

1 **Title:** Speciation and gene flow across an elevational gradient in New Guinea kingfishers

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5 Ethan Linck^{1*}, Benjamin G. Freeman³, and John P. Dumbacher⁴

6 ¹*Department of Biology & Burke Museum of Natural History & Culture, University of*

7 *Washington, Seattle, 98195, USA*

8 ²*Department of Biology & Burke Museum of Natural History & Culture, University of*

9 *Washington, Seattle, 98195, USA*

10 ³*Beaty Biodiversity Research Centre, University of British Columbia, Vancouver, V6T 1Z4, CA*

11 ⁴*Ornithology & Mammalogy, California Academy of Sciences, San Francisco, 94118, USA*

12 *Corresponding Author: ethanblinck@gmail.com

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24 **Abstract:** Closely related species with parapatric elevational ranges are ubiquitous in
25 tropical mountains worldwide. The gradient speciation hypothesis proposes that these series are
26 the result of *in situ* ecological speciation driven by divergent selection across elevation. Direct
27 tests of this scenario have been hampered by the difficulty inferring the geographic arrangement
28 of populations at the time of divergence. In cichlids, sticklebacks, and *Timema* stick insects,
29 support for ecological speciation driven by other selective pressures has come from
30 demonstrating parallel speciation, where divergence proceeds independently across replicated
31 environmental gradients. Here, we take advantage of the unique geography of the island of New
32 Guinea to test for parallel gradient speciation in replicated populations of *Syma* kingfishers that
33 show extremely subtle differentiation across elevation and between historically isolated
34 mountain ranges. We find that currently described high elevation and low elevation species have
35 reciprocally monophyletic gene trees and form nuclear DNA clusters, rejecting this hypothesis.
36 However, demographic modeling suggests selection has likely maintained species boundaries in
37 the face of gene flow following secondary contact. We compile evidence from the published
38 literature to show that while *in situ* gradient speciation in labile organisms such as birds appears
39 rare, divergent selection and post-speciation gene flow may be an underappreciated force in the
40 origin of elevational series and tropical beta diversity along mountain slopes.

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42 *Key words:* gradient speciation, parapatric speciation, ecological speciation, *Syma*

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46 **Introduction:** Series of closely related species with parapatric elevational ranges are
47 ubiquitous in tropical mountains worldwide, contributing to their globally high beta diversity
48 (Diamond, 1972; Jankowski, Ciecka, Meyer, & Rabenold, 2009; Cadena et al., 2012; Terborgh
49 & Weske, 1975). This striking biogeographic pattern is consistent with alternate hypotheses of
50 how speciation proceeds in tropical forest faunas (Moritz, Patton, Schneider, & Smith, 2000).
51 Under the gradient speciation hypothesis, elevational series are the result of local adaptation to
52 divergent elevational niches that leads to the evolution of reproductive isolation *in situ* (i.e.,
53 ecological speciation without geographic isolation; Smith, Wayne, Girman, & Bruford, 1997;
54 Moritz, Patton, Schneider, & Smith, 2000; Nosil, 2012; Caro, Caycedo-Rosales, Bowie, &
55 Cadena, 2013; Beheregaray, Cooke, Chao, & Landguth, 2015). Alternatively, allopatric
56 speciation followed by secondary contact and elevational range displacement through
57 competition could lead to identical distributional patterns (Mayr, 1942; Endler, 1982; Cadena et
58 al., 2012; Freeman, 2015).

59 Despite significant research attention, the origin of elevational series eludes easy
60 synthesis. This is partly due to the difficulty of inferring the geographic mode of speciation and
61 its evolutionary mechanisms from biogeographic and phylogenetic data alone (Endler, 1982;
62 Losos & Glor, 2003). While the gradient speciation hypothesis predicts that lineages with
63 parapatric elevational ranges will be sister to one another—a relationship found in *Ithioma*
64 butterflies (Elias et al., 2009), *Leptopogon* flycatchers (Bates & Zink, 1994) and some Andean
65 amphibians and reptiles (Arteaga et al., 2016; Guayasamin et al., 2017), but not in many other
66 elevational series of Andean birds and mammals (e.g., Patton & Smith, 1992; Dingle, Lovette,
67 Canaday, & Smith, 2006; Cadena et al., 2012; Caro, Caycedo-Rosales, Bowie, & Cadena, 2013;
68 Cadena & Céspedes, 2019)—this pattern alone cannot distinguish divergence with geographic

69 isolation from divergence without geographic isolation. Furthermore, both the lability of species
70 ranges over evolutionary time and extinction can quickly obscure any phylogenetic signal of the
71 geography of speciation (Losos & Glor, 2003). In contrast, population genomic data can provide
72 information on rates of gene flow through time, and by proxy, the geographic mode of
73 divergence (Moyle et al., 2017; Chapman, Hiscock, & Filatov, 2013; Chapman, Hiscock, &
74 Filatov, 2016), but can only reveal the loci underpinning reproductive isolation (and by
75 extension, its selective drivers) under particular circumstances (e.g., with exceptional sampling
76 of hybrid zones).

77 To date, the strongest evidence for ecological speciation of any type has come from
78 studies of parallel divergence on replicated selective gradients (Schluter & Nagle, 1995;
79 Johannesson, 2002). The independent evolution of similar morphs of cichlids (Schliewen, Tautz,
80 & Pääbo, 1994) and sticklebacks (Rundle, Nagel, Boughman, & Schluter, 2000) in response to
81 similar ecological pressures convincingly demonstrates a link between local adaptation and the
82 evolution of *in situ* reproductive isolation. In *Timema* stick insects, parallel adaptation to
83 different host plants in allopatric populations provides a terrestrial analogue at small spatial
84 scales (Nosil, Crespi, & Sandoval, 2002). While this approach evokes studies of phylogenetic
85 relationships in clades with taxa that segregate by both latitude and elevation (e.g., Cadena &
86 Céspedes, 2019), it differs in true geographic independence among replicates, putatively shallow
87 timescales that reduce the probability that extinction and range shifts have obscured evolutionary
88 signal, and phenotypic variance that is shared across similar environments. To our knowledge,
89 only one study has explicitly tested predictions of parallel gradient speciation across elevation
90 (Fuchs, Fjeldså, & Bowie, 2011).

91 Here, we take advantage of the unique geography of the island of New Guinea to perform
92 such a test in replicated populations of interior forest kingfishers in the genus *Syma* that show
93 extremely subtle differentiation across elevation and between geographic replicates. We
94 collected genomic, morphological, and biacoustic data to evaluate species limits and evidence for
95 assortative mating, phylogenetic relationships, and demographic history. Under a hypothesis of
96 parallel gradient speciation, we predicted that highland and lowland populations of *Syma* in
97 isolated mountain ranges that have never been connected by montane forest (Benz, 2011) would
98 be sister to one another and more distantly related to their allopatric congeners. Alternately,
99 under the secondary contact hypothesis, we predicted all highland and lowland populations
100 would be reciprocally monophyletic. We additionally evaluated the role of gene flow during
101 divergence in *Syma*, and compile evidence relevant to these hypotheses from the published
102 literature.

103
104 **Materials and Methods: Study system.** As currently delimited, Yellow-billed and
105 Mountain Kingfishers *Syma torotoro* and *S. megarhyncha* (Aves: Alcedinidae) are putative sister
106 taxa that segregate by elevation (Pratt & Beehler, 2015; Diamond, 1972) across the island of
107 New Guinea. Lowland forest species *S. torotoro* is reportedly smaller with a higher-pitched call,
108 and primarily found below 700 m, while the slightly larger, deeper-voiced *S. megarhyncha* is
109 primarily found above 1100 m to 2200 m or higher in mid-montane forest (Pratt & Beehler,
110 2015). A third population, Yellow-billed Kingfisher subspecies *S. t. ochracea*, may also merit
111 species status; intermediate in size and divergent in call, it is restricted to the oceanic islands of
112 the D'Entrecasteaux Archipelago in southeastern Papua New Guinea. (For the remainder of the
113 manuscript we refer to this taxon as *S. (t.) ochracea* to reflect this uncertainty but will ignore

114 subspecies level taxa in other cases.) All *Syma* taxa spend most of their time in the middle and
115 upper strata of closed-canopy forests, where they feed on a mix of insects and small vertebrates
116 (Pratt & Beehler, 2015).

