

Diversification and divergence in *Myioborus* warblers: insights into evolutionary relationships and plumage genetics

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Abstract

Genomic data can provide valuable insights into the evolutionary history of rapidly diversifying groups and the genetic basis of phenotypic traits. We performed whole-genome resequencing of the warbler genus *Myioborus* to investigate the dynamics of its recent diversification in Neotropical mountains. Mitochondrial and nuclear DNA produced mostly concordant tree topologies and revealed a pattern of north-to-south and low-to-high elevation colonization. Within a young tropical Andes clade (*M. albifrons*, *M. ornatus*, and *M. melanocephalus*), we found topological incongruence between our phylogenies. In general, relationships coincided with plumage types; however, three taxa with striking plumage differences exhibited low mitochondrial divergence. The hybridizing taxa *M. ornatus chrysops* and *M. melanocephalus bairdi* showed very shallow genomic differentiation, with most divergence peaks across the genome shared with other parulid warbler pairs, pointing to shared genomic architecture shaping these regions. However, a unique differentiated region included the gene *CCDC91*, which is associated with melanin-based plumage differences in other birds. We also identified a large region of elevated differentiation on the Z chromosome showing strong signals of reduced recombination. Together, these results highlight the interplay of deep ancestral divergence, recent hybridization, and shared genomic architecture in shaping the evolution of phenotypic and genomic diversity in *Myioborus*.

RESUMEN

Los datos genómicos pueden proporcionar información valiosa sobre la historia evolutiva de grupos que se diversifican rápidamente, así como sobre la base genética de las diferencias fenotípicas entre linajes. Generamos datos de resecuenciación de genomas completos de reinitas del género *Myioborus*, el cual se diversificó recientemente en las montañas del Neotrópico. Encontramos que las filogenias mitocondriales y de UCEs son altamente concordantes y revelan un patrón de colonización de norte a sur y de bajas a altas elevaciones. Dentro de un clado joven de los Andes tropicales (*M. albifrons*, *M. ornatus*, and *M. melanocephalus*), observamos que la estructura genética coincide generalmente con la variación geográfica en el plumaje, aunque tres subespecies con diferencias notables en plumaje presentan baja divergencia mitocondrial. En este complejo, dos taxones que hibridan, *M. ornatus chrysops* y *M. melanocephalus bairdi*, presentan una diferenciación genómica muy baja, con la mayoría de los picos de divergencia a lo largo del genoma compartidos con otros pares de reinitas parúlidas, lo que apunta a una arquitectura genómica compartida que da forma a estas regiones. Sin embargo, una región diferenciada única incluyó el gen *CCDC91*, que se ha asociado con diferencias de plumaje basadas en melanina en otras aves. También identificamos una gran región de diferenciación elevada en el cromosoma Z que mostró señales de recombinación reducida. En conjunto, estos resultados destacan la interacción entre una divergencia ancestral profunda, la hibridación reciente y la arquitectura genómica compartida en la configuración de la diversidad fenotípica y genómica en *Myioborus*.

Keywords: phylogeny, ultra-conserved elements, mitogenome, hybrid zone, Neotropical mountains, *CCDC91*

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Introduction

Genetic data can help infer the evolutionary history of populations and species, and consequently, shed light on the processes that generate biodiversity. Since the development of multi-locus phylogenetics, it has become apparent that different genomic regions often have different genealogies (Degnan & Rosenberg, 2009). This can lead to challenges when trying to establish the most plausible historical relationship among groups (Zhao et al., 2023), but also highlights the complexities of biological diversification. Multiple processes can lead to genealogical differences among loci, such as incomplete lineage sorting, selection, and introgressive hybridization, which are now appreciated as widespread and frequent phenomena across the tree of life (Rheindt & Edwards, 2011; Taylor & Larson, 2019). The unprecedented access we now have to whole-genome data for non-model organisms has allowed us to study evolutionary radiations across the tree of life in a way that considers the complex interplay between divergence and admixture (Edelman et al., 2019; Strykowski & Sorenson, 2017; Svardal et al., 2020).

In addition to reconstructing evolutionary relationships, genomic data also allow novel insights into genetic basis of phenotypic differentiation in evolutionary radiations. For example, when characterizing genome-wide “landscapes of divergence” between phenotypically distinct taxa (Campagna et al., 2017; Lamichhaney et al., 2015; Marques et al., 2022), we often find heterogeneous (i.e., with distinct peaks and valleys) patterns of genomic differentiation. Although the causes of the heterogeneity of landscapes of divergence are multiple and complex (e.g., variation in recombination rates, variation in mutation rates; Ravinet et al., 2017), identifying divergence peaks is a useful first approach to identify candidate genomic regions associated to phenotypes of interest. This approach has proven useful for studying several avian species complexes and has allowed significant progress in identifying candidate genes underlying melanin and carotenoid-based plumage traits (Aguillon et al., 2021; Baiz et al., 2021; Campagna et al., 2017; Funk et al., 2023; Lim et al., 2024; Price-Waldman & Stoddard, 2021; Toews et al., 2016).

