Title: History of divergence and gene flow shaping geographic variation in Andean warblers (*Myioborus*)

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Abstract

Studying how genetic variation is structured across space, and how it relates to divergence in phenotypic traits relevant to reproductive isolation, is important for our overall understanding of the speciation process. We used reduced-representation genomic data (ddRAD-seq) to examine patterns of genetic variation across the full distribution of an Andean warbler species complex (*Myioborus ornatus–melanocephalus)*, which includes a known hybrid zone between two strikingly different plumage forms. Genetic structure largely reflects geographic variation in

head plumage, some of which corresponds to major topographic barriers in the Andes. We also found evidence of isolation by distance shaping genetic patterns across the group's broad latitudinal range. We found that *chrysops* and *bairdi*, two taxa with marked plumage differences that have a known hybrid zone, were characterized by low overall genetic divergence. Based on our cline analyses of both plumage and genomic hybrid indices, this hybrid zone extends for approximately 250 km, where advanced generation hybrids are likely most common. We also identified a slight difference in the centers of the plumage and genomic clines, potentially suggesting the asymmetric introgression of *chrysops*-like plumage traits. By studying genetic variation in a phenotypically complex group distributed across a topographically complex area, which includes a hybrid zone, we were able to show how both geographic features and potentially sexually selected plumage traits may play a role in species formation in tropical mountains

Keywords: *phylogeography, hybrid zone, reduced representation genomic data, geographic variation, Parulidae.*

Introduction

Geographic variation in traits relevant to reproductive isolation is often viewed as a snapshot of the early stages of population divergence (Mayr, 1942; Price, 2008), making it a focus for understanding the process of speciation. To study this process, it is useful to evaluate how phenotypic variation in such traits is related to the underlying population genetic structure (Edwards et al., 2016; Zamudio et al., 2016) A common expectation is that phenotypic and genetic divergence are positively correlated: in the absence of strong divergent selection, extended periods of isolation are typically required for phenotypic differentiation to evolve (Nosil, 2008; Price, 2008; Winger & Bates, 2015). It follows that range disjunctions associated

with topographic and ecological barriers likely to have restricted historical gene flow are expected to be accompanied by genetic and phenotypic discontinuities (Edwards et al., 2016). However, geographic variation in phenotypic traits is not always concordant with genetic structure. This discordance could be driven, for example, by strong selection on some traits across environmental gradients over short time scales (Walsh et al., 2017), or complex histories of selection and gene flow leading to phenotypic convergence (Márquez et al., 2020). A better understanding of how species diversify across space requires studying such histories of isolation, and how these relate to divergence in traits, particularly those that might be important for mate choice and, consequently, reproductive isolation.

Patterns of plumage differentiation—thought to be important for reproductive isolation in birds (Price, 2008; Uy et al., 2018)— among allopatric populations within species are pervasive among Andean birds (Graves, 1988; Remsen, 1984). Although geographic variation in plumage and genetic structure in Andean birds is often associated with current topographic and ecological barriers (Gutiérrez-Pinto et al., 2012; Prieto-Torres et al., 2018), many of these barriers tend to be permeable and unstable. Dispersal over such barriers could potentially lead to introgressive hybridization that connecting otherwise diverging populations (Winger, 2017). Additionally, differentiated populations can come into renewed contact when the barriers themselves change over time. In the particular case of the Andes, climatic fluctuations (Ramírez-Barahona & Eguiarte, 2013) and, potentially, volcanic activity (Sanín et al., 2024) have promoted cycles of fragmentation and reconnection of elevational vegetation belts, creating opportunities for contact among periodically isolated populations (Vuilleumier, 1969). Examining these complex histories of divergence and gene flow in geographically variable and widely distributed Andean taxa

contributes to a more comprehensive understanding of how bird species are formed and maintained in this biodiversity hotspot (Rahbek et al., 2019; Sonne et al., 2022).

Here, we used a reduced representation genomic data set from an Andean bird species complex of *Myioborus* warblers (Parulidae) characterized by striking geographic variation in head color patterns to gain insights into differentiation in geographically isolated populations and the processes that occur after differentiated populations come into secondary contact. This young (<1 my; Cuervo, 2013) species complex inhabits the high Andes from Bolivia to Venezuela, and is characterized by differences in head color patterns despite shallow genetic differentiation in mitochondrial DNA (Céspedes-Arias et al., 2021; Pérez-Emán, 2005). Seven plumage groups have been described as subspecies belonging to two species, *Myioborus ornatus* and *M. melanocephalus*, distributed mostly allopatrically along different sections of the Andes (Cuervo & Céspedes Arias, 2023; Zimmer, 1949). According to this classification scheme, *M. ornatus* includes subspecies *ornatus* and *chrysops*, and *M. melanocephalus* includes subspecies *bairdi, griseonuchus, malaris, melanocephalus* and *bolivianus*. Recent taxonomic revisions, however, suggest that a four-species classification scheme might better capture what is known about genetic and plumage variation in this complex (Cuervo & Céspedes Arias, 2023). Regardless of the species delimitation scheme, analyses based on the ND2 mitochondrial gene have revealed extensive haplotype sharing among groups with conspicuous plumage differences, potentially suggesting a history of rapid plumage evolution or repeated mitochondrial introgression (Céspedes-Arias et al., 2021).

Our previous work in regions where the ranges of *chrysops* and *bairdi* abut, on the border between Colombia and Ecuador, revealed that these two taxa hybridize widely across more than 200 km where individuals with a variety of intermediate head color patterns occur (Céspedes-

Arias et al., 2021). Although hybridization there has been extensive, the existence of a sharp plumage cline, limited to a relatively narrow zone within these taxa's broader ranges (20–30%), suggests the action of some form of selection maintaining their differentiation (Barton & Hewitt, 1985; Brelsford & Irwin, 2009; Harrison, 1986). Here, we leveraged this previous systematic collection and detailed plumage characterization of the *chrysops-bairdi* hybrid zone (Céspedes-Arias et al., 2021) to gain a deeper understanding of hybridization dynamics using genomic data.

We examined patterns of genomic variation within this warbler species complex at two different spatial scales: the entire distribution of the species complex, which corresponds to a broad latitudinal band in the Andes (approximately 3800 km in geographic distance), and the hybrid zone existing between two markedly different plumage groups as outlined above. By focusing on these two different scales, we can address questions related to multiple processes relevant to the formation and maintenance of species. First, leveraging a broad geographic scale sampling of individuals representing all plumage forms in the complex, we asked: (1) How is genetic structure related to geographic distance, topography, and plumage variation along the Andes? At a finer spatial scale, in the hybrid zone between two plumage forms, we asked: (2.1) Does the geographic pattern of introgression mirror plumage variation across the hybrid zone? And (2.2) is there evidence for non-neutral processes such as selection against hybrids based on patterns of genomic variation? Our genome-wide data coupled with dense geographic sampling substantially improves our ability to reconstruct and understand the diversification history of these colorful montane warblers, which was previously limited to studies using only a single mitochondrial gene.

Methods

Study species and sampling

We used tissue samples (*n*=227, Figure 1) collected across the distribution of the *M. ornatus-melanocephalus* complex, by us (Céspedes-Arias et al., 2021), or previously deposited in museum collections (Supplementary Table 1). Samples encompassed all (seven) currently taxonomically recognized plumage forms and a denser sampling in the *bairdi-chrysops* hybrid zone area, along the border between Colombia and Ecuador (Figure 1A). We also obtained data for eight individuals as outgroups corresponding to three other species in *Myioborus*: *M. pictus* (n = 3), *M. miniatus* (n = 3) and *M. brunniceps* (n = 2) which vary in their phylogenetic and geographic proximity to the *M. ornatus-melanocephalus* complex (Lovette et al., 2010; Pérez-Emán, 2005).

The more conspicous differences among plumage groups in the *ornatus-melanocephalus* complex correspond to the presence/absence and extent of colored plumage patches in the head, as illustrated in Figure 1B. We note that there are different degrees of plumage differentiation and diagnosability based on our own assessments (Cuervo & Céspedes Arias, 2023). For example, the subspecies *malaris, bolivianus* and *melanocephalus* are very similar in plumage, and some of the described diagnostic traits do not seem to vary in a way that is discrete and corresponds with these groups (Cuervo & Céspedes Arias, 2023).

We also note that the geographic limits of some groups are also not clearly defined, specifically of the *bairdi* and *griseonuchus* groups which have only subtle differences in in the amount of yellow vs black on the sides of the face, as well as the presence and extent of a black nuchal band behind the rufous crown (Chapman, 1927; Cuervo & Céspedes Arias, 2023; Zimmer, 1949). Although birds from northern Peru (Dept. of Cajamarca) and central Ecuador are clearly distinct (Supplementary Figure 1), it is unclear whether a phenotypic transition is discrete

or clinal across intervening southern Ecuador and there is a lack of sampling across this area to evaluate these possibilities. However, to inform our discussion on patterns of genetic variation, we inspected most of the available specimens collected in this area, either directly or through photographs, to describe variation in these two plumage traits (Supplementary Figure 1).

Laboratory procedures

DNA extraction and library preparation

We extracted genomic DNA from pectoral muscle (n=232) and blood samples (n=3) employing a modified phenol-chlorophorm protocol (Sambrook, 1987), using phase-lock tubes. Each DNA extraction was subsequently purified using homemade Sera-Mag Magnetic Speed-beads for magnetic nucleic acid extraction (MagNA). To obtain reduced representation genomic data, we used double-digest restriction site-associated DNA sequencing (ddRAD) following Peterson et al. (2012), and using modifications from Thrasher et al. (2018). The genomic libraries were then sequenced on one HiSeq2500 Illumina lane (100 bp SE) by the Genomics Core Facility of Cornell University.