117 While Mayr, Rand, and Diamond argued that elevational replacements in New Guinea
118 form through secondary contact of differentiated lineages (Rand, 1936; Mayr, 1942; Diamond,
119 1972), some evidence suggests *Syma megarhyncha* and *S. torotoro* may instead have undergone
120 gradient speciation across elevation. First, both mainland *Syma* are territorial and sedentary,
121 making it plausible that dispersal was sufficiently reduced to be overcome by divergent selection
122 across the steep environmental gradient they inhabit (Endler, 1977). Second, *S. megarhyncha*'s
123 larger size (Beehler & Pratt, 2016) is consistent with predictions of morphological adaptation to
124 a cooler climate (Freeman, 2017). Additionally, geographically isolated populations of *S.*
125 *megarhyncha* in the mountains of the Huon Peninsula and the Central Ranges have fixed
126 differences in bill markings, hinting they may have formed independently through parallel
127 divergence from local *S. torotoro* populations. However, species limits and range-wide variation
128 have never been quantitatively assessed with either phenotypic or genetic data and observed
129 differences might instead reflect phenotypic plasticity or clinal variation of a single widespread
130 lineage (Caro et al., 2013).

131 *Morphological and bioacoustics data.* To evaluate phenotypic support for species limits,
132 we measured bill length, bill depth, tarsus, wing chord, and tail length from 72 museum
133 specimens of *Syma torotoro* ($n=30$), *S. (t.) ochracea* ($n=10$), and *S. megarhyncha* ($n=32$) at the
134 American Museum of Natural History, collected from 1894-1965 and including only individuals
135 of known sex as originally identified by the preparator. Using these data, we performed principal
136 component analyses in R (R Core Team 2018) with log-transformed and normalized variables

137 and used PC1 to build mixture models using the R package mclust v. 5.4.1, which we evaluated
138 with a maximum likelihood classification approach (Scrucca, Fop, Murphy, & Rafferty, 2016).
139 We downloaded all available vocalizations from *S. torotoro* ($n=34$) and *S. megarhyncha* ($n=14$)
140 from xeno-canto and Cornell's Macaulay Library. We filtered these data for quality and
141 quantified 36 standard bioacoustic variables using the warbleR package v. 1.1.14 in R (Araya-
142 Salas & Smith-Vidaurre, 2017), analyzing 278 distinct vocalizations from *S. torotoro* and 106
143 from *S. megarhyncha* in total. We ran PCA with normalized variables on the output and used
144 these data to alternate species delimitation models using the same approach as with our
145 morphological data.

146 *Sampling, library preparation, and DNA sequencing.* To infer population genetic
147 structure and phylogenetic history in *Syma*, we extracted DNA from fresh tissues ($n=6$) and
148 toepad samples from historical museum specimens ($n=34$) from 28 individuals of *S. torotoro*, 2
149 individuals of *S. (t.) ochracea*, and 10 individuals of *S. megarhyncha* ($n=10$), including 3
150 individuals collected in 1928 by Ernst Mayr himself. Though only partially overlapping with
151 samples used in morphometric analyses, these individuals represented the full extent of both
152 species' ranges in New Guinea and Australia (**Table S1**) and included all described subspecies.
153 We extracted DNA from fresh tissues using a Qiagen DNAeasy kit and the manufacturer's
154 recommended protocol. For historical toepad samples (collected 1877-1973), we extracted DNA
155 using either a using a phenol-chloroform and centrifugal dialysis method (Dumbacher &
156 Fleischer, 2001) (for reduced representation sequencing) or a standard salt extraction protocol
157 (for whole genome sequencing). Due to constraints of cost and time, we employed two
158 complementary sequencing approaches. On a subset of samples ($n=20$), we performed reduced
159 representation genome sequencing using a hybridization capture with RAD probes (hyRAD)

160 approach, described in detail elsewhere (Linck, Hanna, Sellas, & Dumbacher, 2017). We sent the
161 remaining samples to the UC Berkeley's Vincent J. Coates Genomic Sequencing Laboratory,
162 where laboratory staff prepared genomic libraries for low coverage whole genome sequencing
163 (WGS) using Illumina TruSeq Nano kits and a modified protocol that enzymatically repaired
164 fragments with RNase and skipped sonication. They then pooled ($n=20$) and sequenced these
165 samples with 150 base pair paired end reads on a single lane of an Illumina HiSeq 4000.

166 *Sequence assembly and variant calling.* We processed demultiplexed reads from both
167 sequencing strategies together with a custom bioinformatic pipeline optimized for handling
168 degraded DNA data and available at https://github.com/elinck/syma_speciation. Briefly, we
169 trimmed raw reads for adapters and low-quality bases using bbduk from BBTools version 38.06
170 suite of bioinformatics tools. We aligned these reads to an unpublished draft genome of
171 Woodland Kingfisher *Halcyon senegalensis* from the Bird 10K Genome Project using bmap
172 with a k-mer value of 12, a maximum indel length of 200 bp, and a minimum sequence identity
173 of 0.65. These highly sensitive alignment parameters were necessary given the clade containing
174 *Syma* diverged from the clade containing *Halcyon* approximately 15 mya (Andersen,
175 McCullough, Mauck, Smith, & Moyle, 2018). We used PicardTools v. 2.17.8 and GATK v. 3.6.0
176 (McKenna et al., 2010) to append read groups and perform local realignment on .bam files. We
177 then used mapDamage 2.0.9 to account for postmortem damage to DNA from historical museum
178 specimens by rescaling quality scores (Jónsson, Ginolhac, Schubert, Johnson, & Orlando, 2013).
179 We performed multisample variant calling using the UnifiedGenotyper tool in GATK v. 3.6.0,
180 and filtered our variant calls for missing data, coverage, and quality with VCFtools 0.1.16
181 (Danecek et al., 2011). We generated multiple SNP datasets by implementing different filters to
182 suit the requirements and goals of different analyses; these settings and their underlying rationale

183 are described in detail below. To complement our nuclear DNA sequences, we assembled near-
184 complete and fully annotated mitochondrial genomes from a majority of individuals using
185 mitofinder v. 1.0.2 (Allo et al., 2020) and a complete mtDNA genome from close relative
186 *Todiramphus sanctus* as a reference (Andersen et al., 2015). We then extracted NADH
187 dehydrogenase 2 (ND2) from these genomes and performed multiple sequence alignment using
188 MAFFT v7.407 under its “-auto” parameter setting package (Katoh & Standley, 2013).

189 *Population structure inference.* We evaluated population genetic structure within and
190 between species using a nonparametric clustering approach. We performed principal component
191 analysis of genotypes (PCA) and identified putative genetic clusters for $k=2$ through $k=4$ using
192 adegenet v. 2.1.1 (Jombart, 2008) and a 95% complete dataset with 66,917 SNPs from 35
193 individuals of *Syma torotoro* ($n=24$), *S. (t.) ochracea* ($n=2$), and *S. megarhyncha* ($n=9$) that
194 passed quality filters. This included individuals from both sequencing approaches with a
195 minimum depth of coverage of 3x per individual, a maximum depth of coverage of 120x per
196 individual, a minimum quality score of 30, and a minimum minor allele frequency of 0.05. These
197 relatively lenient filtering parameters were chosen to permit the inclusion of samples from both
198 sequencing strategies in our initial assessment of probable species limits. We evaluated the best-
199 fit clustering model using the Bayesian Information Criterion, again implemented in adegenet.
200 To ensure our results were not an artifact of different library preparation methods or tissue type,
201 we employed two complementary approaches. First, we performed pairwise Wilcoxon rank sum
202 tests evaluating whether differences in coverage or the proportion of missing data were
203 significantly associated with inferred clusters. Second, we reran PCA using the same filters as
204 above but instead 1) included only whole genome sequence data or 2) excluded all modern

205 tissues. We then compared relationships among samples from these analyses with those observed
206 in the full dataset.

207 *Phylogenetic inference.* As we lacked whole genome sequences from an appropriate
208 outgroup species, we evaluated phylogenetic relationships among using our mitochondrial DNA
209 (ND2) alignment, which was near-complete and did not feature correlations between missing
210 data and library preparation method or tissue type. We inferred a time-calibrated phylogeny in
211 BEAST 2.6.0 (Bouckaert et al., 2019) from the 37 samples of *Syma torotoro* ($n=24$), *S. (t.)*
212 *ochracea* ($n=3$), and *S. megarhyncha* ($n=10$) with sufficiently high quality ND2 sequence data,
213 including Sacred Kingfisher *Todiramphus sanctus* as an outgroup (Andersen et al., 2015). We
214 used a strict molecular clock with a rate of 2.9×10^{-8} following Lerner, Meyer, James, Hofreiter,
215 & Fleischer (2011), a GTR+GAMMA model of nucleotide evolution, and a Yule species tree
216 prior. We ran BEAST for 50 million generations, storing trees every 1000 generations, and
217 assessed mixing, likelihood stationarity and adequate effective sample sizes (ESS) above 200 for
218 all estimated parameters using Tracer v.1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard,
219 2018). We then generated a maximum clade credibility tree using TreeAnnotator v.2.6.0,
220 discarding the first 10% of trees as burnin (Bouckaert et al., 2019).