The parulid warblers of North, Central, and South America comprise a speciose group of colorful passerines that radiated over the last 7 million years (Oliveros et al., 2019; Rabosky & Lovette, 2008). One of the most conspicuous phenotypic differences across the parulid family is plumage color, whereas morphological variation is relatively limited (Leroy et al., 2024; Shutler & Weatherhead, 1990; but see Rosamond et al., 2020). This pattern contrasts with classic adaptive radiations, which are typically characterized by marked differences in size and shape, such as those observed in *Anolis* lizards, Darwin’s finches, African cichlids, Hawaiian honeycreepers, and *Espeletia* plants (Foster et al., 2008; Lerner et al., 2011; Losos, 1990; Pouchon et al., 2018; Seehausen, 2006; Tokita et al., 2017). Because evolutionary radiations produce many species quickly, the resulting lineages are usually genetically compatible, thus allowing for hybridization and gene flow (Abbott et al., 2013; Seehausen, 2004). This phenomenon is true for parulid warblers (Baiz et al., 2021), where several hybrid zones have been described, and numerous interspecies and intergeneric hybrids have been documented (Céspedes Arias et al., 2021; Céspedes

Arias et al., 2025; Irwin et al., 2009; Rohwer & Wood, 1998; Short & Robbins, 1967; Toews et al., 2011, 2020; Vallender et al., 2007, 2016).

Given their recent diversification and extensive hybridization among lineages, warblers likely exhibit variation in gene genealogies across loci, arising from introgression and incomplete lineage sorting (Singhal et al., 2021). Thus, it is necessary to leverage whole-genome data and use phylogenomic approaches to appropriately describe this group’s evolutionary history. In this study, we focused on a lineage of rapidly diversifying warblers, the genus *Myioborus*, composed of 12 colorful, mostly montane, species occurring from the southern United States to Argentina. Based on estimates from a recent phylogeny of Parulidae, *Myioborus* has a crown age of 1.9–2.9 Mya (Bennett et al., 2025). This genus is one of several lineages of resident warblers that have diversified in South and Central America, which as a whole make up more than half of the species diversity in Parulidae (Curson, 2010; Lovette et al., 2010; Winger et al., 2012). Despite their remarkable diversity, comparatively little genomic data has been generated for these tropical resident warblers, in contrast to migratory species that breed in North America (e.g., Baiz et al., 2021; De Zwaan et al., 2021; Toews et al., 2016; Tsai et al., 2024; Zamudio-Beltrán et al., 2024; but see Bennett et al., 2025 and Céspedes Arias et al., 2025).

Our study focuses on *Myioborus* warblers, both to infer the evolutionary relationships among species and geographically circumscribed plumage groups and to describe the landscape of divergence in a young species pair characterized by conspicuous differentiation in color patterns. Most *Myioborus* species have allopatric distributions across mountain ranges or parapatric ranges along elevational gradients (Curson et al., 1994). Ten species occur exclusively in upper montane forest (hereafter, high-elevation or upper montane *Myioborus*), including representatives in Central American cordilleras, the Sierra Nevada de Santa Marta, the Tepuis, and the tropical Andes. This upper montane group is characterized by plumage coloration diversity that is concentrated in the face and crown, with species showing combinations of rufous, black, and yellow crown colors, as well as presence or absence of black, yellow, or white cheeks, lores and eye rings, forming “spectacles” (Figure 1). A previous examination of evolutionary relationships among *Myioborus*, which identified these upper montane taxa as forming a monophyletic group, was based on a few mitochondrial loci and lacked resolution for inferring relationships within this clade, potentially as a result of rapid diversification (Pérez-Emán, 2005). Phylogenetic studies of the entire warbler family have provided insights into the diversification of *Myioborus* taxa, albeit with some limitations due to limited geographic sampling within phenotypically variable species in this upper montane clade (Bennett et al., 2025; Lovette et al., 2010; Zhao et al., 2025). Comprehensive taxonomic and geographic sampling of genomic data within *Myioborus*, especially within this upper montane clade, is necessary to better understand how these species have diversified across Neotropical mountains.

Within this high-elevation group, the species complex formed by *M. ornatus* and *M. melanocephalus* is distributed from northern Bolivia to western Venezuela and displays