Bioinformatics pipeline

Quality assessment, filtering and demultiplexing

We assessed sequence quality using FastQC (Andrews, 2010). We used the *process_radtags* function implemented in STACKS 2.66 (Rochette et al., 2019) to demultiplex the reads and apply quality filters. Specifically, we filtered out all reads with uncalled bases (*--clean*), those with low phred quality scores *(--quality*, *--score-limit* 20) and those that did not pass the Illumina chastity/purity filter (*--filter-illumina*). After demultiplexing, we checked the number of reads

146 available per individual, and only kept those samples with 200,000 or more reads
147 (Supplementary Figure 2). Using this threshold, we discarded 11 individuals, mostly
148 corresponding to the *ornatus* group and tissues collected around ten years prior to this study
149 (range of number of reads for these individuals = 960–150,152). Despite discarding these
150 samples, 23 high-quality samples for the *ornatus* group remained in downstream analyses. After
151 excluding these individuals from downstream analyses, we kept a total of 242,204,894 reads
152 corresponding to 224 individuals (three represented twice in the library).
153
154 ***Integrated assembly and SNP calling***
155 After demultiplexing, we assembled the RAD reads *de-novo* using functions implemented in
156 STACKS 2.66 (Rochette et al., 2019), and later integrated position information for the loci in the
157 catalog from a reference genome (Paris et al., 2017; Rivera-Colón & Catchen, 2022). This
158 integrated approach performs better than directly mapping reads onto a reference genome in
159 some scenarios, for example, if the reference genome is relatively distant to the study species as
160 is our case (Paris et al., 2017). We used the chromosome-level assembly of the *Setophaga*
161 *coronata* genome (Baiz et al., 2021) as reference, the highest quality available genome within
162 Parulidae. The time to the most recent common ancestor between *Setophaga* and *Myioborus* is
163 estimated between 5–7 mya (Barker et al., 2015).
164
165 ***Optimization of de novo assembly parameters***
166 Before assembling the reads, we conducted an optimization of the parameters *M* and *n* used by
167 *denovo_map.pl* (Rochette et al., 2019), for three different assemblies: one including
168 representatives from the outgroups, one that only included individuals within the *ornatus*-

169 *melanocephalus* complex, and one including only samples relevant for the hybrid zone analyses
170 (*chrysops*, individuals in the hybrid zone, *bairdi,* and *griseonuchus*, Figure 1) (see
171 Supplementary Material and Supplementary Table 2 for details).
172
173 ***Full de novo assembly run***
174 We then ran *denovo_map.pl* for the entire dataset with the chosen parameter values, using again
175 the three population maps (n = 224 full assembly, n = 216 ingroup only assembly, and n = 123
176 for the hybrid zone assembly). For the population maps corresponding to the three different
177 assemblies, we classified individuals into one of the following groups: *ornatus*, *chrysops*,
178 "hybrid zone", *bairdi, griseonuchus, malaris, melanocephalus*, and *bolivianus* (populations_2
179 column in Supplementary Table 1), plus *pictus, miniatus*, and *brunniceps* for the assembly
180 including the outgroups. For the population map purposes the hybrid zone group corresponds to
181 a geographic group encompassing populations in and near the hybrid zone, irrespective of the
182 plumage phenotype of the specimens (see the map in Figure 1).
183 After the assemblies were completed, we assessed the per sample coverage and
184 percentage of reads incorporated (Rivera-Colón & Catchen, 2022). The assembly corresponding
185 to only individuals within the *melanocephalus-ornatus* complex resulted in a catalog of 91,650
186 loci, with a mean effective coverage per sample of 59.1x (SD = 13.2x, min = 18.3x, max = 97.4).
187 The "hybrid zone" assembly resulted in a catalog of 70,281 loci, with a mean effective coverage
188 per sample of 61.6x (SD =12.1x, min = 22.5x, max = 99.4x). The assembly including outgroups
189 resulted in a catalog of 97,533 loci, with a mean effective coverage per sample of 59.3x (SD =
190 13.2x, min = 18.1x, max = 98.5x). Four individuals with a mean effective coverage lower than

191 20x in assemblies in which they were included initially were excluded from downstream
192 analyses (see Supplementary Table 1; Supplementary Figure 3).
193
194 ***Mapping to the reference genome***
195 After obtaining the *de novo* catalog, we mapped loci to the reference genome by first indexing
196 the *S. coronata* genome using *bwa index* (Li & Durbin, 2009). Then we used *bwa mem* to align
197 the *de novo* catalog to the reference genome. Finally, we used the *stacks-integrate-alignments*
198 script to generate a catalog with position information (Rivera-Colón & Catchen, 2022; Rochette
199 et al., 2019). After filtering based on mapping quality and alignment coverage (using default
200 values), 60,523 loci (66% of the total number of loci in *de novo* catalog) were retained for the
201 ingroup assembly, 46,036 (66% of the total number of loci in *de novo* catalog) were retained for
202 the "hybrid zone" assembly, and 65,537 (67% of the total number of loci in *de novo* catalog) for
203 the full assembly.
204
205 ***SNP datasets***
206 We obtained different SNP datasets to be used in downstream analyses using the *populations*
207 program from STACKS 2.66 (Rochette et al., 2019). For some analyses, as specified below, we
208 did further filtering of SNPs using *vcftools* (Danecek et al., 2011). For the ingroup assembly, we
209 obtained two different SNP datasets: one including one SNP per locus, and one that included all
210 variant and non-variant sites per locus (i.e., full haplotypes only for fineRADStructure, see
211 below). In both cases we applied the following filters: *--min-samples-overall* 0.8 (locus needs to
212 be present in 80% of all individuals to be retained), *--min-mac* 5 (minimum allele count of 5),
213 and *--max-obs-het* 0.7 (maximum observed heterozygosity of 0.7). For the full haplotype dataset,

214 we used the same parameters and included the *--filter-haplotype-wise* flag. We decided to apply
215 a minimum allele count (*--min-mac* 5) filter to exclude SNPs that may be artifacts of genotyping
216 errors, but kept a relatively low value to avoid excluding all low frequency SNPs which also
217 contain relevant information for population genetic analyses (Linck & Battey, 2019). Details on
218 how the SNPs datasets from the three different assemblies were obtained are in the
219 Supplementary information. In short, we used the same filtering parameters for the hybrid zone
220 assembly and for the assembly including outgroups we used *--min-mac 2* and *--min-samples-per-*
221 *pop* 0.5
222 Based on the assembly for the ingroup only, and after filtering, we obtained 5,054 loci,
223 composed of 73,786 sites, including 22,132 variant sites for the full haplotype dataset. For the
224 ingroup (one SNP per locus) dataset, we obtained the same number of loci and kept 4,800 variant
225 sites.
226
227 **Analyses of diversification within the *ornatus-melanocephalus* complex**
228 We described and visualized genetic structure in order to test its relationship with plumage and
229 geography. To evaluate how genetic structure related to current taxonomic patterns of variation
230 in head color patterns (see Figure 1), we also inferred subspecies-level phylogenies and
231 admixture graphs. Additionally, we estimated the level of differentiation between plumage
232 groups in terms of F_{ST}. Lastly, we used a spatially explicit model to evaluate how genetic
233 variation within the *ornatus-melanocephalus* complex is structured across their range.
234
235 ***Phylogenetic and admixture graph inference***

236 With the goal of inferring the overall relationships among plumage groups, we employed two
237 methods to build phylogenies and infer admixture graphs. For this set of analyses, we excluded
238 all individuals from or adjacent to the hybrid zone (in white, Figure 1) and the *griseonuchus-*
239 *bairdi* transition area (arrows, Figure 1), and used the assembly that includes outgroups (*M.*
240 *pictus, M. miniatus, M. brunniceps*). For phylogenetic approaches, we included all outgroup
241 individuals (total n=110), while for the admixture graph inference, we only included one
242 outgroup (total n=105).
243
244 **raxml-ng:** We used RAxML-NG (Kozlov et al., 2019) to infer maximum-likelihood trees from
245 concatenated ddRAD loci, including both variant and invariant sites. Because individual
246 fragments are short (~150 bp), we concatenated all loci into a supermatrix (Leaché & Oaks,
247 2017). A VCF including invariant sites was generated with *populations* using the parameters --
248 *vcf-all --min-mac* 2 *--min-populations* 10 *--min-samples-per-pop* 0.5, then converted to Nexus
249 with *vcf2phylip*. (https://github.com/edgardomortiz/vcf2phylip). Then, we ran *raxml-ng* using
250 this nexus alignment and the GTR + G model of nucleotide substitution with no partitions. To
251 search for the tree with the highest likelihood, we ran 20 independent runs, 10 of them starting
252 from the maximum parsimony tree and 10 from random trees. From these searches, the tree with
253 the highest likelihood was selected among the 20 slightly different trees. We calculated branch
254 support with 100 bootstrap replicates.
255
256 **snapper:** To infer evolutionary relationships among plumage groups under a phylogenetic
257 framework, we used *snapper* (Stoltz et al., 2021). *Snapper* infers a "species" tree using diffusion
258 models and takes as input biallelic SNPs that are assumed to be unlinked, bypassing the necessity

259 of building individual gene trees, similar to site-based coalescent methods (Bryant et al., 2012).
260 We classified individuals into groups corresponding to the seven subspecies described in Figure
261 1. For this analysis, we generated a SNP dataset from the assembly including outgroups (*--min-*
262 *mac* 2, *--max-obs-het* 0.7, --min-populations 10, *--min-samples-per-pop* 0.5) filtered to only keep
263 biallelic SNPs using *vcftools* and then converted the resulting *vcf* files to nexus using the
264 *vcf2phylip* script. Using the nexus alignments as input, we prepared the *.xml* files in Beauti 2.7.6
265 (Bouckaert et al., 2019). We ran four independent chains with a length of 15,000,000 steps, and a
266 thinning interval of 1,500. We set a gamma prior for the birth rate (λ), and left default values for
267 the remaining priors. We increased the weights of two operators (gamma mover and rate mixer)
268 to 10. Stationarity was assessed using Tracer 1.7.1 (Rambaut et al., 2014), based on a visual
269 exploration of trace plots and ESS values (ESS > 500 was obtained for all parameters.) After
270 exploring trace plots, we set the burn-in at 10%.
271
272 **TreeMix (OrientAGraph):** As an alternative way to describe the historic relationships among
273 plumage groups, we used *OrientAGraph* (Molloy et al., 2021), an approach based on the
274 *TreeMix* algorithm, which implements a graph-based model that uses genome-wide allele
275 frequencies to infer population splits and admixture events (Pickrell & Pritchard, 2012). This
276 method detects evidence of historical introgression between plumage groups and can better
277 describe the history of populations when not fully bifurcating. For input, we applied the same
278 SNP filtering as in the *snapper* analysis, adjusting only *--min-populations* to 8 and excluding
279 SNPs with >30% missing data using *--max-missing* 0.7 in VCFtools. The filtered VCF was
280 converted to TreeMix format with *vcf2treemix.py*.
281 (https://github.com/CoBiG2/RAD_Tools/blob/master/vcf2treemix.py).

282 We ran *OrientAGraph* testing for a range of migration edges (*m* parameter), running 5
283 iterations per *m*, with *m* values ranging from 1 to 6. We used *M. miniatus* (n = 3) as outgroup to
284 root the tree and incorporated a *bairdi-chrysops* known migration event using the *-cor_mig* and -
285 *climb* flags, and a 0.5 migration weight. This was done to account for the high likelihood that
286 there are signs of introgression even away from the hybrid zone. After running all iterations for
287 this range of m values, we used *OptM* (Fitak, 2021) to evaluate the optimal value of *m* in each
288 case, using an approximation based on linear regressions between *m* values and likelihood. We
289 made a small modification to the *optm* function to be able to account for the known migration
290 event. Based on this linear regression criteria, we chose the optimal *m* values and included those
291 results here.
292
293 ***Genetic structure within the ornatus-melanocephalus complex***
294 **PCA:** To summarize genetic variation in the *ornatus-melanocephalus* complex, we conducted a
295 genomic PCA using functions implemented in *gdsmt* and *SNPrelate* (Zheng, 2013) for R. To
296 conduct this PCA, we used only one SNP per locus, including only biallelic SNPs. To account
297 for the effect of missing data on the PCA (Yi & Latch, 2022), we (1) visualized the amount of
298 missing data in our PC plots, to evaluate the possibility that high missing data samples are
299 clustered together and/or have values biased towards the origin, (2) ran an additional PCA only
300 including SNPs with a missing rate < 0.1, and (3) ran an additional PCA using a different *vcf* as
301 input with more strict filtering for missing data when running populations (*--min-samples-overall*
302 *0.9*). We also conducted a PCA for a subset of the individuals to describe in more detail genetic
303 variation in the hybrid zone and adjacent areas (*chrysops*, hybrid zone individuals, *bairdi*, and
304 *griseonuchus*).