221 *Demographic inference.* We inferred the demographic history of the diverging lineages
222 identified in our clustering and phylogenetic analyses using moments v.1.0.0, which uses
223 ordinary differential equations to model the evolution of allele frequencies (Jouganous, Long,
224 Ragsdale, & Gravel, 2017). We used the `joint_sfs_folded()` function in Python package scikit-
225 allele v.1.2.1 (Miles, Ralph, Rae, & Pisupati, 2019) to calculate a folded joint site frequency
226 spectrum (JSFS) using a SNP dataset that included only whole genome sequencing data, a
227 minimum minor allele count of 1, no more than 10% missing data per site, a minimum depth of

228 6x per individual, and a minimum quality score of 30. We chose these filtering parameters to
229 reduce the influence of sequencing error without strongly biasing the JSFS. This dataset did not
230 include *S. t. ochracea*, and had been further thinned to include only variants in approximate
231 linkage equilibrium (defined as sites where $r^2 < 0.1$) using scikit-allel's `locate_unlinked()`
232 function. To account for the remaining missing data, we projected the JSFS down to an effective
233 sample size of 16 x 12 chromosomes for *S. megarhyncha* and *S. torotoro* respectively.

234 We then specified four basic demographic models chosen to represent plausible
235 speciation scenarios in *Syma*, which differed by level and timing of gene flow. These were: an
236 isolation-with-migration model (IM), which permitted gene flow throughout the history of
237 diverging lineages; an isolation-with-ancestral-migration model (AM), which featured an initial
238 period of gene flow followed by a period of isolation; a model of secondary contact (SC), which
239 featured an initial period of isolation followed by a period of gene flow; and a model of strict
240 isolation (SI), which allowed no gene flow following initial divergence. Next, we specified four
241 additional models which differed from each of the above only by allowing exponential
242 population growth in the most recent time period: an isolation-with-migration-and-growth model
243 (IMg), an isolation-with-ancestral-migration-and-growth model (AMg), a secondary contact and
244 growth model (SCg), and a strict isolation and growth model (SIg).

245 After initially optimizing parameters for fitting each model using the “optimize log”
246 method and a maximum of 3 iterations, we ran 9 additional optimizations, randomly sampling
247 starting values from a uniform distribution within the bounds of 1×10^{-4} and 10. We converted
248 parameter values to real units using a genome-wide mutation rate of 2.3×10^{-9} (Smeds,
249 Qvarnström, & Ellegren, 2016), an effective sequence length scaled to reflect our LD-thinned
250 SNP dataset, and a generation time estimate of two years. We generated parameter uncertainty

251 estimates for the best-fit demographic model (selected based on AIC values) by fitting it to 200
252 bootstrapped site frequency spectra generated using the `fs.sample()` function on our data, an
253 approach which is appropriate when sites are unlinked (Jouganous et al., 2017).

254 *Literature review.* Last, we reviewed the published literature for studies explicitly or
255 implicitly testing predictions of the gradient speciation hypothesis in elevational series of birds.
256 We first performed a Web of Science search on 29 May 2020 using the terms “speciation AND
257 elevation* AND bird*”. From the 122 results, we retained 15 studies that met three criteria: they
258 (1) generated novel phylogenetic or population genetic data; (2) involved at least two congeneric
259 species or otherwise classified reciprocally monophyletic lineages with different elevational
260 ranges; and (3) focused on tropical or subtropical taxa. We augmented these results with 16
261 relevant studies missed by the initial search that we were aware of from other contexts or
262 discovered through the cited literature. In sum, our review included studies addressing a total of
263 24 unique taxa (**Table 2**).

264
265 **Results and Discussion:** Despite the aspects of the biology and distribution of *Syma*
266 kingfishers that are consistent with parallel gradient speciation across elevation, phylogenetic
267 and population genetic evidence lead us to unequivocally reject this hypothesis in *Syma*. A
268 maximum likelihood phylogeny from the mitochondrial gene ND2 and a clustering analysis from
269 genome-wide DNA sequence data indicate current species limits are largely correct: allopatric
270 high-elevation populations of Mountain Kingfisher *Syma megahryncha* are indeed each other’s
271 closest relative, as are all sampled populations of Yellow-billed Kingfisher *Syma torotoro*
272 (**Figure 1b,d**). These results were robust to potential artifacts of sample type or sequencing
273 strategy. PCA performed on only whole genome sequencing data or only historic samples

274 recovered qualitatively similar patterns (**Figure S1**), and pairwise Wilcoxon rank sum tests
275 found no statistically significant association between coverage or the proportion of missing data
276 in samples (all $P > 0.05$). Inferred nuclear DNA clusters and mtDNA lineages were also reflected
277 by phenotypic data, occupying divergent though overlapping areas of principal component space
278 (**Figure 1e,f**). The first principal component of all bioacoustic and morphological data was
279 bimodally distributed with respect to *S. torotoro* and *S. megarhyncha*, as were individual trait
280 measurements (**Figure S2a**), a pattern predicted under assortative mating (Cadena, Zapata, &
281 Jiménez, 2018). Strong negative loadings on PC1 for all measured morphological variables are
282 more consistent with general body size differences than divergent selection on a specific
283 ecologically relevant trait (i.e., bill width) (**Table S3**).

284 The clustering analysis and ND2 phylogeny alone do not exclude the possibility that
285 gradient speciation occurred across a single slope followed by population expansion of the
286 montane taxa to additional isolated montane regions. However, our best-fit demographic model
287 of secondary contact (**Table 1**) is difficult to reconcile with speciation at the necessarily small
288 spatial scale of a local mountainside. Furthermore, we note that our ND2 gene tree indicates an
289 unexpected sister relationship between *S. megarhyncha* and the phenotypically distinctive
290 Yellow-billed kingfisher subspecies *S. (t.) ochracea*, suggesting the latter may best be classified
291 as a distinct biological species (**Figure 1b**). Though this relationship is consistent with multiple
292 divergence histories—and reflects only the history of a single, nonrecombining locus—it
293 presents a possible (if unparsimonious) scenario of allopatric speciation on an oceanic island
294 followed by a subsequent reinvasion of the mainland and range displacement.

295 In spite of the lack of evidence for parallel speciation, we emphasize our data nonetheless
296 indicate a prominent role for natural selection in driving the evolution of elevational

297 replacements. Parameter estimates from our model of secondary contact indicate *Syma torotoro*
298 and *Syma megarhyncha* initially diverged in allopatry over 649,700 years ago (SD: 13,672
299 years), a value remarkably similar to the estimate from the time-calibrated ND2 gene tree
300 (**Figure 1b**, 790,020 years ago; 95% CI: 607,421-972,618 years). This period of isolation was
301 followed by a period of secondary contact and gene flow that initiated 250,000 years ago (**Table**
302 **1**). Our best estimate of the effective migration rate from *S. torotoro* to *S. megarhyncha*
303 ($Nm=1.14$; SD: 0.02) is low but above the widely cited rule of thumb that one-migrant-per-
304 generation prevents population stratification (Wright, 1931; Slatkin, 1985; Wang, 2004). This
305 level of migration indirectly implies that some force—which we suggest is divergent selection—
306 prevents gene flow from leading to the collapse of distinct populations (Nosil, 2012). Effective
307 migration from *S. megarhyncha* to *S. torotoro* was considerably lower ($Nm=0.11$; SD=0.007), as
308 expected given the lower inferred census population size of the smaller-ranged Mountain
309 Kingfisher ($N=368,390$; SD=8,528) compared to the Yellow-billed Kingfisher ($N=1,909,985$;
310 SD=26,028).

311 The apparent absence of recently admixed individuals in our dataset is unsurprising given
312 the coarse grain of our sampling, but unfortunately precludes a rigorous assessment of the
313 relative contribution of postzygotic (e.g., hybrid sterility) and prezygotic (habitat choice or
314 assortative mating) isolation. However, the shallow divergence and long duration of gene flow
315 between species leads us to suspect the former mechanism is at the very least only an incomplete
316 reproductive barrier, suggesting extrinsic factors (particularly habitat requirements and selection
317 against maladapted immigrants from different elevations) may be important. Evidence from
318 recent field surveys that the species' elevational range limits may not be truly parapatric in some

319 cases but instead separated by hundreds of meters could further limit effective migration in either
320 direction (Freeman & Freeman, 2014; Sam, Koane, & Novotny, 2014).