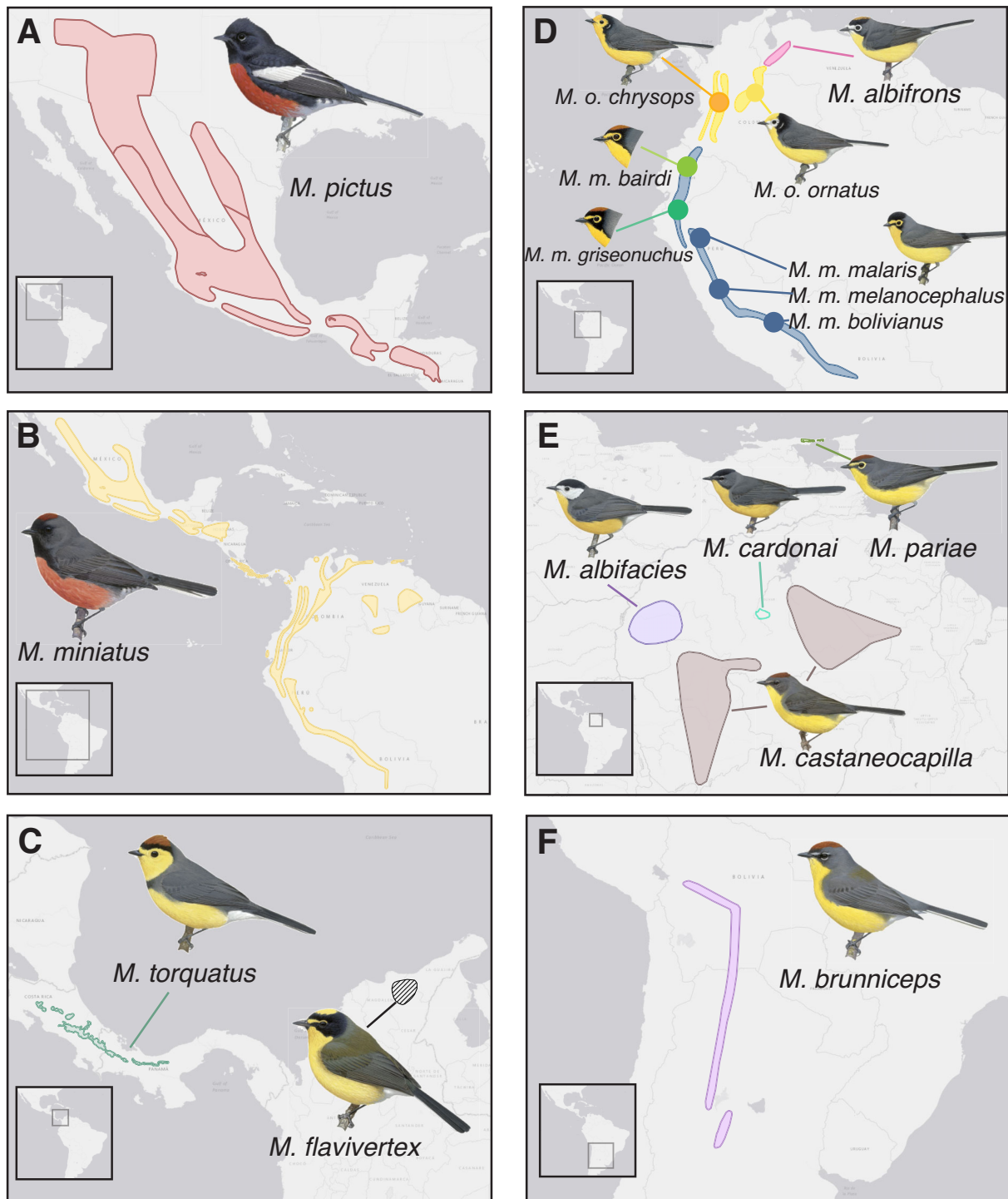


Figure 1. Distribution and plumage diversity in the genus *Myioborus*. Panels are focused on different geographic regions of Central and South America, with some including multiple species. The approximate distribution of species that we sampled is shown as colored polygons; the distribution of the only species in the genus not included in the study is shown in a hatched pattern in panel C (shapefiles from NatureServe). Panel D corresponds to the species *M. albifrons*, and the geographically variable *M. ornatus* and *M. melanocephalus* (sampling sites shown in Figure 3). The color of the polygons represents the nominate subspecies of *M. ornatus* and *M. melanocephalus* and the colored circles represent the approximate areas where each subspecies occurs. Warbler illustrations used with permission, ©Lynx Nature Books and ©Cornell Lab of Ornithology.

striking geographic patterns of plumage variation (Lovette et al., 2010; Pérez-Emán, 2005). These two species comprise between five and seven well-defined plumage groups (Cuervo & Céspedes Arias, 2023). Data from a single mitochondrial locus (Céspedes Arias et al., 2021) suggest that the phenotypically diverse *ornatus*–*melanocephalus* group

is characterized by low genetic differentiation, implicating either rapid recent diversification, introgression, or both. Given the low genetic differentiation within the *ornatus*–*melanocephalus* complex, whole-genome data are needed to better understand how genetic structure correlates with the observed geographic variation in plumage color. Re-

cent work using comprehensive geographic sampling and reduced-representation genomic data suggests that patterns of plumage and genetic differentiation are largely concordant (Céspedes Arias et al., 2025). This study also indicates that both topographic barriers within the cloud forest and isolation by distance contribute to shaping genetic variation within this complex.

Two of the plumage groups within this species complex form an extensive hybrid zone in the Colombia–Ecuador border region, where intermediate plumage phenotypes are common and diverse (Céspedes Arias et al., 2021). Given the low divergence detected in previous genetic surveys in both nuclear and mitochondrial markers (Céspedes Arias et al., 2021; Céspedes Arias et al., 2025), whole-genome resequencing data may permit identification of candidate genes underlying their head plumage differences by detecting genomic regions that show elevated divergence relative to most of the genome.