305 **fineRADStructure:** As a complementary approach to visualize genetic structure within the
306 *ornatus-melanocephalus* complex, we used *fineRADStructure*, which estimates a coancestry
307 matrix and an associated distance tree based on full haplotype information from RADseq data
308 (Malinsky et al., 2018). This method has particularly high resolution in inference of recent
309 shared ancestry, and allows one to describe nested patterns of genetic structure (Malinsky et al.,
310 2018). To run *finestructure* (part of the *fineRADStructure* pipeline), we used the output from the
311 populations program containing both variant and invariant sites (i.e., full haplotypes). No
312 population prior was specified for the run, which was set for 200,000 iterations with a burn-in of
313 100,000 (-x 100,000, -y 100,000), and a thinning interval of 1,000 (-z 1000).
314
315 **Calculation of F_{ST}:** To estimate overall genetic differentiation among plumage groups, we
316 calculated pairwise F_{ST} between subspecies (i.e., plumage and geographic groups). We excluded
317 all individuals from the hybrid zone from this analysis. Given the uncertainty in the *bairdi-*
318 *griseonuchus* limits (see Results), we also excluded individuals from the southern Ecuadorian
319 provinces (as in the *snapper* analyses). We calculated the Weir and Cockerham estimator (Weir
320 & Cockerham, 1984) using *vcftools* (Danecek et al., 2011) in 25,000 bp windows. To test for
321 potential discordance between nuclear and mitochondrial divergence, we evaluated whether
322 SNPs-based pairwise F_{ST} values were correlated with those estimated from the ND2 gene
323 (Céspedes et al. 2021). To assess the correlation between these two matrices, we implemented a
324 Mantel Test based on a Pearson's correlation using the function *mantel* implemented in the R
325 package *vegan* (Oksanen et al., 2007)
326 To test for isolation-by-distance (IBD), we also calculated pairwise windowed F_{ST}
327 between localities as defined in Supplementary Table 1 and evaluated if these are correlated with

328 the pairwise geographic distances. Geographic distances between localities were calculated using
329 the function *rdist.earth* implemented in the R package *fields* (Nychka et al., 2025). We also
330 evaluated the correlation between these matrices using a Mantel test based on a Pearson's
331 correlation as implemented in *vegan*.
332
333 **Admixture:** We employed admixture (Alexander et al., 2009) to estimate individual genetic
334 ancestries using a maximum-likelihood approach at three different geographic scales: whole
335 complex, hybrid zone and adjacent populations (*bairdi, chrysops*, hybrid zone, *griseonuchus*),
336 and hybrid zone only (*bairdi*, *chrysops*, hybrid zone).
337 Prior to running admixture, we calculated relatedness between all individuals and then
338 excluded individuals that appeared closely related. Specifically, we used the *--relatedness2* flag
339 in *vcftools* to export a table of pairwise relatedness values using the KING kinship estimator
340 (Manichaikul et al., 2010). We then identified pairs of individuals with a relatedness higher than
341 0.177 (i.e. likely first-degree relatives) and excluded one of them from admixture analyses. We
342 then transformed the VCF files to *.bed* format by using plink2 (Chang et al., 2015). Additionally,
343 we performed a SNP window linkage-disequilibrium pruning using plink2 *(--indep-pairwise*,
344 followed by *--extract*), on windows of 50 SNPs and using a 0.3 threshold. This was done for both
345 scales of analyses, and then admixture was run on the thinned datasets.
346 For all admixture runs, we included the *–cv* flag, to perform the cross-validation
347 procedure to choose the optimal k (i.e. number of source populations) value. For the whole
348 complex analyses, we ran admixture for values of $k\{1\ldots10\}$, and for the other two scales, we
349 used values of $k\{1\ldots6\}$. For each value of k, we ran 10 iterations starting with a randomly

350 generated seed. We analyzed variation across runs using *pong* (Behr et al., 2016) and generated
351 figures using the *mapmixture* (Jenkins, 2024) package for R.
352
353 **FEEMS:** To more directly evaluate how genetic variation is shaped across the Andes, we used a
354 spatially explicit model based approach: *FEEMS* (Marcus et al., 2021)
355 (https://github.com/VivaswatS/feems). This approach is based on a model of IBD and builds
356 continuous migration surfaces based on a geographic grid, visualizing areas of inferred low and
357 high gene flow (Marcus et al., 2021). To run FEEMS, we prepared four input files: (1) *plink* file
358 with genotype data for all individuals in the *ornatus-melanocephalus* complex (same set as for
359 whole-complex admixture analyses), (2) text file with geographic coordinates for all specimens
360 with genotype data (Supplementary Table 1), (3) a global grid with triangular cells of
361 approximately 7,774 km^2, obtained using the package *dggridR* for R (res=8; Barnes et al. 2024),
362 and (4) outer extent boundary for the grid, which was generated as a polygon that roughly
363 delineates the montane areas occupied by *Myioborus* and encompasses all sampling sites. The
364 chosen resolution of the grid allowed for most individuals in the same sampling site to be
365 assigned to a unique deme, based on preliminary explorations. We used the cross-validation
366 approach (Marcus et al., 2021) to choose the penalty parameters to be used in the optimization
367 procedure (λ and λ_q; https://github.com/VivaswatS/feems/blob/main/docsrc/notebooks/cross-
368 validation.ipynb). After preliminary explorations, the migration surface presented here was
369 generated using $\lambda = 0.17$ and $\lambda_q = 100$, which minimized the cross-validation error (although
370 lower values appeared roughly as adequate, Figure 6).
371
372 **Analysis of genetic variation across the *bairdi* x *chrysops* hybrid zone**

373 ***Calculation of a genomic hybrid index and genetic composition of the hybrid zone***

374 We estimated a genomic hybrid index and interclass heterozygosity using functions implemented
375 in *triangulaR* (Wiens & Colella, 2025). As noted above, the southern parental population was
376 defined based on our own results and includes individuals from central to south Ecuador that do
377 not cluster genetically with *griseonuchus* (see Results). In the SNP dataset for this analysis, we
378 included all SNPs per locus.

379 To identify ancestry-informative SNPs, we chose an allele frequency difference of 0.5
380 between parental populations. Higher thresholds resulted in very few (i.e., less than 100) or no
381 SNPs being retained, and there were no alleles fixed in each parental population. Then, to
382 describe the genetic composition of the hybrid zone, we used the values of genome-wide hybrid
383 indices and observed interclass heterozygosity for all individuals, and plotted these in a triangle
384 plot (Cronemberger et al., 2020; Liu et al., 2022; Lopez et al., 2021).

385

386 ***Geographic cline analyses***

387 To describe the geographic extent of the hybrid zone and evaluate whether genetic and plumage
388 variation were concordant, we fitted geographic clines using *HZAR* (Derryberry et al., 2014). We
389 fitted and compared clines for genomic hybrid indices—corresponding to admixture proportions
390 and estimated using *gghybrid*—and plumage hybrid indices estimated previously for these
391 individuals (Céspedes-Arias et al., 2021). For each of these "traits", we fit a model that estimates
392 cline width (w) and center (c) but has fixed values for mean and variance of the trait values at the
393 cline extremes (μL, μR, $varL$, $varR$). Both models were run with a tune value of 1.5, and for each
394 of these we ran 3 chains of 2 million iterations discarding the first 10% as burn-in. All runs were
395 performed randomizing starting parameters. To evaluate whether plumage and genomic clines

396 are coincident and concordant, we compared the 95% confidence interval of the center and width
397 estimates, which were calculated aggregating the three independent chains for each model.
398

399 ## Results

400 **Evolutionary relationships among plumage groups as inferred from phylogenies and**
401 **admixture graphs**

402 We employed two different phylogenetic approaches to describe evolutionary relationships
403 among the seven plumage groups that define the study taxa. Using a maximum-likelihood
404 approach with all loci concatenated (21,304 variant sites), we found that most individuals of the
405 same subspecies clustered together (Figure 1, panel C), yet we did not observe complete
406 reciprocal monophyly in most groups. The *chrysops* group was the exception, as it appeared as a
407 clade nested within the *ornatus* population. Notably, however, support values were generally
408 low, implying that not many sites are phylogenetically informative as expected for a scenario of
409 recent divergence and/or ongoing gene flow. Generally, we observed that individuals from
410 geographically adjacent subspecies are intermixed in the tree.

411 The *snapper* subspecies-level tree (Figure 2A), inferred using 3,105 biallelic SNPs,
412 showed strong support for a monophyletic "southern" group, encompassing three *M.*
413 *melanocephalus* subspecies (*malaris, melanocephalus, bolivianus*) characterized by having
414 spectacles and a black crown. The taxon *griseonuchus*, from north of the Marañón River Valley,
415 characterized by having a reddish crown that is phenotypically very similar to *bairdi*, was
416 recovered as sister to a strongly supported clade including the more northerly groups from
417 Colombia and Ecuador. Within this expanded "northern" group, *chrysops* was recovered as sister
418 to *ornatus*, and these, in turn, are sister to *bairdi* (Figure 2A). Note that, unlike the maximum-

419 likelihood tree, we *a priori* assigned individuals to groups for these analyses, which can bias the
420 phylogenetic estimation given that the limits among these populations are not fully defined.
421 Overall, we found stronger signals of admixture events between the northern relative to
422 the southern group (Figure 2B). Depending on the *m* value, we recovered slightly different
423 topologies, though we note that these incongruencies were restricted to the relationships between
424 the northern group taxa, namely *griseonuchus, bairdi*, *chrysops*, and *ornatus* taxa (Figure 2B).
425 Here we present the inferences for m = 1 and m = 3 as these were the optimal values based on
426 the linear regressions fitting the likelihood vs *m* curves (Supplementary Figure 1). Both graphs
427 consistently recovered a monophyletic "southern" group (*malaris*, *melanocephalus* and
428 *bolivianus*), which was consistent with the topology of the *snapper* tree. In the m = 1 graph
429 (Figure 2B), *griseonuchus* and *bairdi* appeared as sister, and this clade was, in turn, sister to the
430 southern group. In contrast, in the m=3 graph, *bairdi* was sister to *chrysops*, and thus a migration
431 edge between these taxa was not estimated despite the known migration event parameter. Two
432 edges with high migration weight were inferred, between *griseonuchus* and *bairdi,* and
433 *griseonuchus* and the *bairdi-chrysops* node. An additional edge, with low migration weight, was
434 inferred between the outgroup and the *bolivianus-melanocephalus* group (Figure 2). We note,
435 however, that the outgroup has a very low sample size (n = 3) and thus do not find this inference
436 to be very reliable.
437
438 **Genetic structure within the *ornatus-melanocephalus* complex**
439 The PCA, based on 4,800 biallelic SNPs, showed that individuals generally cluster in relation to
440 plumage groups and geographic origin (Figure 3), but some phenotypically distinct groups were
441 very close in the PC1-2 space (particularly *chrysops, ornatus* and *bairdi*). As in the phylogenetic