321 Ultimately, our assessment of speciation in *Syma* is consistent with trends revealed by a
322 review of the literature on the origin of elevational series of tropical birds (**Table 2**). Of the 24
323 taxa included in our review, 17 concluded secondary contact was the exclusive mechanism
324 behind the formation of elevational replacements. While seven taxa that showed patterns
325 consistent with gradient speciation between at least two species, only a single study suggested
326 gradient speciation was more common than allopatric divergence within elevational zones in a
327 particular clade. All putative cases of gradient speciation lacked corroborating population
328 genomic evidence. Of the eight taxa that had been explicitly tested for gene flow between
329 elevational replacements, six detected it at appreciable levels. Though this figure is likely
330 inflated by ascertainment bias, it nonetheless suggests hybridization between elevational
331 replacements may be more than previously appreciated (e.g., Cadena & Céspedes, 2019). We
332 believe that while these findings confirm that divergence in allopatry likely generates the
333 overwhelming majority of elevational series and support a shift away from *in situ* gradient
334 speciation in labile organisms such as birds, they should reinforce a focus on divergent selection
335 in the origin of tropical beta diversity. As the discovery of gene flow between diverging lineages
336 at some point in the speciation continuum becomes the norm rather than the exception (Nosil,
337 2008; Kumar et al. 2017; Schumer et al., 2018; Edelman et al., 2019), we suggest that ecological
338 and phenotypic differences that are evident during early divergence are likely to be adaptive and
339 to function as drivers of the speciation process.

340

341 **Data availability:** All code used in this study can be found at
342 https://github.com/elinck/syma_speciation. Processed data are available from Dryad [doi:
343 10.5061/dryad.4f4qrfj9b], and sequence data are available from the NCBI SRA [accession:
344 pending].

345

346 **References:**

- 347 Arctander, P., & Fjeldså, J. (1994). Andean tapaculos of the genus *Scytalopus* (Aves,
348 Rhinocryptidae): a study of speciation using DNA sequence data. In V. Loeschcke, J. Tomiuk, &
349 S. K. Jain, (Eds.), *Conservation Genetics* (pp. 205–225). Basel, Switzerland: Birkhäuser Verlag.
350 Allo, R., Schomaker-Bastos, A., Romiguier, J., Prosdociami, F., Nabholz, B., & Delsuc, F.
351 (2020). MitoFinder: Efficient automated large-scale extraction of mitogenomic data in target
352 enrichment phylogenomics. *Molecular Ecology Resources*, early view.
353 Andersen, M.J., Shult, H.T., Cibois, A., Thibault, J.C., Filardi, C.E., & Moyle, R.G.
354 (2015). Rapid diversification and secondary sympatry in Australo-Pacific kingfishers (Aves:
355 Alcedinidae: *Todiramphus*). *Royal Society Open Science*, 2, 140375.
356 Andersen, M.J., McCullough, J.M., Mauck III, W.M., Smith, B.T., & Moyle, R.G. (2018)
357 A phylogeny of kingfishers reveals an Indomalayan origin and elevated rates of diversification
358 on oceanic islands. *Journal of Biogeography*, 45, 269–281. Araya-Salas, M., & Smith-Vidaurre,
359 G. (2017). warbleR: an r package to streamline analysis of animal acoustic signals. *Methods in*
360 *Ecology and Evolution*, 8, 184–191.
361 Arteaga, A., Pyron, R.A., Peñafiel, N., Romero-Barreto, P., Culebras, J., Bustamente, L.
362 ... Guayasamin, J.M. (2016). Comparative phylogeography reveals cryptic diversity and
363 repeated patterns of cladogenesis for amphibians and reptiles in northwestern Ecuador. *PLoS*
364 *ONE*, 11, e0151746.
365 Bates, J.M., & Zink, R.M. (1994). Evolution into the Andes: species relationships in the
366 flycatcher genus *Leptopogon*. *The Auk*, 111, 507-515.
367 Beehler, B.M., & Pratt, T.K. (2016). *Birds of New Guinea: Distribution, Taxonomy, and*
368 *Systematics*. Princeton, NJ: Princeton University Press.
369 Beheregaray, L.B., Cooke, G.M., Chao, N.L., & Landguth, E.L. (2015). Ecological
370 speciation in the tropics: insights from comparative genetic studies in Amazonia. *Frontiers in*
371 *Genetics*, 5.
372 Benham, P.M., Cuervo, A.M., McGuire, J.A., & Witt, C.C. (2015). Biogeography of the
373 Andean metal-tail hummingbirds: contrasting evolutionary histories of treeline-specialist and
374 habitat-generalist clades. *Journal of Biogeography*, 42, 763–777.
375 Benz, B.W. (2011). *Deciphering the evolutionary history of the montane New Guinea*
376 *avifauna: comparative phylogeography and insights from paleodistributional modeling in a*
377 *dynamic landscape* (Unpublished doctoral dissertation). University of Kansas, Lawrence, KS.
378 Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M.,
379 Gavryushkina, A., ... Drummond, A.J. (2019). BEAST 2.5: An advanced software platform for
380 Bayesian evolutionary analysis. *PLoS Computational Biology*, 15(4), e1006650.

- 381 Bourgeois, Y.X.C., Bertrand, J.A.M., Delahaie, B., Holota, H., Thébaud, C., & Milá, B.
382 (2020). Differential divergence in autosomes and sex chromosomes is associated with
383 intra-island diversification at a very small spatial scale in a songbird lineage. *Molecular*
384 *Ecology*, 29, 1137–1153.
- 385 Cadena, C.D., Klicka, J., & Ricklefs, R.E. (2007). Evolutionary differentiation in the
386 Neotropical montane region: molecular phylogenetics and phylogeography of *Buarremon* brush
387 finches (Aves, Emberizidae). *Molecular Phylogenetics and Evolution*, 44, 993–1016.
- 388 Cadena, C.D., Kozak, K.H., Gómez, J.P., Parra, J.L., McCain, C.M., Bowie, R.C.K., ...
389 Graham, C.H. (2012). Latitude, elevational climatic zonation and speciation in New World
390 vertebrates. *Proceedings of the Royal Society B: Biological Sciences*, 279, 194–201.
- 391 Cadena, C. D., Zapata, F., & Jiménez, I. (2018). Issues and perspectives in species
392 delimitation using phenotypic data: Atlantean evolution in Darwin's finches. *Systematic Biology*,
393 67, 181-194.
- 394 Cadena, C.D., Pérez-Emán, J.L., Cuervo, A.M., Céspedes, L.N., Epperly, K.L., & Klicka,
395 J.T. (2019). Extreme genetic structure and dynamic range evolution in a montane passerine bird:
396 implications for tropical diversification. *Biological Journal of the Linnean Society*, 126, 487-506.
- 397 Cadena, C. D. & Céspedes, L.N. (2020). Origin of elevational replacements in a clade of
398 nearly flightless birds: most diversity in tropical mountains accumulates via secondary contact
399 following allopatric speciation. In: V. Rull & A. Carnaval (Eds.), *Neotropical Diversification*
400 (pp. 635–659). Berlin: Springer.
- 401 Cadena, C.D., Cuervo, A.M., Céspedes, L.N., Bravo, G.A., Krabbe, N., Schulenberg,
402 T.S., ... Fjeldså, J. (2020). Systematics, biogeography, and diversification of *Scytalopus*
403 tapaculos (Rhinocryptidae), an enigmatic radiation of Neotropical montane birds. *The Auk*, 137,
404 ukz077.
- 405 Caro, L.M., Caycedo-Rosales, P.C., Bowie, R.C.K., Slabbekoorn, H., & Cadena, C.D.
406 (2013). Ecological speciation along an elevational gradient in a tropical passerine bird? *Journal*
407 *of Evolutionary Biology*, 26, 357–374.
- 408 Chapman, M.A., Hiscock, S.J., & Filatov, D.A. (2013). Genomic divergence during
409 speciation driven by adaptation to altitude. *Molecular Biology and Evolution*, 30, 2553–2567.
- 410 Chapman, M.A., Hiscock, S.J., & Filatov, D.A. (2016). The genomic bases of
411 morphological divergence and reproductive isolation driven by ecological speciation in *Senecio*
412 (Asteraceae). *Journal of Evolutionary Biology*, 29, 98–113.
- 413 Chaves, J.A., Pollinger, J.P., Smith, T.B., & LeBuhn, G. (2007). The role of geography
414 and ecology in shaping the phylogeography of the speckled hummingbird (*Adelomyia*
415 *melanogenys*) in Ecuador. *Molecular Phylogenetics and Evolution*, 43, 795–807.
- 416 Cowles, S.A., & Uy, J.A.C. (2019). Rapid, complete reproductive isolation in two closely
417 related *Zosterops* White-eye bird species despite broadly overlapping ranges. *Evolution*, 73,
418 1647–1662.
- 419 Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., ... Durbin,
420 R. (2011) The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158.
- 421 Diamond, J.M. (1972). *Avifauna of the Eastern Highlands of New Guinea*. Cambridge,
422 MA: Nuttall Ornithological Club.
- 423 Dingle, C., Lovette, I.J., Canaday, C., & Smith, T.B. (2006). Elevational zonation and the
424 phylogenetic relationships of the *Henicorhina* wood-wrens. *The Auk*, 123, 119–134.