Here, we obtained whole-genome resequencing data representing 11 of the 12 species in *Myioborus* (excluding *M. flavivertex*, a species endemic to the Santa Marta mountains of Colombia). We inferred the evolutionary relationships among species and plumage groups and evaluated topological incongruence between the nuclear and mitochondrial genome resulting from introgression or incomplete lineage sorting. We next addressed cases of tree incongruence, both with respect to the findings of previous studies (Bennett et al., 2025; Lovette et al., 2010; Zhao et al., 2025) and among our different tree-building methods. Additionally, we described genetic variation in both nuclear and mitochondrial genomes among the tropical Andes mountain species (*M. ornatus*, *M. melanocephalus*, and *M. albifrons*). Finally, we focused on the youngest taxa in the genus, *M. o. chrysops* and *M. m. bairdi*, which hybridize extensively, and identified genomic features of divergence between them, including a gene putatively associated with differences in plumage coloration.

Methods

Study species and sampling

We obtained tissue samples from 56 individuals (Table S1) comprising 11 out of 12 species in the genus *Myioborus*: *M. pictus* ($n = 5$), *M. miniatus* ($n = 5$), *M. torquatus* ($n = 5$), *M. pariae* ($n = 1$), *M. castaneocapilla* ($n = 5$), *M. cardonai* ($n = 2$), *M. albifacies* ($n = 1$), *M. brunniceps* ($n = 4$), *M. albifrons* ($n = 2$), *M. ornatus* ($n = 11$), and *M. melanocephalus* ($n = 14$). Of the 56 samples, 41 were loaned from museum collections (IAvH, PUCE, AMNH, FMNH, KU, LSUMZ, MSB, UWBM, and YPM; Table S1). The remaining 15 samples, from the hybridizing taxa *M. ornatus chrysops* and *M. melanocephalus bairdi*, were obtained from a previous sampling effort described in Céspedes Arias et al. (2021).

A high proportion of the samples analyzed in this study correspond to the polytypic species *M. ornatus* and *M. melanocephalus*, which allowed us to have a good representation of their taxonomic diversity and geographic variation in plumage pattern and color. The southern species *M. melanocephalus* traditionally comprises five subspecies (from south to north; Figure 1): *bolivianus*, *melanocephalus*, *malaris*, *griseonuchus*, and *bairdi* (also known as *ruficoronatus*, but see Cuervo & Céspedes Arias, 2023). These taxa

differ in their crown color (either black or rufous) and the extent of black in the lores and forehead that delineate “spectacles” characteristic of the species (for detailed descriptions see Cuervo & Céspedes Arias, 2023). The northern species, *M. ornatus*, lacks these “spectacles” and instead has a yellow forehead and either yellow cheeks (subspecies *chrysops*) or white cheeks (subspecies *ornatus*) (Figure 1). We had at least one sample from each of these subspecies, with higher sample sizes ($n = 9$ each) for *M. o. chrysops* and *M. m. bairdi*, the two taxa that hybridize extensively in the Colombia–Ecuador border region. We sampled from across the subspecies’ parental ranges and along the hybrid zone, including several individuals that were determined to be of partially admixed ancestry by Céspedes Arias et al., 2025 (see Table S1). However, all birds included in the study had parental plumage phenotypes (Céspedes Arias et al., 2021).

Sequencing and data processing

We used ethanol-preserved or frozen tissues from vouchered specimens to extract genomic DNA and prepare genomic libraries. For the 18 *M. o. chrysops* and *M. m. bairdi* samples, we conducted laboratory work in the Fuller Evolutionary Biology Lab at the Cornell Lab of Ornithology. From these samples, we extracted genomic DNA following a modified phenol–chloroform protocol (Sambrook, 1987), using phase-lock tubes. We prepared individually barcoded libraries using the TruSeq Nano DNA Library Prep Kit (Illumina, California, USA) with a 350 bp insert size. These samples were then pooled and sequenced on a single Illumina NextSeq 500 (2×150 bp) lane at the Cornell Institute of Biotechnology.

For the remaining 39 samples, laboratory work was conducted in the Toews Lab at Pennsylvania State University. We extracted genomic DNA from frozen tissues using Qiagen DNeasy Blood & Tissue Kits. We prepared libraries using Illumina DNA Prep Kits with a 350-bp target insert size. Samples were pooled, together with samples from other projects, and subsequently sequenced (2×150 bp) across five Illumina NextSeq P3 flowcells on a NextSeq 2000 sequencer at the Genomics Core Facility in the Huck Institutes of Life Sciences at Penn State.