442 analyses, one can distinguish clearly the "southern" group, including all individuals from
443 populations south of the Marañón River Valley (*bolivianus, melanocephalus, malaris,* Figure 3),
444 which occupied a wide range of PC1 values (Figure 3). There was also an identifiable northern
445 cluster (*chrysops, bairdi*, and hybrid zone individuals) that was, however, not fully distinct from
446 a third cluster comprised of *griseonuchus* individuals. Within this northern cluster, the plumage
447 groups *ornatus, chrysops, bairdi,* and individuals from the hybrid zone ("northern", hereafter)
448 appeared very close in PC1-2 space. As expected, individuals from the hybrid zone (in gray,
449 Figure 3) fell intermediate between their parental populations. The *ornatus* group appeared as a
450 more defined cluster when considering PC3 (Supplementary Figure 5). Notably, some
451 individuals putatively classified as *bairdi*, collected in the Ecuadorian provinces of Loja and
452 outlining ridges in the Amazon, appeared to cluster with *griseonuchus* (Figure 2). We observed a
453 similar pattern in the hybrid zone plus *griseonuchus* PCA (Supplementary Figure 6). We
454 obtained similar results when using a SNP set of the whole complex obtained with a stricter
455 filtering for the PCA (Supplementary Figure 7).

456 Two main clusters are supported in the coancestry matrix resulting from
457 *fineRADstructure*: populations occurring from south Ecuador to Bolivia (*griseonuchus*
458 plus "southern" group), and those occurring from Ecuador to Colombia ("northern" group)
459 (Figure 3C), which contrasted with the topology inferred by *snapper*. Within the first group,
460 *griseonuchus* and the southern group appear as differentiated clusters. The few exceptions to this
461 pattern are a few putatively *bairdi* individuals that clustered with *griseonuchus*, but were
462 collected in the southern edge of the distribution of this plumage group (where it is replaced by
463 *griseonuchus,* Figures 1 and 3) in the Cordillera del Cutucú and Loja Province of Ecuador. We
464 detected two main groups within the "northern" group (Ecuador and Colombia): one

465 corresponded to *ornatus* (occurring in the Eastern Andes of Colombia) and the other to the
466 hybridizing taxa in the complex and individuals from the hybrid zone.

467 Admixture analyses including all individuals in the *ornatus-melanocephalus* complex
468 revealed genetic breaks along the distribution of these warblers that were overall consistent with
469 fineRADStructure (Figure 4, A). For example, when assuming K = 2 and K = 3 the "southern"
470 group appeared as an identifiable cluster. For K = 2, which is the optimal K value based on
471 cross-validation error values (Supplementary Figure 8, top panel), the group *griseonuchus* also
472 clustered with the southern group (Figure 4, A1). For K = 3, a "southern" plus *griseonuchus*
473 group can be also identified, and there was an additional genetic break roughly located in the
474 described *bairdi-chrysops* hybrid zone in southern Colombia (Figure 4, B1).

475 The results of an admixture analyses focused on the hybrid zone and adjacent
476 populations, when assuming K = 2, showed a genetic break that clearly coincides with the
477 *chrysops-bairdi* hybrid zone (Figure 4, B and C), as previously identified based on plumage
478 variation (see map in Figure 1). However, when also considering *griseonuchus*, the transition
479 between these genetic groups is less sharp and displaced to the south (Figure 4, panel B). In both
480 cases there was some east-west differentiation, with localities to the west of the Ecuadorian
481 Andes showing a higher mean admixture proportion corresponding to the southern cluster
482 (Figure 4, B1 and B3). When using K = 3 for the analyses including *griseonuchus* (a *K* that
483 corresponds to the number of identifiable plumage groups), two genetic clusters were identified
484 in Ecuador, and one in Colombia, and one of these genetic breaks did again roughly correspond
485 to the *bairdi-chrysops* hybrid zone (Figure 4, B2). In both cases the *K* value with the lowest
486 cross-validation is *K* = 1 (Supplementary Figure 7).

487 Values of pairwise F_{ST} between populations showed an overall correspondence with
488 geographic distance: subspecies that are far apart in space show higher genetic differentiation
489 (e.g., *ornatus* vs *bolivianus,* Figure 5). We observed higher pairwise differentiation between taxa
490 belonging to the "southern" versus "northern" groups, which is consistent with all other analyses
491 describing genetic structure. In contrast, there was very low differentiation among the three
492 subspecies occurring south of the Marañón River Valley ("southern" group), which all have
493 similar head coloration. However, there was also relatively low levels of differentiation within
494 the "northern" plus *griseonuchus* group, which encompasses striking plumage diversity,
495 particularly between the two hybridizing taxa, *bairdi* and *chrysops, and bairdi* and *griseonuchus*
496 (Figure 5). There was a positive correlation (Figure 5, Mantel statistic r = 0.75, p-value = 0.001)
497 between pairwise F_{ST} calculated based on our ddRAD SNPs and the ND2 mitochondrial gene
498 (Céspedes-Arias et al., 2021). Lastly, as expected if patterns of genetic variation reflect IBD, we
499 found a positive correlation between pairwise F_{ST} between localities and geographic distance
500 (Figure 5, Mantel statistic r = 0.65, p-value = 0.001). Using the same classification scheme for
501 individuals as that to calculate F_{ST}, we estimated genetic diversity statistics. We found that
502 nucleotide diversity is very similar among groups, ranging from 0.076 to 0.091 (Supplementary
503 Table 3).
504 The migration surfaces inferred by FEEMS suggest that there are areas within the
505 distribution of these warblers in which genetic differentiation cannot be fully explained by IBD
506 (i.e., areas of inferred low gene flow; Figure 6). These areas of low migration coincided mostly
507 with two geographic breaks that dissect the distribution of the complex (Figure 1, Figure 6),
508 specifically the Magdalena River Valley and the Marañón River Valley, and with the hybrid
509 zone. In contrast, most of the range south of the North Peruvian low showed mid to high values

510 of inferred effective migration, which can indicate that genetic structure along this area is well
511 explained by IBD alone. We acknowledge that low sampling density in certain regions,
512 particularly in central and southern Peru, may have influenced the inferred migration surface
513 (Figure 6). However, cross-validation results indicate that the selected regularization parameter
514 provides a good fit while accounting for unobserved nodes (Supplementary Figure 9). With that
515 caveat in mind, the FEEMS results are broadly consistent with the patterns of genetic structure
516 revealed by our other analyses.
517
518 **Hybrid zone**
519 To describe the geographic extent of hybridization and the genetic composition of the *bairdi* x
520 *chrysops* hybrid zone, we identified ancestry-informative markers to estimate genomic hybrid
521 indexes and interclass heterozygosity for individuals across the hybrid zone and parental *bairdi*
522 and *chrysops* populations. Using a relatively low threshold for allele frequency difference (0.5),
523 we identified only 29 sites that were ancestry informative from the total of 14,301, and none of
524 these were fixed between the parental populations. This result complicates the interpretation of
525 the triangle plots (see below) and highlights the low level of genetic differentiation between
526 these two hybridizing taxa. Because of the scarcity of ancestry informative sites in our ddRAD
527 data set, which can affect the accuracy of genomic hybrid index estimations, we opted to conduct
528 cline analyses with admixture proportions (with K=2) instead.
529 We found that the head coloration and genomic hybrid index, defined as the admixture
530 proportions in Figure 4 (panel C3) had different centers but similar widths (Figure 7, panel A).
531 The admixture proportion cline had a width of 281.6 km (95% confidence interval [CI]: 245.0–
532 319.8km), compared to 240.6 km for head coloration (95% confidence interval [CI]: 277.5–

533 311.1 km). Given the substantial overlap in confidence intervals, we do not interpret this 41 km
534 difference in width as significant. However, we found a 37 km difference in the estimates of the
535 center between the plumage and admixture proportion clines, for which the 95% confidence
536 intervals did not overlap. For admixture proportions the cline was centered around 348.8 km
537 (95% confidence interval [CI]: 335.4–362.7 km) in our sampling transect, while the plumage
538 cline was centered around 311.1 km (95% confidence interval [CI]: 300.7–322.6 km). Therefore,
539 based on our *HZAR* analyses, the plumage cline appeared to be displaced towards the south (i.e.,
540 towards Tungurahua, Ecuador, the origin of the sampling transect), implying that the *chrysops*-
541 like plumage traits may be introgressing into *bairdi*. However, there was an overall strong
542 correlation between the plumage hybrid indices and admixture proportion across the hybrid zone
543 and allopatric populations (Figure 7, panel B; linear model; *p-value* < 0.001, *m* = 0.9, *R-squared*
544 = 0.8). We identified the outliers of this linear regression as those individuals with residuals >2
545 SD units from the mean, finding that most were below the regression line (Figure 7).
546 Specifically, we identified six individuals that had a more *chrysops*-like plumage from the hybrid
547 zone area (i.e., lower) relative to what the model predicts based on the admixture proportion,
548 which was consistent with the cline analysis results that shows that *chrysops*-like plumage is
549 introgressing into *bairdi*.
550 To describe the genetic composition of the *bairdi-chrysops* hybrid zone, we built triangle
551 plots (interclass heterozygosity vs hybrid index) based on the 29 ancestry-informative markers
552 that we identified (Figure 7, panel C). The individuals from the hybrid zone are distributed
553 towards the center of the triangle, many below the line that defines what value combinations are
554 possible under Hardy-Weinberg Equilibrium which is likely a product of the low allele
555 frequency difference threshold. We note that individuals from populations away from the hybrid

556 zone (Figure 7, in green and orange) are not located in the bottom corners of the triangle plot,
557 which is expected given that the allele frequency difference threshold is lower than one. If
558 interpreted considering the caveats described above, the distribution of individuals in the triangle
559 is suggestive of a hybrid zone mostly composed of advanced generation hybrids, without many
560 F1s.