- 425 DuBay, S.G., & Witt, C.C. (2012). An improved phylogeny of the Andean tit-tyrants
426 (Aves, Tyrannidae): more characters trump sophisticated analyses. *Molecular Phylogenetics and*
427 *Evolution*, 64, 285–296.
- 428 DuBay, S.G., & Witt, C.C. (2014). Differential high-altitude adaptation and restricted
429 gene flow across a mid-elevation hybrid zone in Andean tit-tyrant flycatchers. *Molecular*
430 *Ecology*, 23, 3551–3565.
- 431 Dumbacher, J.P., & Fleischer, R.C. (2001). Phylogenetic evidence for colour pattern
432 convergence in toxic pitohuis: Müllerian mimicry in birds? *Proceedings of the Royal Society B:*
433 *Biological Sciences*, 268, 1971–1976.
- 434 Elias, M., Joron, M., Willmott, K., Silvaa-Brandão, K.L., Kaiser, V., Arias, C.F., ...
435 Jiggins, C.D. (2009). Out of the Andes: patterns of diversification in clearwing butterflies.
436 *Molecular Ecology*, 18, 1716–1729.
- 437 Endler, J.A. (1982). Problems in distinguishing historical from ecological factors in
438 biogeography. *American Zoologist* 22, 441–452.
- 439 Edelman, N.B., Fransen, P.B., Miyagi, M., Clavijo, B., Davey, J., ... Mallet, J. (2019).
440 Genomic architecture and introgression shape a butterfly radiation. *Science* 366, 594–599.
- 441 Freeman, B.G. (2015). Competitive interactions upon secondary contact drive elevational
442 divergence in tropical birds. *The American Naturalist* 186, 470–479.
- 443 Freeman, B.G., & Freeman, A.M.C. (2014). The avifauna of Mt. Karimui, Chimbu
444 Province, Papua New Guinea, including evidence for long-term population dynamics in
445 undisturbed tropical forest. *Bulletin of the British Ornithologists' Club*, 134, 30–51.
- 446 Freeman, B.G. (2017). Little evidence for Bergmann's rule body size clines in passerines
447 along tropical elevational gradients. *Journal of Biogeography*, 44, 502–510.
- 448 Fuchs, J., Fjeldså, J., & Bowie, R.C.K. (2011). Diversification across an altitudinal
449 gradient in the Tiny Greenbul (*Phyllastrephus debilis*) from the Eastern Arc Mountains of
450 Africa. *BMC Evolutionary Biology*, 11, 1–17.
- 451 Gabrielli, M., Nabholz, B., Leroy, T., Milá, B., & Thébaud, C. (2020). Within-island
452 diversification in a passerine bird. *Proceedings of the Royal Society B*, 287, 1–9.
- 453 Garcia-Moreno, J., Arctander, P., & Fjeldså, J. (1998). Pre-Pleistocene differentiation
454 among chat-tyrants. *The Condor*, 100, 629–640.
- 455 Garcia-Moreno, J., Arctander, P., & Fjeldså, J. (1999a). A Case of rapid diversification in
456 the Neotropics: phylogenetic relationships among *Craniroleuca* spinetails (Aves, Furnariidae).
457 *The Auk*, 116, 702–711.
- 458 Garcia-Moreno, J., Arctander, P., & Fjeldså, J. (1999b). Strong diversification at the
459 treeline among *Metallura* hummingbirds. *Molecular Phylogenetics & Evolution*, 12, 273–281.
- 460 Garcia-Moreno, J., Ohlson, J., & Fjeldså, J. (2001). MtDNA sequences support
461 monophyly of *Hemispingus* tanagers. *Molecular Phylogenetics & Evolution*, 21, 424–435.
- 462 Guayasamin, J.M., Hutter, C.R., Tapia, E.E., Culebras, J., Peñafiel, N., Pyron, R.A., ...
463 Arteaga, A. (2017). Diversification of the rainfrog *Pristimantis ornatissimus* in the lowlands and
464 Andean foothills of Ecuador. *PLoS ONE* 12, e0172615.
- 465 Halfwerk, W., Dingle, C., Brinkhuizen, D.M., Poelstra, J.M., Komdeur, J., &
466 Slabbekoorn, H. (2016). Sharp acoustic boundaries across an altitudinal avian hybrid zone
467 despite asymmetric introgression. *Journal of Evolutionary Biology*, 29, 1356–1367.
- 468 Jankowski, J.E., Ciecka, A.L., Meyer, N.Y., & Rabenold, K.N. (2009). Beta diversity
469 along environmental gradients: implications of habitat specialization in tropical montane
470 landscapes. *Journal of Animal Ecology*, 78, 315–327.

- 471 Johannesson, K. (2001). Parallel speciation: a key to sympatric divergence. *Trends in*
472 *Ecology & Evolution*, 16, 148–153.
- 473 Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic
474 markers. *Bioinformatics*, 24, 1403–1405.
- 475 Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F., & Orlando, L. (2013).
476 mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters.
477 *Bioinformatics*, 29, 1682–1684.
- 478 Jouganous, J., Long, W., Ragsdale, A.P., & Gravel, S. (2017). Inferring the joint
479 demographic history of multiple populations: beyond the diffusion approximation. *Genetics*, 206,
480 1549–1567.
- 481 Katoh, K., & Standley, D.M. (2013). MAFFT multiple sequence alignment software
482 version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30,
483 772–780.
- 484 Kumar, V., Lammers, F., Bidon, T., Pfenninger, M., Kolter, L., Nilsson, M.A., & Janke,
485 A. (2017). The evolutionary history of bears is characterized by gene flow across species.
486 *Scientific Reports*, 7, 46487.
- 487 Lerner, H.R., Meyer, M., James, H.F., Hofreiter, M., & Fleischer, R.C. (2011).
488 Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian
489 honeycreepers. *Current Biology*, 21, 1838–1844.
- 490 Linck, E.B., Hanna, Z.R., Sellas, A., & Dumbacher, J.P. (2017). Evaluating hybridization
491 capture with RAD probes as a tool for museum genomics with historical bird specimens.
492 *Ecology and Evolution*, 7, 4755–4767.
- 493 Losos, J.B., Glor, R.E. (2003). Phylogenetic comparative methods and the geography of
494 speciation. *Trends in Ecology and Evolution*, 18, 220–227.
- 495 Mayr, E. (1942). *Systematics and the origin of species, from the viewpoint of a zoologist*.
496 Cambridge, MA: Harvard University Press.
- 497 Miles, A., Ralph, P., Rae, S., & Pisupati, R. (2019). cggh/scikit-allel: v1.2.1 (Version
498 v1.2.1). Zenodo. <http://doi.org/10.5281/zenodo.3238280>.
- 499 McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytzky, A., ...
500 DePristo, M.A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing
501 next-generation DNA sequencing data. *Genome Research*, 20, 1297–1303.
- 502 Morales-Rozo, A., Tenorio, E.A., Carling, M.D., & Cadena, C.D. (2017). Origin and
503 cross-century dynamics of an avian hybrid zone. *BMC Evolutionary Biology*, 17, 1–18.
- 504 Moritz, C., Patton, J.L., Schneider, C.J., & Smith, T.B. (2000). Diversification of
505 rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics*,
506 31, 533–563.
- 507 Moyle, R.G., Manthey, J.D., Hosner, P.A., Rahman, M., Lakim, M., & Sheldon, F.H.
508 (2017). A genome-wide assessment of stages of elevational parapatry in Bornean passerine birds
509 reveals no introgression: implications for processes and patterns of speciation. *PeerJ*, 5, e3335.
- 510 Norman, J.A., Rheindt, F.E., Rowe, D.L., & Christidis, L. (2007). Speciation dynamics in
511 the Australo-Papuan *Meliphaga* honeyeaters. *Molecular Phylogenetics and Evolution*, 42, 80–91.
- 512 Nosil, P. (2008). Speciation with gene flow could be common. *Molecular Ecology*, 17,
513 2103–2106.
- 514 Nosil, P. (2012). *Ecological Speciation*. Oxford Series in Ecology and Evolution. Oxford,
515 England, UK: Oxford University Press.