We trimmed adapters and collapsed overlapping reads with AdapterRemoval 2.17 (Schubert et al., 2016). The number of raw reads per sample ranged from 11.2 to 47.5 million (mean = 23,773,417; SD = 6,790,475; see Table S1 for per-sample values). We then aligned the reads to the chromosome-level *Setophaga coronata* reference genome (Baiz et al., 2021) using Bowtie2 v2.5.1 (Langmead & Salzberg, 2012). The resulting SAM files were then converted to BAM and sorted using SAMtools v1.18 (Li et al., 2009), and polymerase chain reaction (PCR) duplicates were marked using Picard v3.1.0 (Broad Institute, 2019). The depth of coverage of the resulting files ranged from 2.4 to 8.8X (mean = 3.9; SD = 1.05; Table S1), and the alignment rates ranged from 0.84 to 0.89 (mean = 0.87; SD = 0.01; Table S1). We called variants separately for each chromosome using bcftools v1.18 (Danecek et al., 2021) *mpileup* and *bcftools call*, specifying a ploidy of two for autosomes and either one or two for the Z chromosome of females and males, respectively. We then left-aligned and normalized indels using bcftools *norm*. Next, we filtered the resulting variant call format (VCF) files using bcftools as fol-

lows: we set a minimum genotype quality of 10 and a minimum read depth of three. We then examined the per-sample distribution of read depths at a large subset of sites across the genome and set maximum read depth filters for each sample accordingly. These cutoffs ranged from 10 for the lowest-coverage samples to 30 for the highest-coverage samples. Precise cutoffs for all samples are available in Dryad (DOI: 10.5061/dryad.j3tx95xt3). All filtered sites were set to uncalled, and we retained all sites in the final concatenated VCF file, which consisted of 953.5 million sites, 100.0 million SNPs, and 3.9 million indels. We repeated this procedure for the mitochondrial genome but without setting a cutoff for depth, which was universally high for all samples, and setting a ploidy of one. The mtDNA dataset contained 16,809 sites and 3,504 SNPs.

Phylogenetic analysis

From our all-sites mtDNA VCF file, we obtained consensus fasta sequences for each sample using SAMtools *faidx* to extract the mitochondrial genome from the reference genome and bcftools *consensus*, applying SNPs and indels and inserting Ns for uncalled or missing sites. We next aligned the individual fasta files using MAFFT v7.520 (Katoh et al., 2002). We used the mtDNA alignment to infer a phylogenetic tree with IQ-TREE v2.2.5 (Minh et al., 2020), specifying ModelFinder Plus (-m MFP), and collapsing near-zero-length branches (-czb). We assessed branch support by using 1,000 replicates of an approximate likelihood-ratio test (-alrt 1000). For this and all phylogenetic analyses, we excluded *M. m. bairdi* and *M. o. chrysops* individuals collected near the hybrid zone, which included some identified as putative hybrids by Céspedes Arias et al., (2025).

In addition to the mitochondrial DNA tree, we inferred phylogenetic relationships using ultra-conserved elements (UCEs), which have proven useful to resolve phylogenetic relationships both at deep and shallow time scales (Carter et al., 2023). We used a set of 4,016 UCEs that were previously identified in the *S. coronata* assembly (Baiz et al., 2021; Faircloth et al., 2012). To extract the UCE sequences, we subset the filtered whole-genome VCF using bcftools *view*, then created fasta files for each sample containing 2-kb regions of UCEs and flanking sequence using samtools *faidx* and bcftools *consensus* as described above, additionally inserting IUPAC ambiguity codes for heterozygous sites. We next aligned, trimmed, filtered, and concatenated the UCE loci using phyluce v1.7.3 (Faircloth, 2016). We first aligned the UCE fasta files with *phyluce_align_seqcap_align*, specifying MAFFT as the aligner, and then did internal trimming with GBlocks (Castresana, 2000). All 4,016 UCE loci were present for all taxa, so we retained all loci for analyses. Finally, we concatenated the UCE alignments and produced a single phylip file using *phyluce_align_concatenate_alignments*.

We estimated the *Myioborus* phylogeny in IQ-TREE using the concatenated phylip alignment, using the flag *-m MFP* to determine the best fitting molecular evolution model and 1,000 approximate likelihood ratio test (aLRT) replicates to assess branch support (-alrt 1000). The best fitting model was TVM + F + I + R6.

In addition to the tree based on a concatenated UCE dataset, we inferred “gene” trees in IQ-TREE 2 using the individual trimmed UCE alignments and the same sequence

evolution model as the concatenated dataset and 1,000 aLRT replicates for branch support. We used the set of resulting trees as input to weighted TREE-QMC (Han & Molloy, 2025), a quartet-based gene tree-species tree reconciliation method.

We visualized discordance between the mitochondrial and the quartet-based UCE trees using the *cophylo* function implemented in the phytools library v2.0 (Revell, 2024) for R (R Core Team, 2025).