561 **Discussion**

562 We used reduced-representation genomic data to study genetic variation at two different spatial
563 scales: the full distribution of a geographically variable warbler complex (*M. ornatus-*
564 *melanocephalus*) and a known hybrid zone between two of these plumage forms. At both scales,
565 our set of thousands of SNPs allowed us to explore genetic structure with a resolution that
566 mtDNA loci could not provide in previous studies (Céspedes-Arias et al., 2021; Pérez-Emán,
567 2005). We found evidence of isolation-by-distance driving genetic structure across the
568 latitudinally extensive range of these warblers, and genetic divergence increased where ranges
569 are separated by known geographic barriers. These clear genetic breaks coincided with observed
570 differences in head plumage coloration among populations. Notably, one transition between
571 groups includes a geographically restricted hybrid zone between *bairdi* and *chrysops*, likely
572 comprised primarily of advanced generation hybrids, where differences in genetic ancestry and
573 head coloration are correlated.

574 Below we discuss how our findings relate to the history of divergence and gene flow
575 among these populations, with a focus on how genetic variation imperfectly covaries with
576 plumage traits, and how it is structured across geography. We then discuss how genetic variation
577 is structured across the hybrid zone, how it relates to patterns of variation in head coloration in

578 this area, and whether there is evidence of selection against hybrids maintaining this seemingly
579 extensive hybrid zone.
580
581 **Geographic variation in plumage color and genetic structure**
582 Our qualitative assessments based on historical plumage descriptions and examinations of study
583 skins suggest that for these *Myioborus*, patterns of geographically clustered phenotypic variation
584 coincide with phylogeographic structure uncovered in the present study. First, we found that
585 individuals belonging to the same subspecies usually form genetic clusters, and that all major
586 genetic breaks within the species complex coincide with a transition between clearly
587 differentiated plumage groups, although we note that we lack data quantifying the degree of
588 plumage differentiation between subspecies (e.g. Winger and Bates 2015). In addition,
589 subspecies with subtle plumage differentiation (Cuervo and Céspedes Arias 2023), specifically
590 those occurring south of the Marañón River Valley (Figure 1), all characterized by black crown
591 and spectacles, show little genetic differentiation from one another.
592 Previous examinations of genetic variation in mitochondrial loci (Céspedes-Arias et al.,
593 2021; Pérez-Emán, 2005) found extensive haplotype sharing and little genetic differentiation
594 among the subspecies *ornatus* and the hybridizing *bairdi* and *chrysops*, which are all
595 characterized by striking plumage differences (see Figure 1). In contrast, in our analyses of
596 ddRAD data, which encompass thousands of SNPs across the genome, these subspecies are each
597 identifiable genetic clusters, albeit with shallow genetic differentiation. Taken together, we
598 interpret these results as evidence of rapid and recent diversification of head plumage patches in
599 the *ornatus-melanocephalus* complex, as seen in other Neotropical taxa (Campagna et al., 2012,
600 2017). Our results also highlight the usefulness of reduced representation genomic data to study

601 the evolutionary history of young radiations (Aguillon et al., 2018; Toews, Campagna, et al.,
602 2016), in which few loci might be insufficient to detect genetic differentiation and describe
603 evolutionary relationships among phenotypically distinct populations (Friis et al., 2016;
604 Stryjewski & Sorenson, 2017). We note that lack of differentiation in mtDNA between
605 phenotypically distinct groups could also be the result of mitochondrial introgression (Irwin et
606 al., 2009; Toews & Brelsford, 2012), although the strong positive correlation of F_{ST} for ND2 and
607 our ddRAD loci does not support a scenario of extensive mitochondrial introgression in the
608 group.

609 Genetic structure in the region of the Andes spanning from central Ecuador to northern
610 Peru (north of the Marañón River Valley) is complex and not clearly associated with a single
611 topographic feature. This area also broadly corresponds to the transition between *griseonuchus*
612 and *bairdi* plumage (see Figure 1, Supplementary Figure 1), two subspecies characterized by a
613 rufous crown and spectacles. Despite their overall similarity, these two groups show
614 differentiation in at least two head plumage traits: the presence of a black band behind the rufous
615 crown and the extent of black in the cheeks and lores (Zimmer 1949; Curson et al. 1994; World
616 and International 2019; Cuervo and Céspedes Arias 2023). Our qualitative assessments of
617 plumage variation (Supplementary Figure 1), however, suggest that the variation in these traits
618 might not be completely discrete. Moreover, we found that individuals from Loja and the
619 Cordillera del Cutucú (Ecuador), show substantial variation in both putatively diagnostic
620 plumage traits. Therefore, these individuals, which are genetically clustered with *griseonuchus,*
621 do not uniformly resemble the individuals from Cajamarca, Peru (Supplementary Figure 1).
622 Because of limited sampling in the area, we are unable to draw stronger conclusions, but our data
623 suggest that there is some discordance between plumage and genetic variation, and evidence of

624 introgression based on admixture graphs. Despite some differentiation in at least these two
625 plumage traits, our genetic results are concordant with substantial historical or contemporary
626 gene flow among populations in this area. This suggests that the geographic range of *bairdi* is
627 potentially outlined by two hybrid zones, one to the north with *chrysops* (Céspedes-Arias et al.,
628 2021), and to the south with *griseonuchus.* Evaluating this hypothesis of hybrid zones at the
629 range edges, as observed in barn swallows (Scordato et al., 2017), will require a systematic
630 collection of specimens across the *bairdi-griseonuchus* boundary.
631
632 **Genetic structure across space: the influence of geographic distance and topographic**
633 **features along the Andes**
634 Several of our analyses support a strong correlation between genetic variation and geographic
635 location of samples and support an important role of isolation-by-distance driving genetic
636 structure in *Myioborus* along the Andes. Patterns of IBD are close to ubiquitous in nature
637 (Aguillon et al., 2017; Seeholzer & Brumfield, 2018; Vos & Velicer, 2008), but see Meirmans,
638 2012), and are often taken as the null model for genetic differentiation if organisms have limited
639 dispersal given the scale of their range, as is the case for these small warblers with a
640 latitudinally-expansive distribution range. However, IBD does not account fully for the observed
641 patterns of genetic variation. Specifically, we found evidence of discrete genetic—and
642 phenotypic—clusters, and of reduced migration (relative to IBD) across some areas along the
643 Andes, which in turn correspond to lowland valleys such as the Marañón and the Magdalena
644 River Valleys. These deep, lowland gaps interrupt the continuity of humid high-elevation forest
645 and scrub (Graham et al., 2010) that these *Myioborus* inhabit in the present, and also coincide
646 with genetic and phenotypic breaks within other taxa that inhabit similar elevation bands

(Gutiérrez-Pinto et al., 2017; Martínez-Gómez et al., 2023; Winger & Bates, 2015). These two topographic features were identified in a comparative study as being associated with allopatric events across multiple Andean bird genera (Hazzi et al., 2018). These genetic breaks, clearly tied to topographic features, also correspond with striking discontinuities in head coloration traits (see Figure 1).

Other areas associated with clear genetic breaks observed in the inferred migration surface were not clearly associated with topographic features that interrupt the present-day continuity of humid, high-elevation forests. Specifically, low migration is inferred in a large area ranging from the border between Ecuador and Colombia down to southern Ecuador. We suggest that this pattern emerges results partially because this broad area includes the *chrysops-bairdi* hybrid zone, in which genetic differentiation relative to distance is expected to be higher than what is expected under pure IBD, as two somewhat differentiated subspecies come into contact. The extension of this area of low inferred migration to south Ecuador is consistent with our fineRADStructure, PCA and admixture results that suggest that there is substantial genetic differentiation between individuals in close proximity in this area. Specifically, we found that birds from isolated mountain spurs such as the Cordillera del Cutucú (southeastern Ecuador) and Cordillera del Condor (Peru-Ecuador border) clustered genetically with birds from the main Andes in Cajamarca (northwestern Peru), and Loja (southern Ecuador), instead of the more geographically proximate individuals from the eastern Ecuadorian Andes in Morona-Santiago (see Figure 2). The Cordillera del Cutucú and Cordillera del Cóndor are outlying mountain ranges in the Amazonian foothills, separated from the main Andes by the valleys of the rivers Upano and Zamora, respectively (Pozo-Zamora et al., 2022; Robbins et al., 1987). Our results suggest that these valleys, and surrounding lowland areas, have likely acted as dispersal barriers

670 for these higher-elevation warblers. On the other hand, the patterns of genetic variation described
671 here indicate that there has likely been substantial gene flow connecting the populations of
672 warblers inhabiting isolated peaks in these mountain ranges (separated by the Santiago River
673 Valley; Robbins et al. 1987; Pozo-Zamora et al. 2022), and the eastern slope of the Andes in
674 northern Peru.
675
676 **Hybrid zone dynamics**
677 Based on our reduced-representation genomic data set, the hybridizing *chrysops* and *bairdi* are
678 characterized by very shallow genetic divergence, which contrasts with their striking plumage
679 differentiation. Moreover, across thousands of SNP sites, we only identified 29 ancestry
680 informative sites with substantial allele frequency differences between parental populations. This
681 scarcity of ancestry informative markers limits our ability to draw definitive conclusions about
682 the genetic composition of this hybrid zone but highlights the low level of genetic differentiation
683 between *chrysops* and *bairdi*.
684 This hybrid zone likely formed by secondary contact of two formerly isolated plumage
685 forms. The disconnection and subsequent reconnection of habitat that could have shaped this
686 process may have followed elevational shifts in the cloud forest belt during the Pleistocene
687 (Flantua et al., 2014; Ramírez-Barahona & Eguiarte, 2013), or potentially been driven by
688 volcanic activity that modified topographic connectivity (Sanín et al., 2024). An investigation of
689 the evolutionary origins of this hybrid zones will require both a better understanding of the
690 patterns of connectivity of cloud forest in the area where the *chrysops-bairdi* hybrid zone is
691 centered, as well as an estimation of the time since secondary contact, which could be obtained
692 based on inferences of ancestry tract length in whole genome data (Pool & Nielsen, 2009).

Our geographic cline analyses using plumage and genomic data suggest that the hybrid zone extends for over 200 km, similar to the findings from its original description based on face color traits (Céspedes-Arias et al., 2021). We do not find statistically significant differences in the width of the admixture proportion (i.e., genomic) and plumage geographic clines. Taken together, plumage and genomic patterns of variation thus suggest that hybridization between *chrysops* and *bairdi* has been extensive, albeit still restricted to a fraction of the entire distribution of these taxa (approximately 30% of our sampling transect) which is continuous at least from central Ecuador to the north of the Central Andes of Colombia. Therefore, the geographic extent of hybridization is unlikely to be constrained by physical or ecological barriers that limit dispersal beyond the hybrid zone (e.g., DeRaad et al., 2023), raising the question of what factors are restricting the hybrid zone to this area.

The lack of information on natal dispersal distances as well as a the wide range of plausible times since secondary contact constrains our ability to generate a direct comparison of the observed cline widths with that expected under neutral diffusion (Barton and Gale 1993). With the available data we are thereby unable to evaluate whether the *chrysops* x *bairdi* hybrid zone corresponds to a stable tension zone, in which the width of clines is constrained by selection against hybrids. A second possibility is that the observed sharp genomic and plumage clines are not maintained by selection, but instead that this is a young and potentially expanding hybrid zone, and that the differentiation between *chrysops* and *bairdi* might become eroded over time (Kearns et al., 2018).