- 516 Nosil, P., Crespi, B.J., & Sandoval, C.P. (2002). Host-plant adaptation drives the parallel
517 evolution of reproductive isolation. *Nature*, 417, 440–443.
- 518 Päckert, M., Martens, J., Sun, J.-H., Severinghaus, L.L., Nazarenko, A.A., Ting, J., ...
519 Tietze, D.T. (2012). Horizontal and elevational phylogeographic patterns of Himalayan and
520 Southeast Asian forest passerines (Aves: Passeriformes). *Journal of Biogeography*, 39, 556–573.
- 521 Parra, J.L., Remsen, J.V., Jr., Alvarez-Rebolledo, M., & McGuire, J.A. (2009). Molecular
522 phylogenetics of the hummingbird genus *Coeligena*. *Molecular Phylogenetics and Evolution*, 53,
523 425–434.
- 524 Patton, J.L., & Smith, M.F. (1992). mtDNA phylogeny of Andean mice: a test of
525 diversification across ecological gradients. *Evolution* 46, 174–183.
- 526 Pratt, T.K., & Beehler, B.M. (2015). *Birds of New Guinea: Second Edition*. Princeton,
527 NJ: Princeton University Press.
- 528 R Core Team. (2018). R: A language and environment for statistical computing.
- 529 Rambaut, A., Drummond, A.J., Xie, D., Baele, G., & Suchard, M.A. (2018). Posterior
530 summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67, 901–904.
- 531 Rand, A.L. (1936). Altitudinal variation in New Guinea birds. *American Museum*
532 *Novitates*, 890, 1–14.
- 533 Ribas, C.C., Moyle, R.G., Miyaki, C.Y., & Cracraft, J. (2007). The assembly of montane
534 biotas: linking Andean tectonics and climatic oscillations to independent regimes of
535 diversification in *Pionus* parrots. *Proceedings of the Royal Society B*, 274, 2399–2408.
- 536 Rundle, H.D., Nagel, L., Boughman, J.W., & Schluter, D. (2000). Natural Selection and
537 Parallel Speciation in Sympatric Sticklebacks. *Science* 287, 306–308.
- 538 Sam, K., Koane, B., & Novotny, V. (2014). New avian records along the elevational
539 gradient of Mt. Wilhelm, Papua New Guinea. *Bulletin of the British Ornithologists' Club*, 134,
540 116–133.
- 541 Schluter, D., & Nagle, L. (1995). Parallel speciation by natural selection. *The American*
542 *Naturalist*, 146, 292–301.
- 543 Schumer, M., Xu, C., Powell, D., Durvasula, A., Skov, L., Holland, D., ... Przeworski,
544 M. (2018). Natural selection and location recombination rates shape the genome evolution of
545 swordtail hybrids. *Science*, 260, 656–660.
- 546 Scrucca, L., Fop, M., Murphy, T.B., & Raftery, A.E. (2016). mclust 5: clustering,
547 classification and density estimation using Gaussian finite mixture models. *The R Journal*, 8,
548 289–317.
- 549 Schlieffen, U.K., Tautz, D., & Pääbo, S. (1994). Sympatric speciation suggested by
550 monophyly of crater lake cichlids. *Nature*, 368, 629–632.
- 551 Sedano, R.E., & Burns, K.J. (2010). Are the Northern Andes a species pump for
552 Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves:
553 Thraupini). *Journal of Biogeography*, 37, 325–343.
- 554 Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and*
555 *Systematics*, 16, 393–43.
- 556 Smeds, L., Qvarnström, A., & Ellegren, H. (2016). Direct estimate of the rate of germline
557 mutation in a bird. *Genome Research*, 26, 1211–1218.
- 558 Smith, T.B., Wayne, R.K., Girman, D.J., & Bruford, M.W. (1997). A role for ecotones in
559 generating rainforest biodiversity. *Science*, 276, 1855–1857.
- 560 Terborgh, J., & Weske, J.S. (1975). The role of competition in the distribution of Andean
561 birds. *Ecology*, 56, 562–576.

562 Voelker, G., Semenov, G., Fadeev, I.V., Blick, A., & Drovetski, S.V. (2015). The
563 biogeographic history of *Phoenicurus* redstarts reveals an allopatric mode of speciation and an
564 out-of-Himalayas colonization pattern. *Systematics and Biodiversity*, 13, 296–305.

565 Wang, J. (2004). Application of the one-migrant-per-generation rule to conservation and
566 management. *Conservation Biology*, 18, 332–343.

567 Winger, B.M., Hosner, P.A., Bravo, G.A., Cuervo, A.M., Aristizábal, N., Cueto, L.E., &
568 Bates, J.M. Inferring speciation history in the Andes with reduced-representation sequence data:
569 an example in the bay-backed antpittas (Aves; Grallariidae; *Grallaria hypoleuca* s. l.). *Molecular*
570 *Ecology*, 24, 6256–6277.

571 Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, 16, 97–259.

572 Wu, Y., Colwell, R.K., Han, N., Zhang, R., Wang, W., Quan, Q., ... Lei, F. (2014).
573 Understanding historical and current patterns of species richness of babblers along a 5000 m
574 subtropical elevational gradient. *Global Ecology and Biogeography*, 23, 1167–1176.

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577 **Table 1.** Demographic model test results.

Model	Log-likelihood	AIC
Secondary contact (SC)	-564.062	1140.124
Isolation-with-ancestral-migration (AM)	-590.0233	1192.047
Strict isolation and growth (SIg)	-611.0493	1228.099
Isolation-with-migration and growth (IMg)	-617.116	1244.232
Isolation-with-migration (IM)	-631.8897	1273.779
Strict isolation (SI)	-637.185	1280.37
Isolation-with-ancestral-migration and growth (AMg)	-653.5472	1319.094
Secondary contact and growth (SCg)	-716.0587	1444.117

578

579 **Table 2.** Previous studies addressing the origin of elevational series of tropical birds using
580 molecular data. The column “Speciation mode” describes the inferred model of divergence for
581 each clade; when multiple species pairs in a study demonstrated conflicting histories, we
582 describe the more common process but acknowledge heterogeneity using the word “mostly”. In
583 some cases, we have assigned speciation mode based on our interpretation of the data rather than
584 an explicitly stated conclusion by the original authors. The column “Gene flow” indicates
585 whether gene flow between elevational replacements was tested for, and if so, whether or not it
586 was detected.

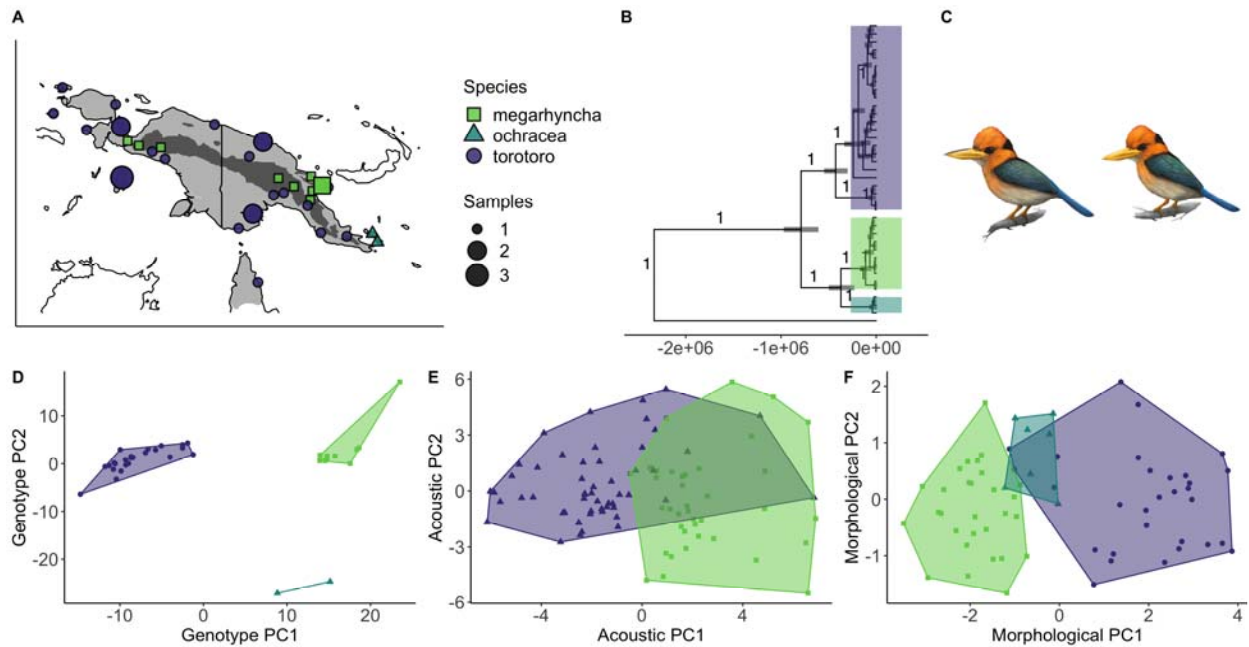
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Citations	Taxon	Region	Speciation mode	Gene flow
Bates & Zink, 1994	<i>Leptogon</i> flycatchers	Andes	Gradient speciation	N/A
Arctander & Fjeldsa, 1994; Cadena et al., 2020	<i>Scytalopus</i> tapaculos	Andes	Mostly secondary contact	N/A
Garcia-Moreno et al., 1998	<i>Ochthoeca</i> chat-tyrants	Andes	Secondary contact	N/A
Garcia-Moreno et al., 1999a	<i>Cranioleuca</i> spinetails	Andes	Secondary contact	N/A
Garcia-Moreno et al., 1999b; Benham et al., 2014	<i>Metallura</i> hummingbirds	Andes	Secondary contact	N/A
Garcia-Moreno et al., 2001	<i>Hemispingus</i> tanagers	Andes	Secondary contact	N/A
Burns & Naoki, 2004	<i>Tangara</i> tanagers	Andes	Secondary contact	N/A
Dingle et al., 2006; Caro et al., 2013; Halfwerk et al., 2016; Cadena et al., 2019	<i>Henicorhina</i> wood wrens	Andes	Secondary contact	Yes
Ribas et al., 2007	<i>Pionus</i> parrots	Andes	Secondary contact	N/A
Cadena, 2007	<i>Buarremon</i>	Andes	Secondary contact	N/A