Genetic variation in the tropical Andes group (*albifrons-ornatus-melanocephalus*)

To further explore genomic variation in the Tropical Andean (*ornatus-melanocephalus-albifrons*) clade, where we had a better geographic sampling, including all major plumage groups, we conducted a PCA in ANGSD v0.941 (Korneliussen et al., 2014). First, we calculated genotype likelihoods using the in ANGSD, specifying the GATK likelihood model (-GL 2). We retained SNPs with a *p*-value less than or equal to 1e-6 (-SNP_pval 1e-6), which produced a beagle file of ~28.2 M sites. We then used the ANGSD python script *pcangsd.py* to estimate the covariance matrix. Using the *eigen* function in R, we calculated eigenvalues and eigenvectors. In addition, we built a network of mitochondrial haplotypes in the *M. albifrons-ornatus-melanocephalus* complex using the function haploNet from pegas (Paradis, 2010) in R.

To generate a PCA for the *ornatus-melanocephalus* complex only, we followed the same procedure described above but excluding those individuals corresponding to *M. albifrons*. This PCA was based on ~25.9M sites.

We also summarized genetic differentiation among taxa represented by at least two samples by calculating pairwise genome-wide F_{ST} using PLINK v1.9 (Chang et al., 2015) – *make-pgen* and –*fst*.

Genomic landscape of divergence between hybridizing *Myioborus* species

To describe the landscape of divergence between the hybridizing taxa *M. o. chrysops* and *M. m. bairdi* (Céspedes Arias et al., 2021), we used data from individuals with parental phenotypes from both taxa (*n* = 9 for each), collected across their ranges, including several near the hybrid zone. We included individuals with parental phenotypes but potentially admixed ancestry to increase our ability to detect peaks of divergence specifically associated with plumage differentiation and potentially identify genomic regions underlying associated with plumage differences. We calculated windowed F_{ST} using the realSFS program from ANGSD. First, we generated the site frequency spectrum from allele frequency likelihoods (realSFS), and then we prepared the data for the calculation of F_{ST} (realSFS *fst index*). Finally, we calculated F_{ST} in non-overlapping sliding windows of 10 kbp (realSFS *fst stats2*).

Divergence peaks between taxa can result from multiple factors in addition to regions underlying differentiated traits. Processes that may operate deeper in evolutionary time and generate regions of reduced recombination or low genetic diversity can produce common “landscapes of divergence” (Cruickshank & Hahn, 2014). We had the advantage of working in a bird family for which such information is available for many other related species. For instance, Baiz et al.

(2021) studied nine independent species pairs of *Setophaga* and found that 43% of divergence peaks were shared across two or more pairs, likely the result of divergence acting upon an ancient and shared genomic substrate.

To separate divergence peaks that are likely to underlie unique trait differences between the hybridizing pair *M. o. chrysops* and *M. m. bairdi* from those that are likely attributable to other evolutionary processes operating at deeper time scales (Burri, 2017; Burri et al., 2015), we used the divergence landscape described by Baiz et al. (2021) to mask F_{ST} outliers in the *Myioborus* genome. Specifically, we identified which of the top 0.1% of 10-kb *Myioborus* F_{ST} windows, calculated separately for the Z chromosome and autosomes, fell outside the 1 Mb regions that Baiz et al. (2021) determined to contain an overrepresentation of elevated F_{ST} windows between one or more sister pairs of *Setophaga* warblers. We also plotted windowed F_{ST} in *Myioborus* alongside the non-hybridizing sister species *S. striata* and *S. castanea* from Baiz et al. (2021) to aid in visualization.

Because we found a notable pattern of divergence between *M. o. chrysops* and *M. m. bairdi* on the Z-chromosome suggestive of localized regions of reduced recombination, we conducted a local PCA to examine patterns of local genomic structure. Local PCAs were performed on each chromosome using the R package *lostruct* (Li & Ralph, 2019) with non-overlapping 10,000 bp windows, to remain comparable with F_{ST} calculations. Only scaffolds with a length ≥ 500 kbp were included (autosomes 1 to 29 and the Z chromosome), and the first two principal components were retained for each window. From principal component pairwise distance matrices, we performed multidimensional scaling and calculated the first two dimensions in R (function *cmdscale*). The resulting metrics, MDS1 and MDS2, summarize the pairwise distances between each sample in PC space per window. Similar values on each chromosome therefore reflect regions of the chromosome that share similar genetic structure among the samples. We identified outlier windows by chromosome because each MDS dimension is calculated independently per chromosome. Here, outliers were defined as those with MDS1 values three or more scaled absolute deviation units away from the chromosome median. Although this approach is primarily descriptive, empirical data indicate that large-scale heterogeneity in local genetic structure is often driven by structural variants (e.g., inversions) as well as by overall variation in recombination rates (Li & Ralph, 2019). This framework has been applied, e.g., to identify putative structural variants using short-read data (Hale et al., 2021; Recuerda et al., 2025).

To further investigate patterns of genetic variation in the Z chromosome, we used the *lostruct* outlier windows to guide a more targeted analysis. We ran *PCAngsd* (Korneliusson et al., 2014) as above in four blocks of 30,000 SNPs each: the larger MDS outlier region and three “control” regions. The control regions were selected haphazardly, to include two regions close to but not overlapping the MDS outliers and another from roughly the center of the chromosome.