Using a range of plausible dispersal distances inferred from another *Myioborus* species (Céspedes-Arias et al., 2021; Mumme, 2015), we can approximate expectations under neutrality (Barton & Gale, 1993). For a cline width of 281 km, the neutral diffusion model predicts that

716 ~14,337 generations would be required assuming a dispersal distance of 935 m, or ~53,278
717 generations with a dispersal distance of 485 m. In principle, building upon our systematic
718 collection of specimens across the *chrysops* x *bairdi* hybrid zone, the alternative of an expanding
719 hybrid zone could be revisited by comparing plumage and genomic clines based on specimens
720 collected across different time points. However, given that the expansion of this hybrid zone
721 under neutral diffusion with plausible dispersal distances is expected to be slow (i.e. 0.5 km over
722 50 generations), time between sampling periods would need to be very long to detect changes.
723 We found that cline centers are not fully coincident, as that of the plumage cline is
724 displaced to the south of the genomic cline center, implying that the *chrysops*-like plumage is
725 introgressing into *bairdi.* Assuming that most of our ddRAD loci can be considered neutral
726 markers, our results indicate that head coloration might be under selection across this hybrid
727 zone (Gay et al., 2008). Specifically, the observed pattern would be consistent with a scenario in
728 which the *chrysops*-like plumage is favored across the hybrid zone, which would lead to the
729 asymmetric introgression of this phenotype towards the south. Asymmetric introgression of
730 plumage traits has been observed in other avian hybrid zones (Baldassarre et al., 2014; Lim et al.,
731 2024; Semenov et al., 2021), and in two of these cases, behavioral evidence supported the idea
732 that these traits provide fitness advantages in the context of mate choice (Baldassarre & Webster,
733 2013; Long et al., 2024; Stein & Uy, 2006).
734 Even though the difference in plumage and genomic cline center is statistically
735 significant, we found it to be relatively small (38 km; approximately 4% of the entire sampling
736 transect). This contrasts with much more marked differences in center estimations found in other
737 hybridizing birds like *Motacilla* wagtails (Semenov et al., 2021) and *Malurus* fairy-wrens
738 (Baldassarre et al., 2014). We acknowledge that this minor difference could be an artifact of the

739 way in which both of our hybrid indexes were calculated (e.g. with the scoring system giving
740 equal weight to pre-defined patches; Céspedes-Arias et al., 2021), and potentially low sampling
741 sizes in some of the localities. However, this slight difference could be the result of selection on
742 the head plumage patches. Even though the role of these color patches has not been studied in
743 these warblers yet, it is plausible that they may be relevant for intraspecific communication and
744 mate choice as seen in head plumage patterns in many other bird species (e.g., Marchetti 1992;
745 Andersson et al. 1998; Scott and Deag 1998; Rémy et al. 2010). Future behavioral studies could
746 evaluate these hypotheses of selection favoring *chrysops*-like head color patterns across this
747 hybrid zone.

748 Besides describing the geographic extent of hybridization, we used our reduced-
749 representation genomic data set to gain insights into the genetic composition of the *chyrsops x*
750 *bairdi* hybrid zone. However, as noted above, the scarcity of ancestry informative sites in our
751 dataset complicates the interpretation of these results, as it compromises the accuracy of genomic
752 hybrid indexes and interclass heterozygosity estimations (Gompert et al., 2024; Wiens & Colella,
753 2025). Setting a low allele frequency threshold, which was necessary in our case, can also result
754 in a tendency of individuals to cluster near the center of triangle plots (Wiens & Colella, 2025).
755 Bearing these caveats in mind, we interpret our triangle plot results as suggestive of a hybrid
756 zone composed mostly of advanced generation hybrids, with no F1s sampled, given the lack of
757 individuals with intermediate hybrid indexes and very high interclass heterozygosity (Capblancq
758 et al., 2020; DeRaad et al., 2023; Natola et al., 2021). This is consistent with the existence of a
759 high diversity of plumage phenotypes in the hybrid zone, as well as the lack of contemporary
760 geographic contact between parental *chrysops* and *bairdi* populations (i.e., we do not observe
761 “pure" individuals of both species in the same sites) (Céspedes-Arias et al., 2021). Given the

762 shallow genetic divergence between *chrysops* and *bairdi*, a more detailed examination of the
763 genetic composition of this hybrid zone will necessitate whole-genome sequencing, capturing
764 more ancestry informative sites. Specifically, as in other examples of hybridizing taxa with very
765 little genomic divergence, whole-genome sequencing may be needed to identify these regions
766 and sites that are highly differentiated and study hybridization dynamics in detail (Aguillon et al.,
767 2021; Poelstra et al., 2014; Toews, Taylor, et al., 2016).
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785 **Figures**

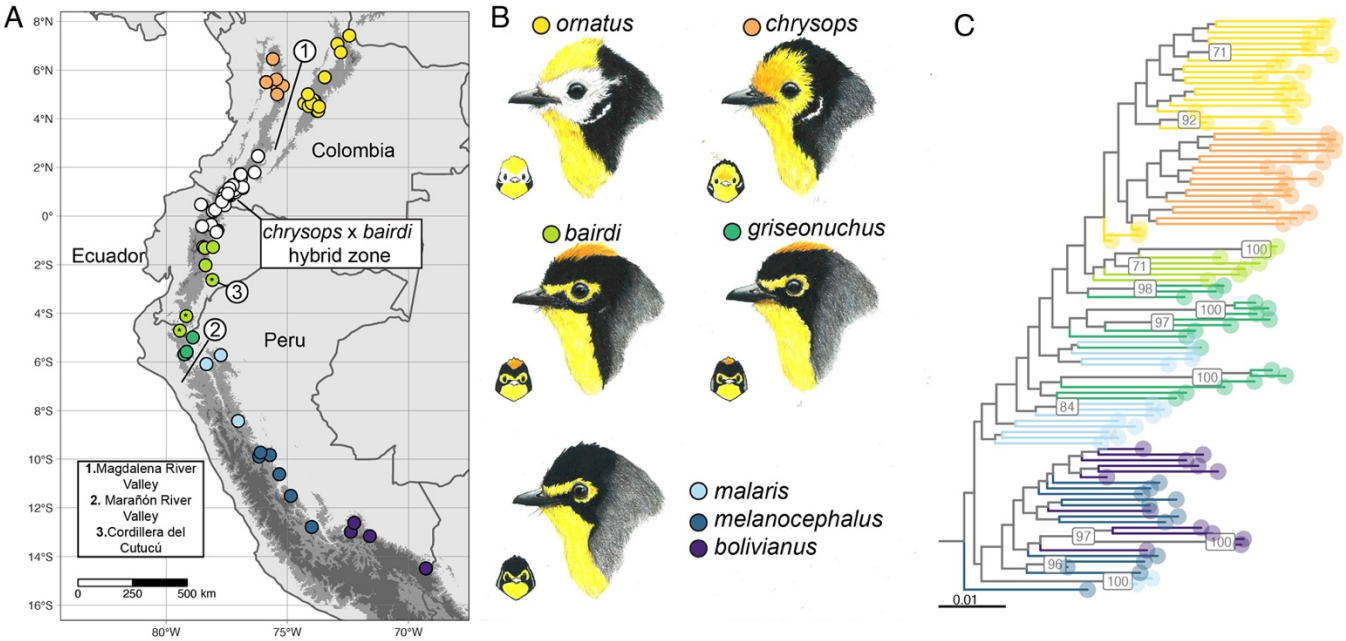

Figure 1: Sampling localities of specimens included in the study **(A)**, which encompass all major plumage groups **(B)**. The color of the dots corresponds to study taxa. The white dots correspond to areas around the *bairdi-chrysops* hybrid zone as identified based on plumage patterns (Céspedes-Arias et al., 2021). The subspecies *malaris, melanocephalus*, and *bolivianus* are here represented by one illustration. In the map, elevation is denoted by the gray scale in the background, with the elevation in which these *Myioborus* occur shown in an intermediate shade of gray. The localities marked with an asterisk correspond to those in Loja and outlining ridges in Morona-Santiago that are discussed in the main text, and numbers mark important topographic features. **(C)** Maximum-likelihood phylogeny estimated with our dataset of concatenated ddRAD loci, showing how overall relationships somewhat reflect geography (outgroups are cropped out of the figure here). The branches are colored by subspecies as in the map, with only bootstrap values over 70 shown (hybrid zone samples not included in the analysis). Illustrations: Andrés Montes Rojas.