	brush-finches			
Chaves et al., 2007	<i>Adelomyia melanogenys</i> hummingbird lineages	Andes	Possibly gradient speciation	Yes
Norman et al., 2007	<i>Meliphaga</i> honeyeaters	Australasia	Mostly secondary contact	N/A
Parra et al., 2009	<i>Coeligena</i> hummingbirds	Andes	Secondary contact	N/A
Sedano & Burns, 2010	93 tanager species	Andes	Mostly secondary contact	N/A
Fuchs et al., 2011	<i>Phyllastrephus debilis</i> lineages	Afrotropics	Secondary contact	Yes
Päckert et al., 2011	Seven passerine groups	Himalaya	Secondary contact	N/A
Dubay & Witt, 2012; Dubay & Witt, 2014	<i>Anareites</i> tit-tyrants	Andes	Secondary contact	Yes
Wu et al., 2014	Leiotherichinae babblers	Himalaya	Secondary contact	N/A
Winger et al., 2014	<i>Grallaria</i> antpittas	Andes	Secondary contact	N/A
Voelker et al., 2015	<i>Pheonicurus</i> redstarts	Himalaya	Mostly secondary contact	N/A
Moyle et al., 2017	Five pairs of passerine taxa	Borneo	Secondary contact	No
Morales-Rozo et al., 2017	<i>Ramphocelus</i> tanagers	Andes	Secondary contact	Yes
Cowles & Uy, 2019	<i>Zosterops</i> white-eyes	Australasia	Secondary contact	No
Gabrielli et al., 2020; Bourgeois et al., 2020	<i>Zosterops bourbonicus</i> white-eyes	Reunion Island	Possibly gradient speciation	Yes

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591 **Figure 1.** A) Sampling localities for *Symbia* kingfishers across New Guinea and Australia, color-
592 coded by genotype PC1 and scaled by number of individuals. B) A time-calibrated ND2
593 phylogeny supports reciprocal monophyly of *S. torotoro* and a clade with *S. megarhyncha* and *S.*
594 *(t.) ochracea*, both themselves monophyletic. C) Illustration of *S. megarhyncha* (top) and *S.*
595 *torotoro* (bottom), by Kevin Epperly. D). Principal component analysis of genotypes, clustered
596 by the best fit *k*-means result ($K=3$) and color-coded by the mean PC1 value for all individuals in
597 a given cluster. E) Principal component analysis of bioacoustic parameters, color-coded by
598 species. F) Principal component analysis of morphological data, color-coded by species.

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Supplemental material.

Table S1. Sampling, sequencing, and analysis information. WP=Indonesian New Guinea; PNG=Papua New Guinea.

Specimen	Species	Subspecies	Locality	Tissue source	Date collected	Sequencing strategy	PCA	mtDNA phylogeny	Demographic inference
AMNH:Birds:293714	torotoro	torotoro	Ifaar, WP	toepad	1928	hyRAD	yes	yes	no
AMNH:Birds:293715	torotoro	torotoro	Kepaur, WP	toepad	1897	hyRAD	yes	yes	no
AMNH:Birds:300723	torotoro	torotoro	Kepaur, WP	toepad	1897	hyRAD	yes	yes	no
AMNH:Birds:300723	torotoro	torotoro	Misol Island, WP	toepad	1900	hyRAD	yes	yes	no
AMNH:Birds:329542	torotoro	torotoro	Wasior, WP	toepad	1928	hyRAD	yes	yes	no
AMNH:Birds:437798	torotoro	torotoro	Amberbaki, WP	toepad	1877	hyRAD	yes	yes	no
AMNH:Birds:637429	torotoro	torotoro	Humbolt Bay, WP	toepad	1928	hyRAD	yes	yes	no
AMNH:Birds:637441	torotoro	torotoro	Mt. Mori, WP	toepad	1899	hyRAD	yes	yes	no
AMNH:Birds:637445	torotoro	torotoro	East Sepik Province, PNG	toepad	2003	hyRAD	yes	yes	no
AMNH:Birds:637446	torotoro	torotoro	Waigeu Island, WP	toepad	1900	hyRAD	yes	yes	no
AMNH:Birds:637450	torotoro	tentelare	Aru Islands, WP	toepad	1896	hyRAD	yes	yes	no
AMNH:Birds:637464	torotoro	tentelare	Aru Islands, WP	toepad	1900	hyRAD	yes	yes	no
AMNH:Birds:637464	torotoro	ochracea	Normanby Island, PNG	toepad	1934	hyRAD	yes	yes	no
CAS:Birds:7131	torotoro	pseuestes	Gulf Province, PNG	muscle	2002	hyRAD	yes	yes	no
KU:Birds:5215	torotoro	pseuestes	Gulf Province, PNG	muscle	2003	hyRAD	yes	yes	no
KU:Birds:5464	torotoro	pseuestes	Gulf Province, PNG	muscle	2003	hyRAD	yes	yes	no
KU:Birds:626	torotoro	meeki	Varirata National Park, PNG	muscle	2011	hyRAD	yes	yes	no
KU:Birds:6927	torotoro	meeki	Mt. Suckling, PNG	muscle	2011	hyRAD	yes	yes	no

NHMUK:Birds:1911.12.20.822	torotoro	pseustes	Satakwa River, WP	toepad	1911	hyRAD	yes	yes	no
NHMUK:Birds:1911.12.20.823	torotoro	pseustes	Mimika River, WP	toepad	1913	hyRAD	yes	yes	no
AMNH:Birds:301861	megarhyncha	wellsi	Weylendgep Kunupi, WP	toepad	1931	WGS	yes	yes	yes
AMNH:Birds:302859	megarhyncha	wellsi	Mt. Derimapa, WP	toepad	1930	WGS	yes	yes	yes
AMNH:Birds:339957	megarhyncha	wellsi	Bernhard Camp, WP	toepad	1939	WGS	yes	yes	yes
AMNH:Birds:808986	megarhyncha	megarhyncha	Okapa, PNG	toepad	1965	WGS	yes	yes	yes
ANWC:Birds:B02192	megarhyncha	megrhyncha	Western Highlands, PNG	toepad	1963	WGS	yes	yes	yes
ANWC:Birds:B04293	megarhyncha	megarhyncha	Morobe, PNG	toepad	1966	WGS	yes	yes	yes
ANWC:Birds:B25307	megarhyncha	megarhyncha	Wagau, PNG	toepad	1973	WGS	yes	yes	yes
ANWC:Birds:B25652	megarhyncha	sellamontis	Huon Peninsula, PNG	toepad	1973	WGS	yes	yes	yes
ANWC:Birds:B26140	megarhyncha	sellamontis	Huon Peninsula, PNG	toepad	1973	WGS	yes	yes	yes
YPM:Birds:91444	megarhyncha	sellamontis	Huon Peninsula, PNG	toepad	1969	WGS	yes	yes	yes
AMNH:Birds:329539	torotoro	ochracea	Fergusson Island, PNG	toepad	1928	WGS	yes	yes	yes
AMNH:Birds:329540	torotoro	ochracea	Fergusson Island, PNG	toepad	1928	WGS	yes	yes	yes
AMNH:Birds:426095	torotoro	flavirostris	Fly River, PNG	toepad	1936	WGS	yes	yes	yes
AMNH:Birds:426096	torotoro	flavirostris	Fly River, PNG	toepad	1936	WGS	yes	yes	yes
AMNH:Birds:426121	torotoro	meeki	Wassi Kussi River, PNG	toepad	1937	WGS	yes	yes	yes
AMNH:Birds:637460	torotoro	tentelare	Aru Islands, WP	toepad	1900	WGS	yes	yes	yes
AMNH:Birds:637471	torotoro	meeki	Simbang, PNG	toepad	1899	WGS	yes	yes	yes
AMNH:Birds:637511	torotoro	flavirostris	Cape York Peninsula, AU	toepad	1913	WGS	yes	yes	yes
ANWC:Birds:B07293	torotoro	torotoro	East Sepik Province, PNG	toepad	1966	WGS	yes	yes	yes
ANWC:Birds:B07499	torotoro	torotoro	East Sepik Province, PNG	toepad	1966	WGS	yes	yes	yes

