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

Running Title: Speciation in New Guinea kingfishers

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

After initially optimizing parameters for fitting each model using the "optimize log" method and a maximum of 3 iterations, we ran 9 additional optimizations, randomly sampling starting values from a uniform distribution within the bounds of $1x10^{-4}$ and 10. We converted parameter values to real units using a genome-wide mutation rate of $2.3x10^{-9}$ (Smeds, Qvarnström, & Ellegren, 2016), an effective sequence length scaled to reflect our LD-thinned SNP dataset, and a generation time estimate of two years. We generated parameter uncertainty

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

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

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

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

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

In spite of the lack of evidence for parallel speciation, we emphasize our data nonetheless 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 formational 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. Thought 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

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

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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.
587

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

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

588

589

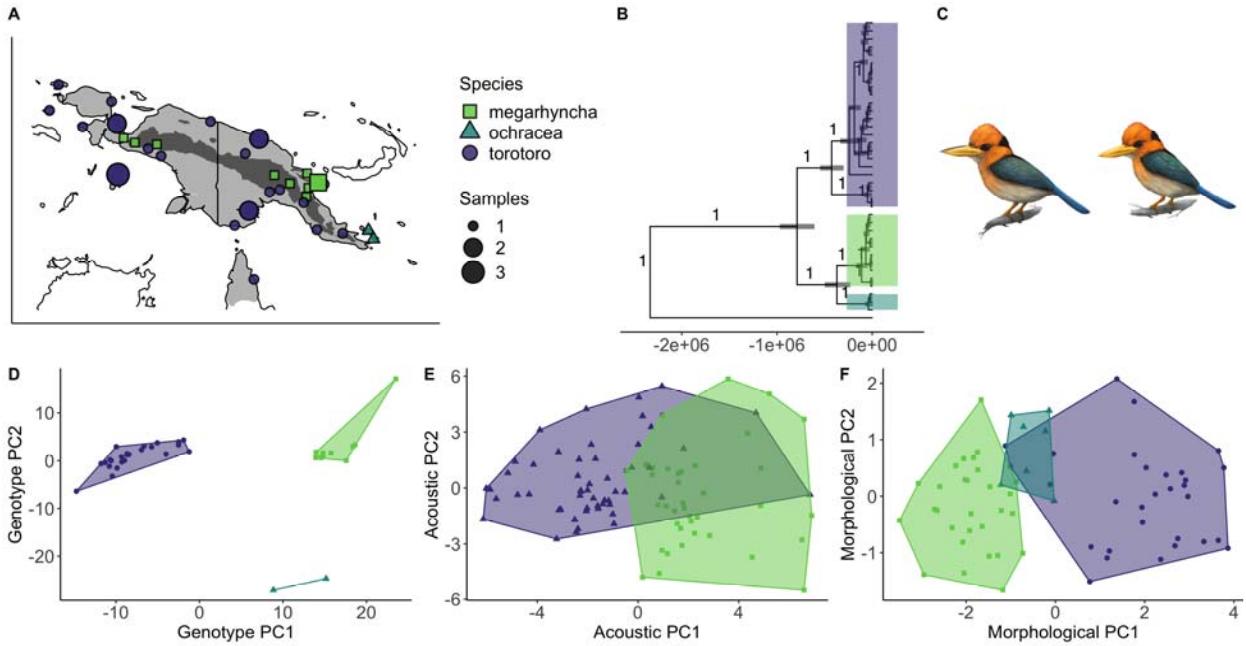

Figure 1. A) Sampling localities for *Syma* kingfishers across New Guinea and Australia, color-coded by genotype PC1 and scaled by number of individuals. B) A time-calibrated ND2 phylogeny supports reciprocal monophyly of *S. torotoro* and a clade with *S. megarhyncha* and *S. (t.) ochracea*, both themselves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

599 **Supplemental material.**

600

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

602

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	pseuestes	Satakwa River, WP	toepad	1911	hyRAD	yes	yes	no
NHMUK:Birds:1911.12.20.823	torotoro	pseuestes	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.
606

Sample subset	Missing data (±SD)	Sequencing coverage (±SD)
WGS libraries	0.007 (±0.010)	6.022x (±3.567x)
hyRAD libraries	0.015 (±0.013)	4.427x (±2.795x)
Modern tissues	0.007 (±0.009)	5.452x (±3.321x)
Toepad tissues	0.011 (±0.013)	4.427x (±3.235x)