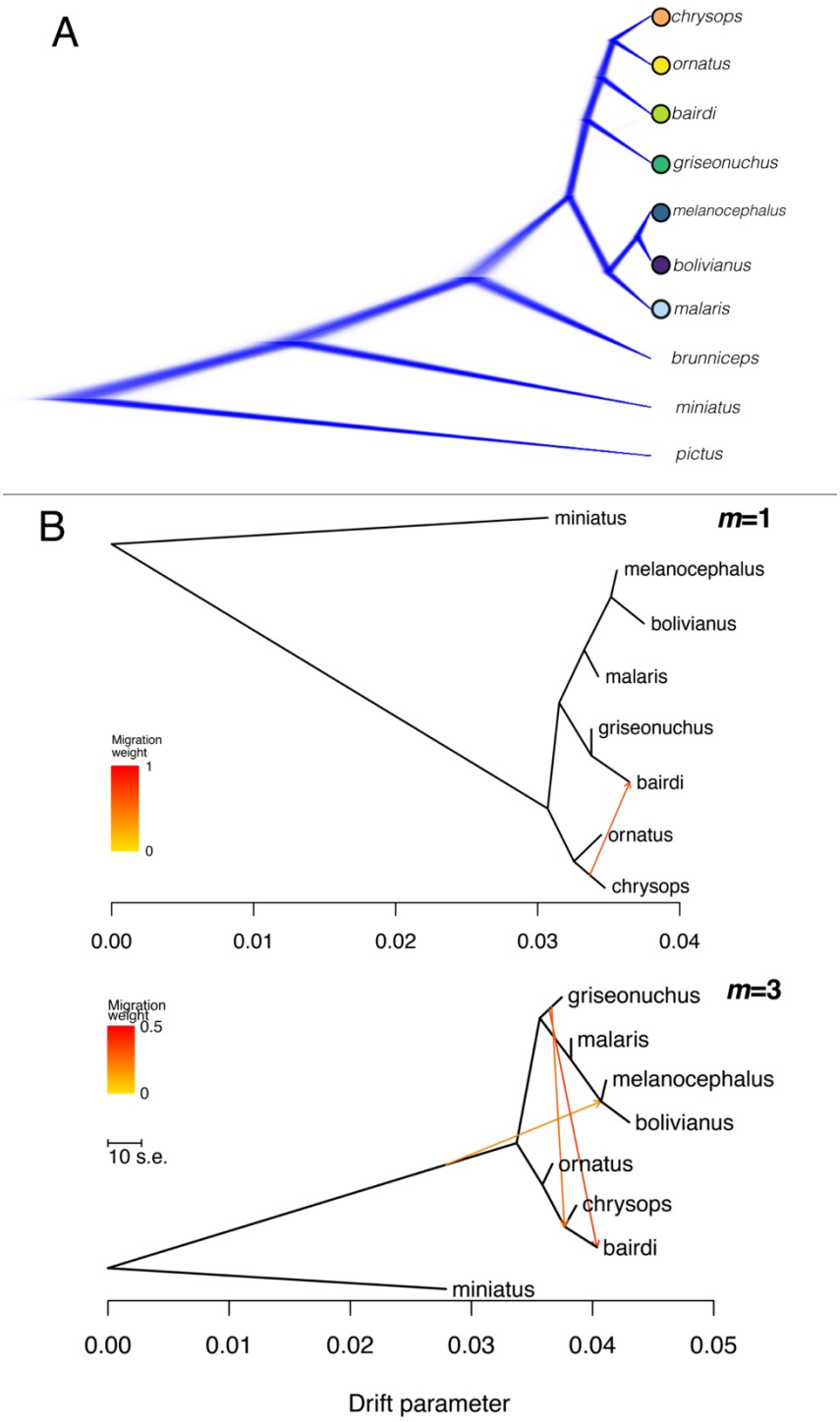

Figure 2. Evolutionary relationships within groups in the *Myioborus ornatus-melanocephalus* complex as inferred by snapper and *OrientAGraph*. **(A)** The main figure shows the phylogenetic relationships between taxa, with the blue topology corresponding to the most frequent in the posterior distribution, followed by red. **(B)** Admixture graphs from *OrientAGraph* with 1(top) and 3 (bottom) migration edges (*m*). The migration edges are shown as colored arrows, and the hue denotes the migration weight.

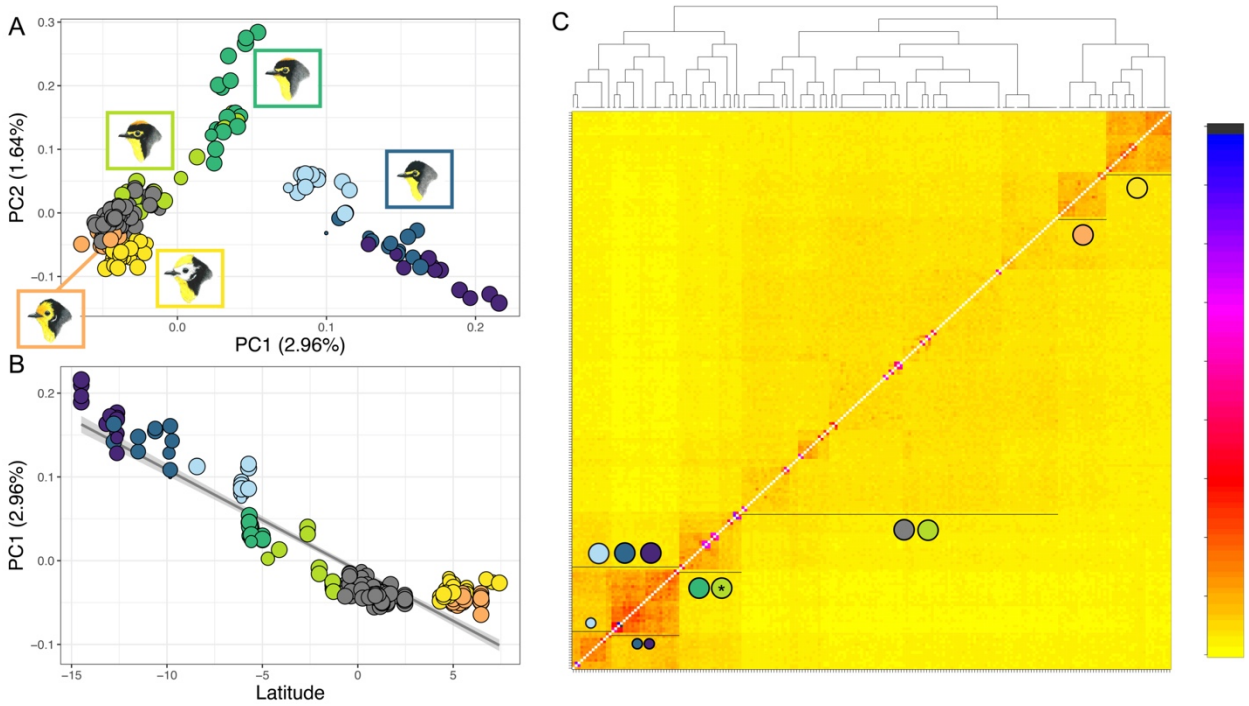

Figure 3. Genetic structure within the *ornatus-melanocephalus* complex. (**A)** The distribution of individuals in the PC1/PC2 space showing clustering based on plumage groups, which are denoted by the color of the dots. **(B)** Strong correlation between PC1 and latitude. In both cases (A and B) the size of the dot is inversely proportional to the proportion of missing data. **(C)** fineRADStructure coancestry matrix, and associated distance tree (above) showing clusters of individuals that overall correspond to plumage groups, which are denoted by the colored dots that correspond to groups as in Figure 1. The asterisk highlights a small number of putatively *bairdi* individuals, all from the south end of Ecuador, that genetically cluster with *griseonuchus* (from northern Peru). The color scale denotes values of coancestry, with blue and black tones corresponding to very high levels of coancestry.

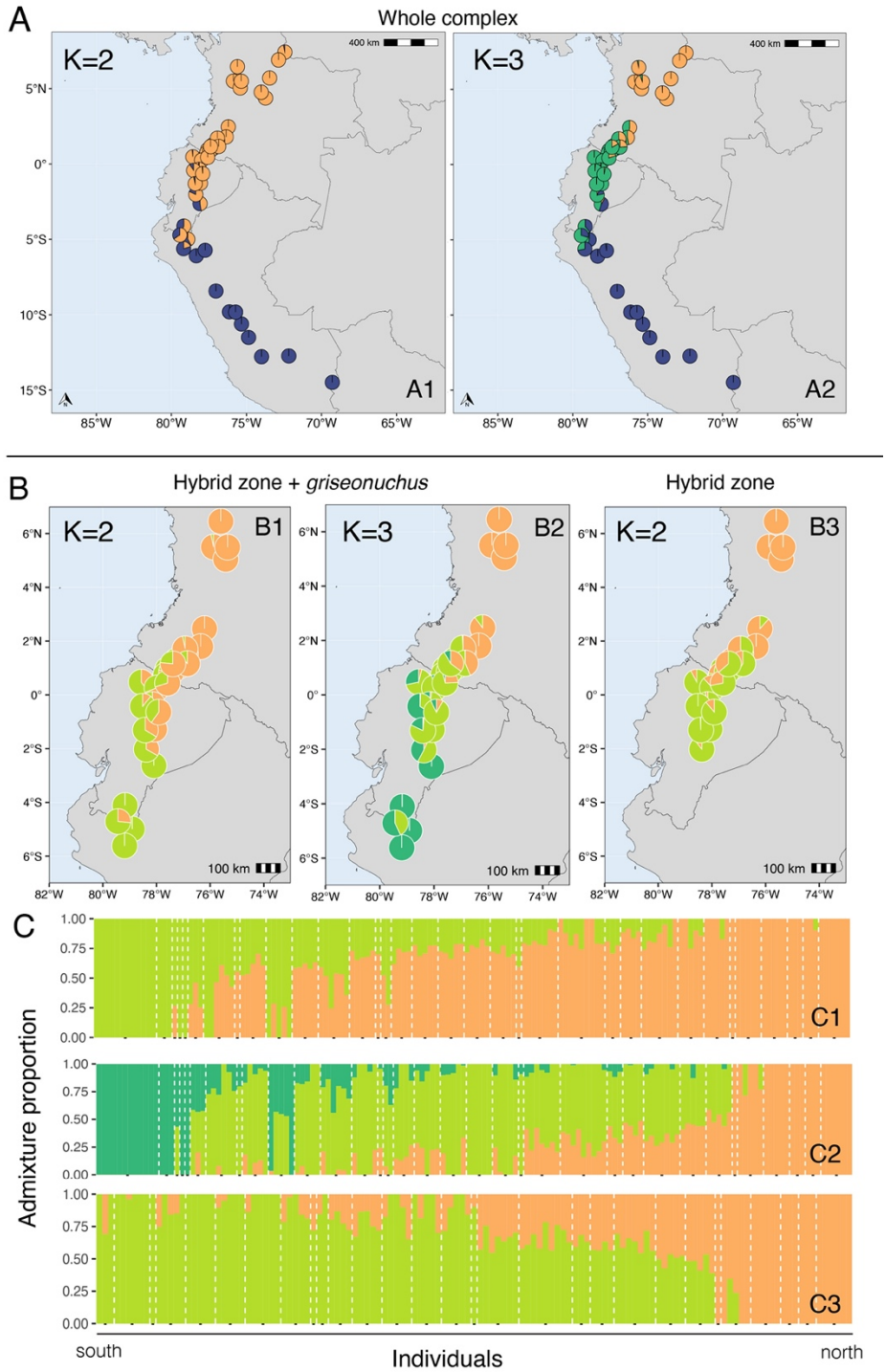

Figure 4. Genetic clusters (**A**) across the entire distribution of the *ornatus*-*melanocephalus* complex and (**B**) the hybrid zone and adjacent populations as shown by the mean admixture proportions per locality. The dataset and *K* value for each plot is specified at the top of each map. **(C) Admixture prop**barplots showing the admixture proportions per individual, which are organized into localities from south to north (localities are divided by white dotted lines).