603 **Table S2.** The proportion of missing data (genotypes) and mean fold sequencing coverage by
604 library preparation method and tissue type. Missing data rates calculated from the 95% complete
605 SNP matrix used in *k*-means clustering analyses.
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Sample subset	Missing data (\pmSD)	Sequencing coverage (\pmSD)
WGS libraries	0.007 (\pm 0.010)	6.022x (\pm 3.567x)
hyRAD libraries	0.015 (\pm 0.013)	4.427x (\pm 2.795x)
Modern tissues	0.007 (\pm 0.009)	5.452x (\pm 3.321x)
Toepad tissues	0.011 (\pm 0.013)	4.427x (\pm 3.235x)

607 **Table S3.** PCA loadings of log-transformed morphological variables for all *Syma* taxa.
608
609

Trait	PC1	PC2	PC3
Bill (from nostril)	-0.4249207	0.33505714	0.22963905
Bill width	-0.4367190	0.27088119	0.08777821
Bill depth	-0.4389784	0.14310077	0.30742375
Tarsus	-0.3046142	-0.85803722	0.37960606
Wing chord	-0.4467254	0.05852709	-0.16954328
Tail length	-0.3790295	-0.23287113	-0.81988160

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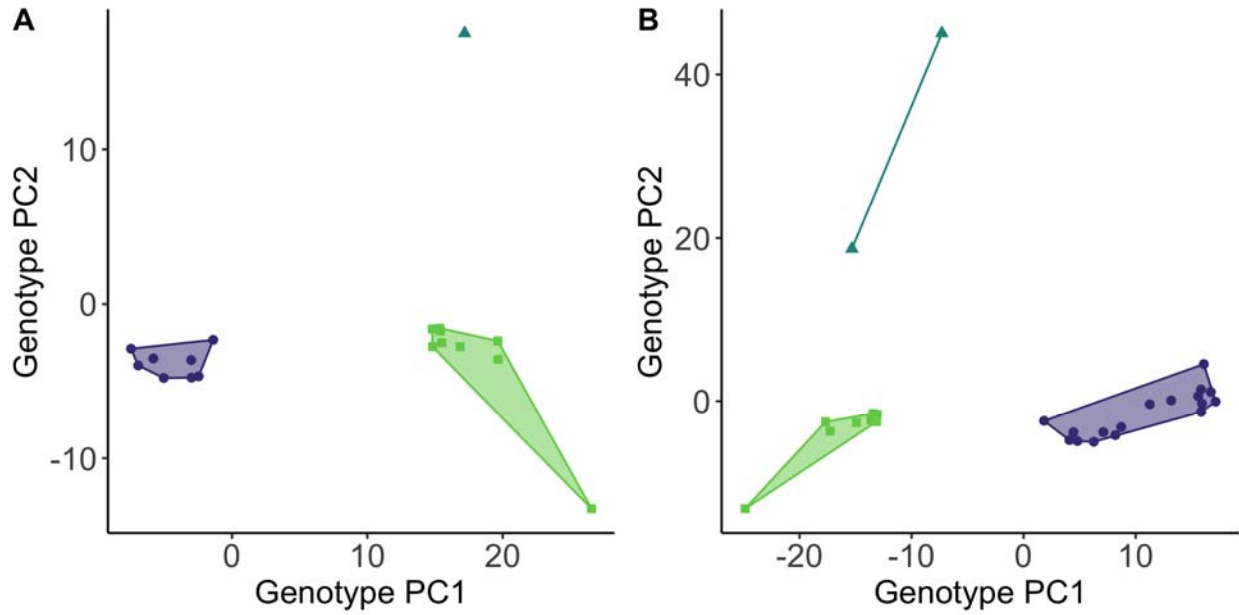
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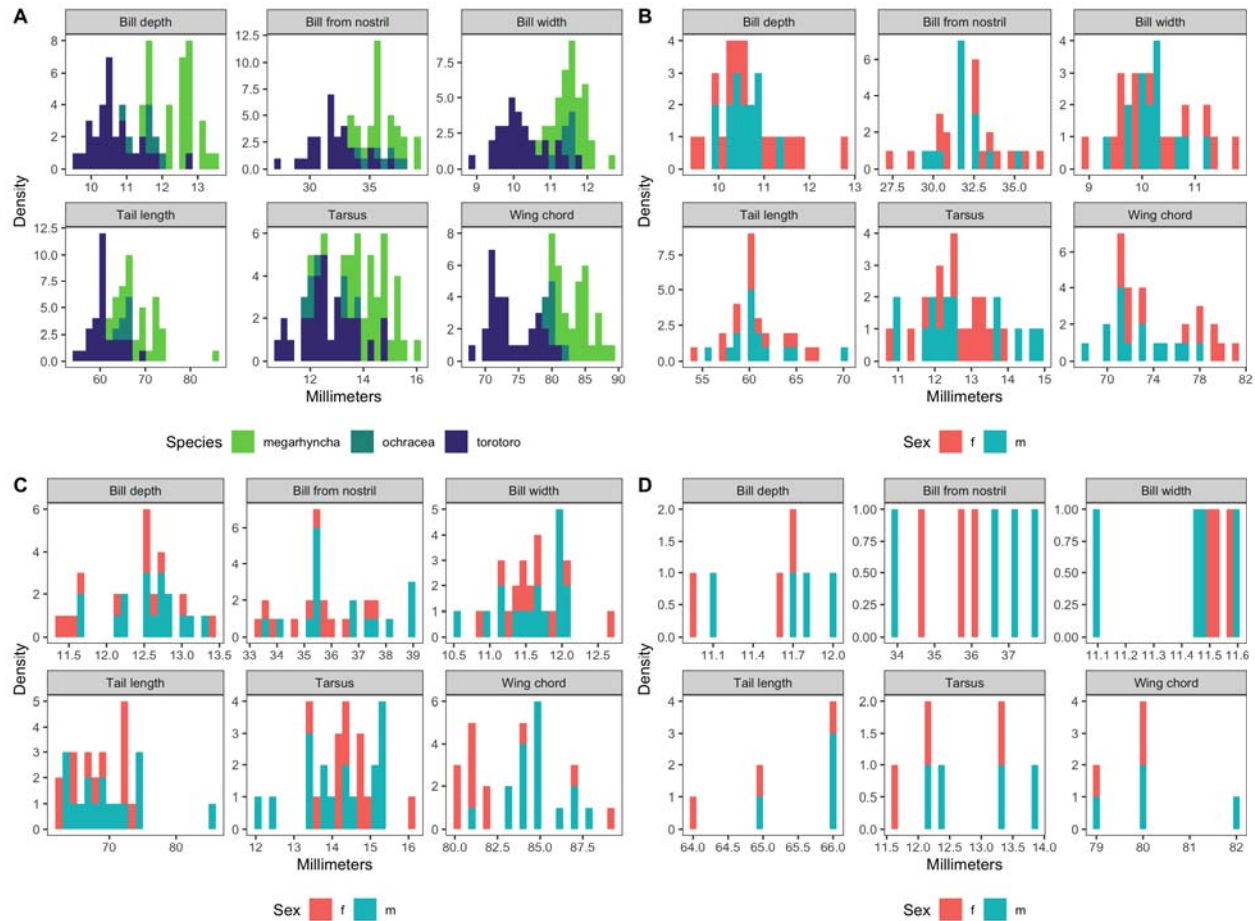
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625 **Figure S1.** Principal component analysis of genotypes from A) whole-genome sequence data
626 alone or B) only historic samples regardless of sequencing method, color-coded to match *k*-
627 means results in Figure 1D. Note the single *S. (t) ochracea* individual high on PC2 in panel A.
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630 **Figure S2.** A) The distribution of log-transformed morphological trait measurements by taxon.
 631 B-D) The distribution of log-transformed morphological trait measurements by sex for *S.*
 632 *toroto*, *S. megarhyncha*, and *S. (t.) ochracea*, respectively. Note the limited sample size of
 633 *S. (t.) ochracea*.
 634