We note that when comparing F_{ST} and MDS1 values, windows were matched by their midpoints, calculated as the average of the start and end positions and rounded down to the nearest kilobase. Although both metrics were calculated using 10 kb windows, a small number of minor mismatches in

the start and end positions sometimes occurred due to differences in how *lostruct* and *PCAngsd* handle window boundaries. Matching by midpoint ensured that corresponding genomic regions were aligned.

Results

Phylogenetic relationships in *Myioborus* inferred using mitochondrial genomes and UCEs

The topologies of the mtDNA and both the UCE phylogenies (i.e., the concatenated UCE tree and the quartet-based tree inferred using *TREE-QMC*) were mostly congruent (Figure 2, Figure S3) but with some discordance, especially among recently diverged species. In all three phylogenies, the earliest two diverging branches contained *M. pictus* and *M. miniatus*, respectively, with *M. miniatus* sister to all upper montane *Myioborus*. All three methods also supported a deep divergence between North and South American *M. miniatus* (Figures S1–S3).

Within the upper montane *Myioborus* group, the mtDNA and UCE trees consistently recovered two well-supported clades: a tropical Andes clade containing *M. albifrons*, *M. ornatus*, and *M. melanocephalus*, and a Tepui region and coastal Venezuela clade containing *M. pariae*, *M. castaneocapilla*, *M. cardonai*, and *M. albifacies* (Figure 2, Figures S1–S3). This major upper montane clade showed some uncertainty in the placement of *M. brunniceps* (subtropical Andes) and *M. torquatus* (Central America). The mtDNA tree positioned *M. brunniceps* as sister to all other high elevation species, though with low aLRT branch support (16%; Figure 2B, Figure S1). In contrast, the UCE trees placed *M. torquatus* as sister to all other high-elevation species, with strong support. The concatenated UCE tree recovered a tropical Andes plus *M. brunniceps* clade with fairly low aLRT support (43%; Figure 2C, Figure S2), whereas the quartet-based UCE tree placed *M. brunniceps* as sister to the tropical Andes plus Tepui and coastal Venezuela clade with fairly strong support (69% of supporting quartets vs. 16% for the next most likely topology; Figure S3).

Within the Tepui and coastal Venezuela clade, the three trees placed *M. pariae*, which inhabits the Paria peninsula, as sister to the rest of the species in the clade, which all inhabit the Tepuis. Among the three Pantepui species, the two UCE trees placed *M. cardonai* as sister to *M. albifacies* with high support, while the mtDNA tree placed *M. cardonai* as sister to *M. castaneocapilla*, also with high support. In the tropical Andean group, the two UCE trees produced the same topology but differed from the mtDNA tree in the placement of both *M. ornatus* and *M. melanocephalus*, which inhabit the eastern Andes of Colombia to the Táchira depression in Venezuela (Figures 1, 2) and *M. melanocephalus griseonuchus* from northwestern Peru. The UCE trees placed *M. o. ornatus* as sister to *M. albifrons* and placed *M. m. griseonuchus* as sister to the southern subspecies *M. m. melanocephalus*, *M. m. malaris*, and *M. m. bolivianus* (hereafter, the “nominated group”), while the mtDNA tree placed *M. m. griseonuchus* as sister to a clade with very short branch lengths containing *M. o. ornatus*, *M. o. chrysops*, and *M. m. bairdi* (Figure 2, Figure S1).

