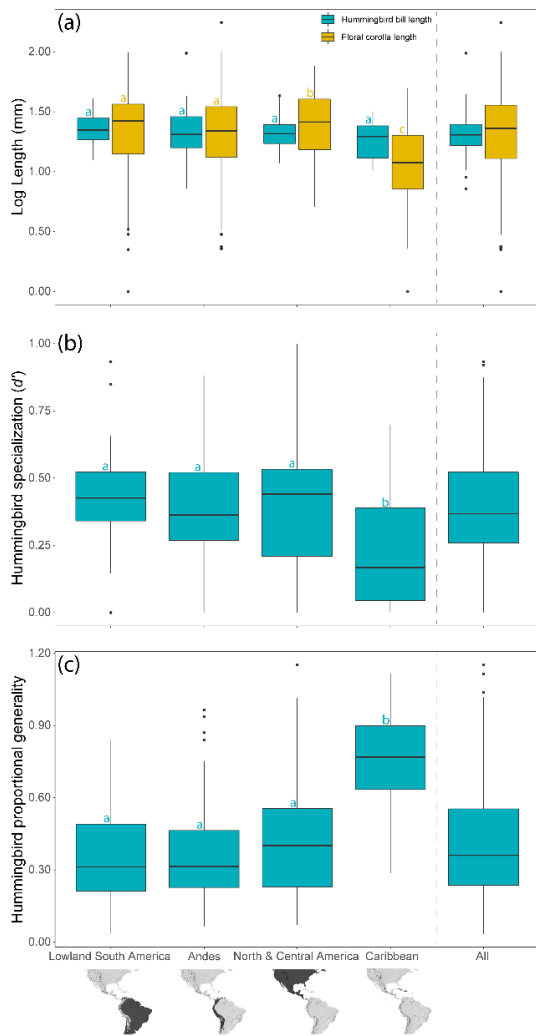
774

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).



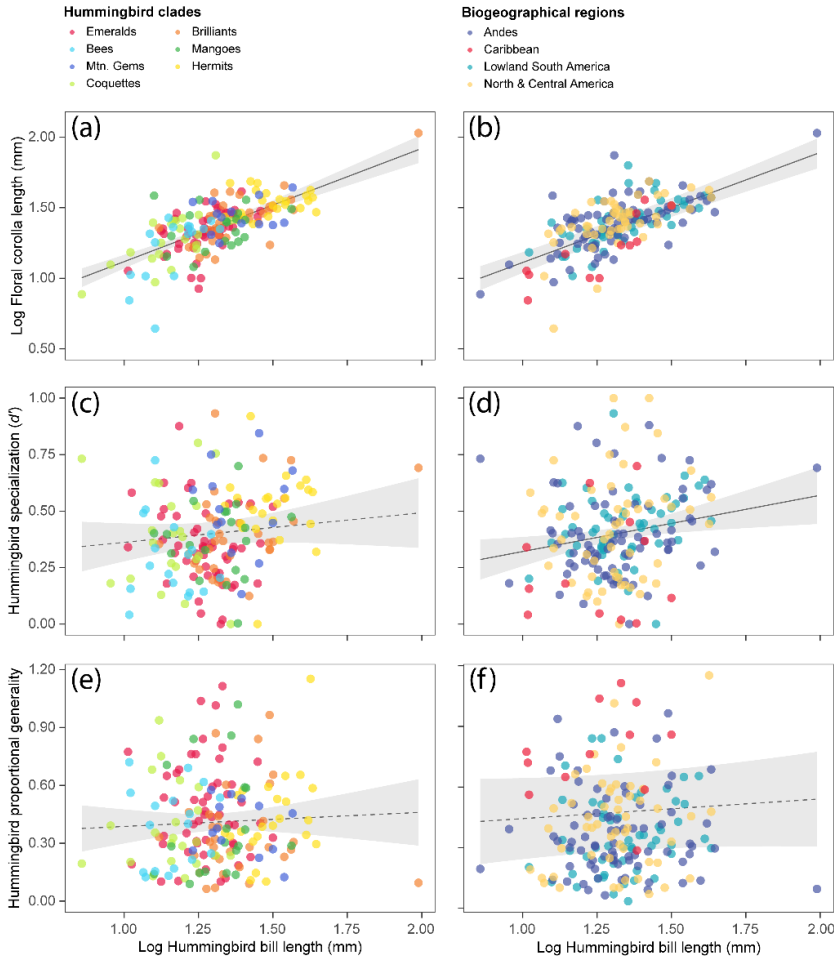
786

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.