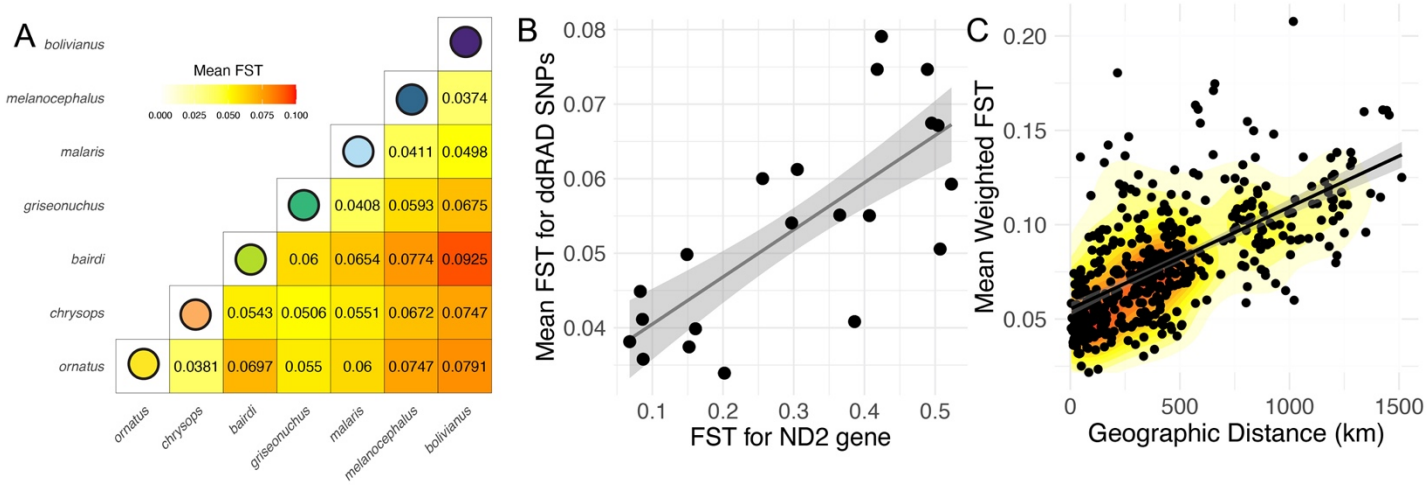

Figure 5. Genetic differentiation among Andean *Myioborus* taxa. **(A)** Mean pairwise F_{ST} values between subspecies (i.e., plumage and geographic groups). **(B)** Correlation between pairwise F_{ST} calculated with ddRAD SNPs (this study) and the ND2 mitochondrial gene from the same set of samples (Cespedes-Arias *et al* 2021). **(C)** Positive correlation between geographic distance and F_{ST} between localities supports a role of isolation-by-distance shaping genetic variation in the *ornatus-melanocephalus* complex.

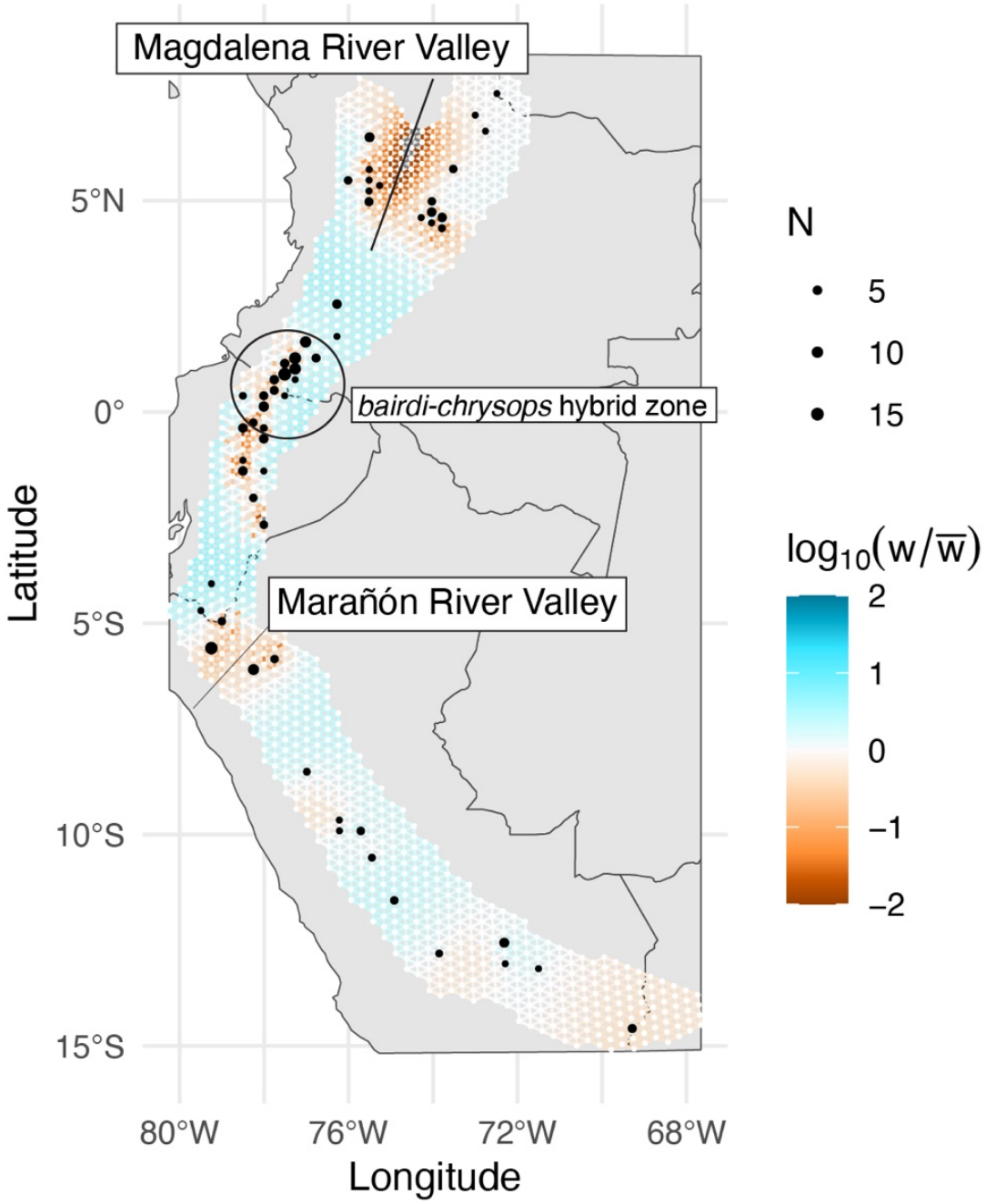

Figure 6. Estimated effective migration graph across the entire distribution of the *Myioborus ornatus-melanocephalus* complex, highlighting areas of restricted gene flow. The edges of the grid are colored by estimated effective migration, from burnt orange (low migration) to blue (high migration). In the grid, the black dots correspond to the sampled nodes and the size of the dot correspond to the sample size (*N*). We highlight three areas of low migration, two geographic barriers and the hybrid zone where genetically distinct taxa have come into contact.

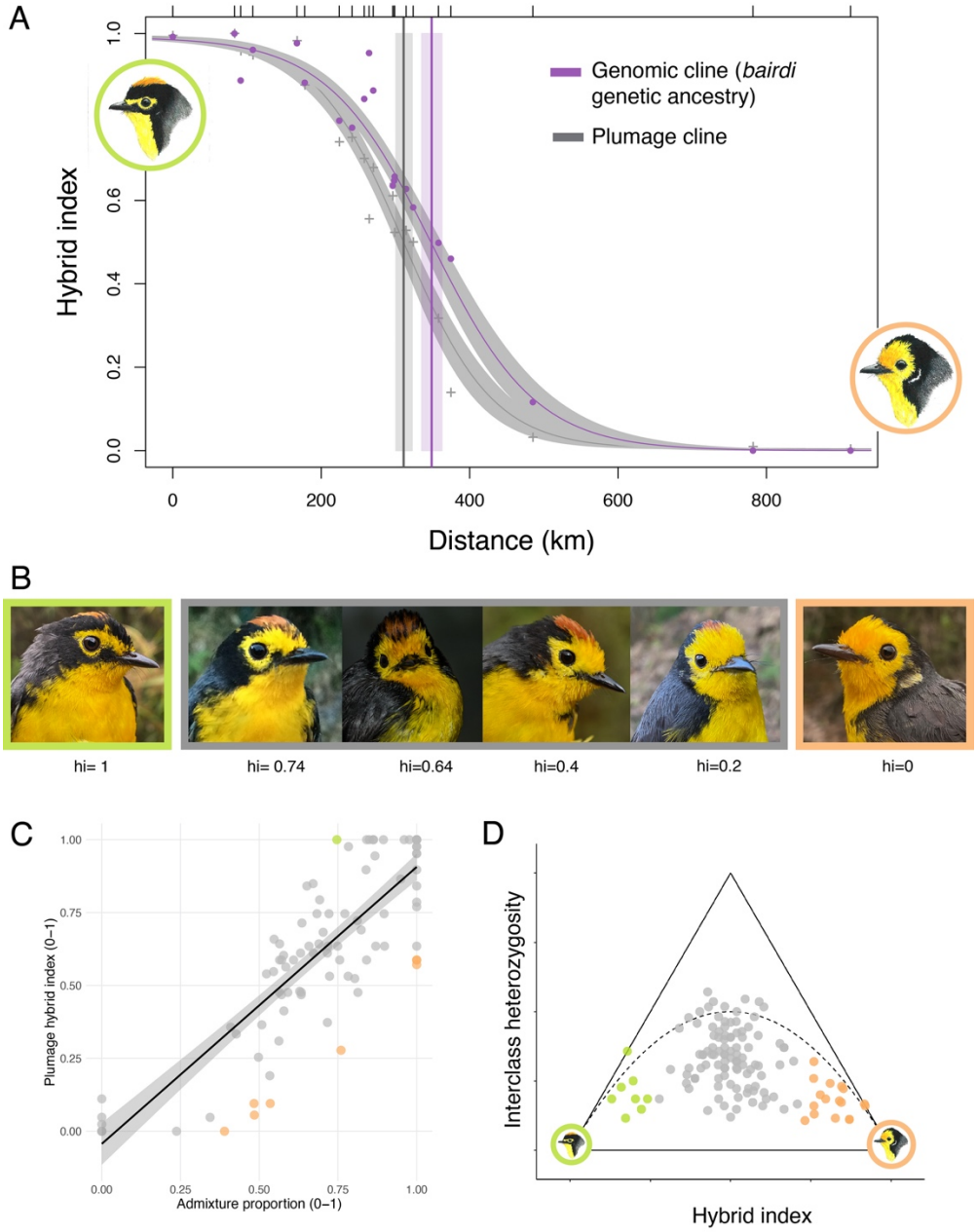

Figure 7. Genetic and plumage variation across the *bairdi x chrysops* hybrid zone. **(A)** The plumage and genomic clines have different estimated centers. **(B)** Photographs of specimens collected for which we calculated a plumage hybrid index, showing a sample of the phenotypic diversity across the hybrid zone, and "parental" phenotypes at both extremes. Photos: Andrés Cuervo, Paulo Pulgarín, David Ocampo, and Laura Céspedes. **(C)** We observed an overall a strong correlation between the genomic hybrid index (*bairdi* admixture proportion) and the plumage hybrid index. Most individuals that deviate from this relationship (outliers marked in color) are individuals with a more chrysops-like plumage than expected given the admixture proportion. **(F)** Triangle plot showing the genetic composition of the hybrid zone. The x axis corresponds to a genomic hybrid index as calculated using 29 ancestry informative markers. Parentals are shown in lime green and orange.

Author contributions: L.N.C.A, A.M.C., C.D.C. and L.C. conceived the study; L.N.C.A. and A.M.C. conducted fieldwork with substantial logistical support from C.D.C. and E.B.; L.N.C.A. conducted lab work with support from L.C. and I.L.; L.N.C.A. conducted the bioinformatic processing of data and data analyses with substantial input from L.C. L.N.C.A. wrote the original draft, with major contributions from L.C. All authors contributed to reviewing and editing the manuscript.

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