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

Running Title: Speciation in New Guinea kingfishers

Article Type: Short Communication

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

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

Washington, Seattle, 98195, USA

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

Washington, Seattle, 98195, USA

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

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

*Corresponding Author: ethanblinck@gmail.com

Acknowledgements: We thank generations of New Guinean landowners and field assistants without whose help this paper would have been impossible to write. For tissue loans, we thank L. Joseph at ANWC, K. Zykowski at YPM, R. Moyle and M. Robbins at KUMNH, P. Sweet at AMNH, and R. Prys-Jones at NHMUK-Tring. For help arranging fieldwork, we thank A. Mack, G. Kapui, J. Robbins, N. Gowep, B. Beehler, L. Dabek, N. Whitmore, F. Dem, S. Tulai, B. Iova., and V. Novotny. We thank A. Wiebe for assistance measuring specimens and thank C.J. Battey for his many contributions. This work was supported by NSF Doctoral Dissertation Improvement Grant #1701224 to J. Klicka and E.B.L., a NDSEG Fellowship to E.B.L., and by NSF DEB #0108247 to J.P.D

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 contact 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

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

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

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

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

Morphological and bioacoustics data. To evaluate phenotypic support for species limits, we measured bill length, bill depth, tarsus, wing chord, and tail length from 72 museum specimens of *Syma torotoro* ($n=30$), *S. (t.) ochracea* ($n=10$), and *S. megarhyncha* ($n=32$) at the American Museum of Natural History, collected from 1894-1965 and including only individuals of known sex as originally identified by the preparator. Using these data, we performed principal 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 DNeasy kit and the manufacturer's recommended protocol. For historical toepad samples (collected 1877-1973), we extracted DNA using either 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 bbdut 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

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

Population structure inference. We evaluated population genetic structure within and between species using a nonparametric clustering approach. We performed principal component analysis of genotypes (PCA) and identified putative genetic clusters for $k=2$ through $k=4$ using adegenet v. 2.1.1 (Jombart, 2008) and a 95% complete dataset with 66,917 SNPs from 35 individuals of *Syma torotoro* ($n=24$), *S. (t.) ochracea* ($n=2$), and *S. megarhyncha* ($n=9$) that passed quality filters. This included individuals from both sequencing approaches with a minimum depth of coverage of 3x per individual, a maximum depth of coverage of 120x per individual, a minimum quality score of 30, and a minimum minor allele frequency of 0.05. These relatively lenient filtering parameters were chosen to permit the inclusion of samples from both sequencing strategies in our initial assessment of probable species limits. We evaluated the best-fit clustering model using the Bayesian Information Criterion, again implemented in adegenet. To ensure our results were not an artifact of different library preparation methods or tissue type, we employed two complementary approaches. First, we performed pairwise Wilcoxon rank sum tests evaluating whether differences in coverage or the proportion of missing data were significantly associated with inferred clusters. Second, we reran PCA using the same filters as 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 TreeAnnotator 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 1×10^{-4} and 10. We converted parameter values to real units using a genome-wide mutation rate of 2.3×10^{-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 criteria: 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

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

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

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

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

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].

References:

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

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

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

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

- Voelker, G., Semenov, G., Fadeev, I.V., Blick, A., & Drovetski, S.V. (2015). The biogeographic history of *Phoenicurus* redstarts reveals an allopatric mode of speciation and an out-of-Himalayas colonization pattern. *Systematics and Biodiversity*, 13, 296–305.
- Wang, J. (2004). Application of the one-migrant-per-generation rule to conservation and management. *Conservation Biology*, 18, 332–343.
- Winger, B.M., Hosner, P.A., Bravo, G.A., Cuervo, A.M., Aristizábal, N., Cueto, L.E., & Bates, J.M. Inferring speciation history in the Andes with reduced-representation sequence data: an example in the bay-backed antpittas (Aves; Grallariidae; *Grallaria hypoleuca* s. l.). *Molecular Ecology*, 24, 6256–6277.
- Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, 16, 97–259.
- Wu, Y., Colwell, R.K., Han, N., Zhang, R., Wang, W., Quan, Q., ... Lei, F. (2014). Understanding historical and current patterns of species richness of babblers along a 5000 m subtropical elevational gradient. *Global Ecology and Biogeography*, 23, 1167–1176.

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	<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 antpittas</i>	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

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.

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	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

Table S2. The proportion of missing data (genotypes) and mean fold sequencing coverage by library preparation method and tissue type. Missing data rates calculated from the 95% complete SNP matrix used in *k*-means clustering analyses.

Sample subset	Missing data ($\pm$ SD)	Sequencing coverage ($\pm$ SD)
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)

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

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

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 measurements by sex for *S. tororoto*, *S. megarhyncha*, and *S. (t.) ochracea*, respectively. Note the limited sample size of *S. (t.) ochracea*.