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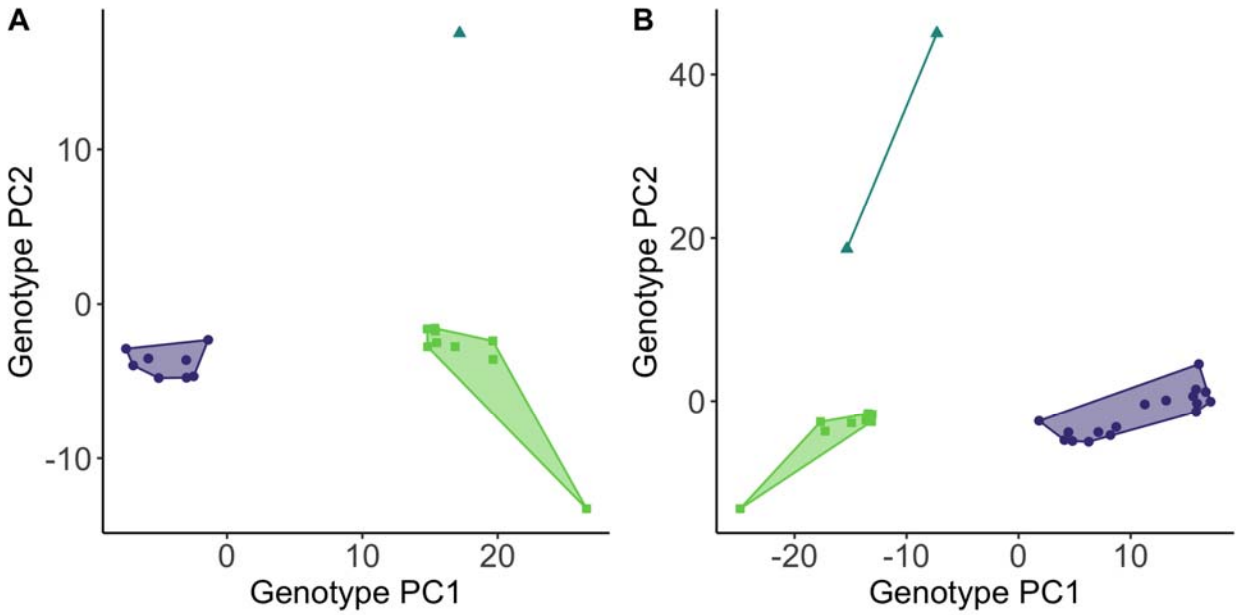

Figure S1. Principal component analysis of genotypes from A) whole-genome sequence data alone or B) only historic samples regardless of sequencing method, color-coded to match *k*-means results in Figure 1D. Note the single *S. (t) ochracea* individual high on PC2 in panel A.

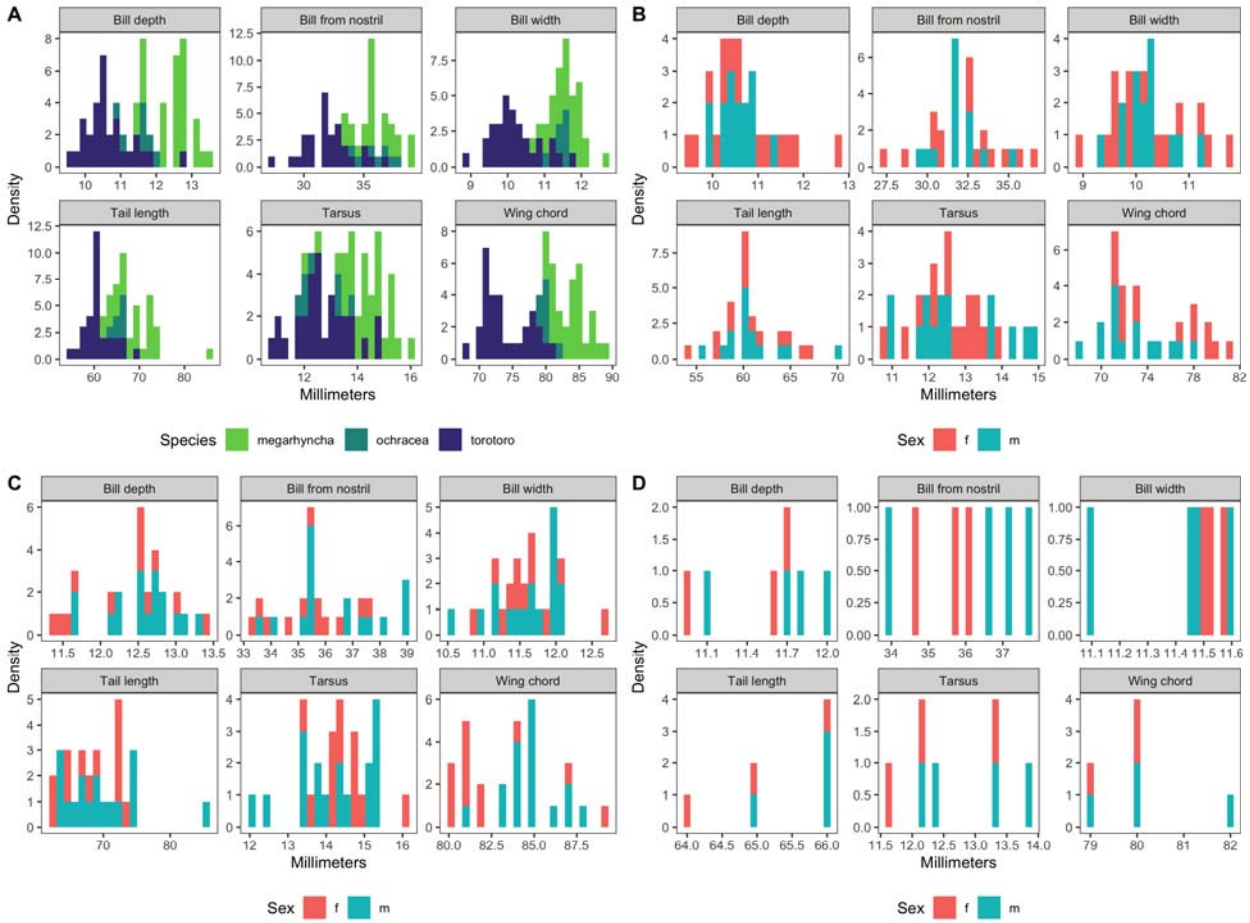

Figure S2. A) The distribution of log-transformed morphological trait measurements by taxon. B-D) The distribution of log-transformed morphological trait measureements by sex for *S. tororoto*, *S. megarhyncha*, and *S. (t.) ochracea*, respectively. Note the limited sample size of *S.(t.) ochracea*.