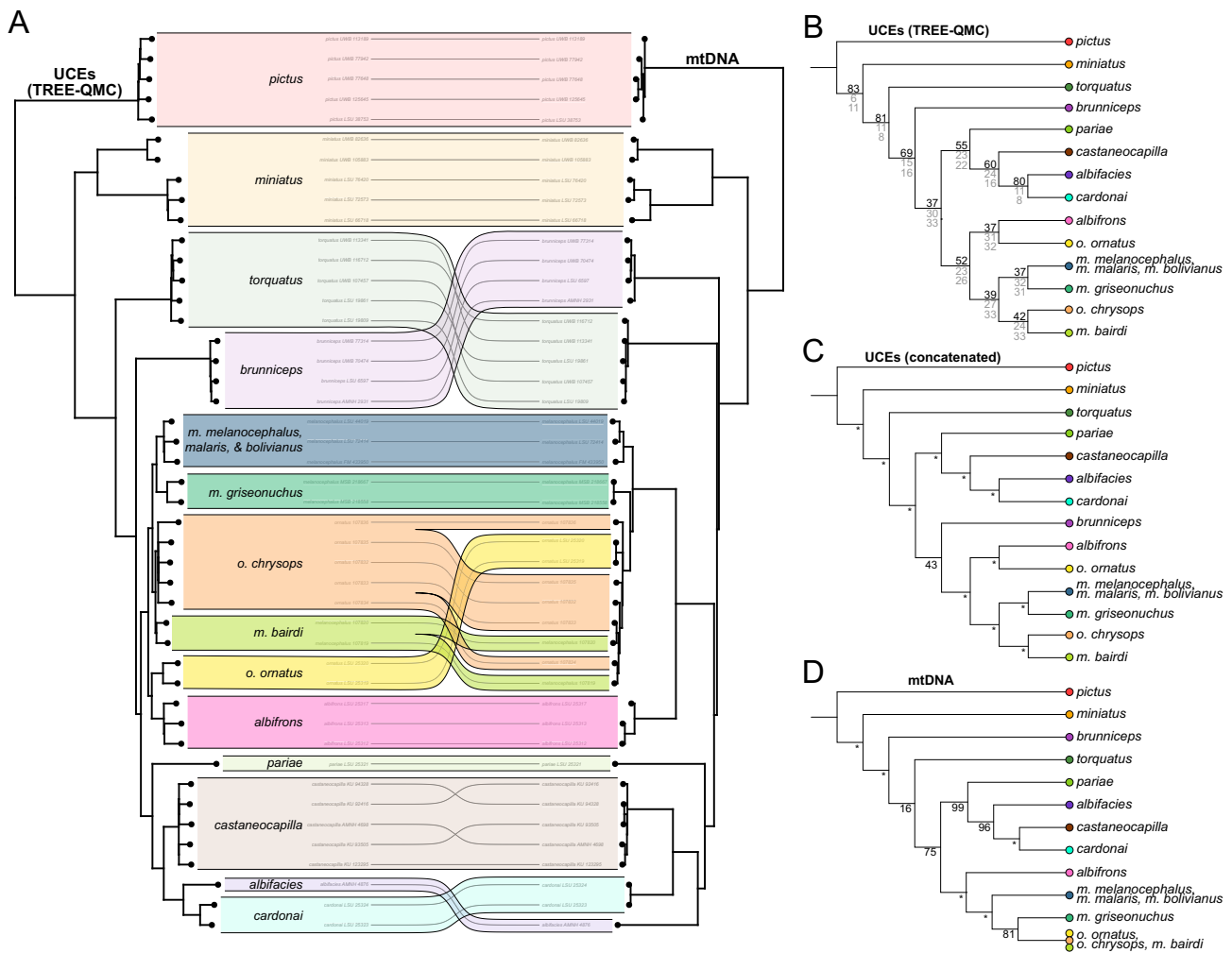


Figure 2. Phylogenetic hypothesis for *Myioborus*. (A) Cophylogeny comparing the quartet-based UCE and the mtDNA phylogenies. (B–D) Simplified topologies with not-to-scale branch lengths of the quartet-based UCE, concatenated UCE, and mtDNA trees. Support values in (B) represent support for the best arrangement in black and the two alternates in gray. Support values in (C) and (D) are from 1,000 aLRT replicates. Values greater than 99 are represented by an asterisk (*).

Genetic variation in the tropical Andean group (*ornatus*–*melanocephalus*–*albifrons*)

Our phylogenetic analyses produced discordant topologies between mitochondrial and UCE trees in the Andean clade made up of *M. albifrons*, *M. ornatus*, and *M. melanocephalus*. For this reason and because we had comprehensive sampling of geographic variation among these species (Figure 3A), we further focused on mitochondrial and nuclear genetic variation in the group. The haplotype network of the mitochondrial genome showed clusters corresponding to the major clades of the mtDNA tree (Figure 3B). Specifically, *M. albifrons* individuals cluster together further from the other taxa; the southern *M. melanocephalus* nominate group and *M. m. griseonuchus* each form distinct clusters; and *M. o. ornatus*, *M. o. chrysops*, and *M. m. bairdi* cluster together, including both putatively pure parental individuals and those with some admixed ancestry.

In the genome-wide PC1/PC2 space, the pattern of clustering observed among samples was generally consistent with the relationships obtained from UCEs (Figure 3C): PC1 separated *M. albifrons* individuals from the other samples,

with *M. o. ornatus* positioned between the two clusters, whereas PC2 separated the northern hybridizing taxa (*M. o. chrysops* and *M. m. bairdi*), *M. o. ornatus*, and *M. albifrons* from the *M. melanocephalus* subspecies further south (*M. m. griseonuchus* and the *M. melanocephalus* nominate group) (Figure 3C). *M. o. chrysops* and *M. m. bairdi* were distinguishable by PC2, including those with admixed ancestry and closer affinities to one or the other. The PC2/PC3 and PC3/PC4 spaces did not cluster samples as clearly by plumage groups, but suggest some clustering of individuals collected in the same locality (Figure S4). Lastly, when excluding *M. albifrons* from the PCA, we found a similar clustering by subspecies across the PC1/PC2 space, with a clear separation among the *M. melanocephalus* nominate group, *M. o. ornatus* and the two hybridizing taxa across the PC1 (Figure S5).

Genome-wide differentiation as measured by F_{ST} varied among these taxa and other *Myioborus* species. Consistent with phylogenetic results, tropical Andes taxa showed lower differentiation among themselves (range = 0.07–0.29) than with other *Myioborus* species (Figure S6). The two hybridizing taxa, *M. m. bairdi* and *M. o. chrysops*, exhib-

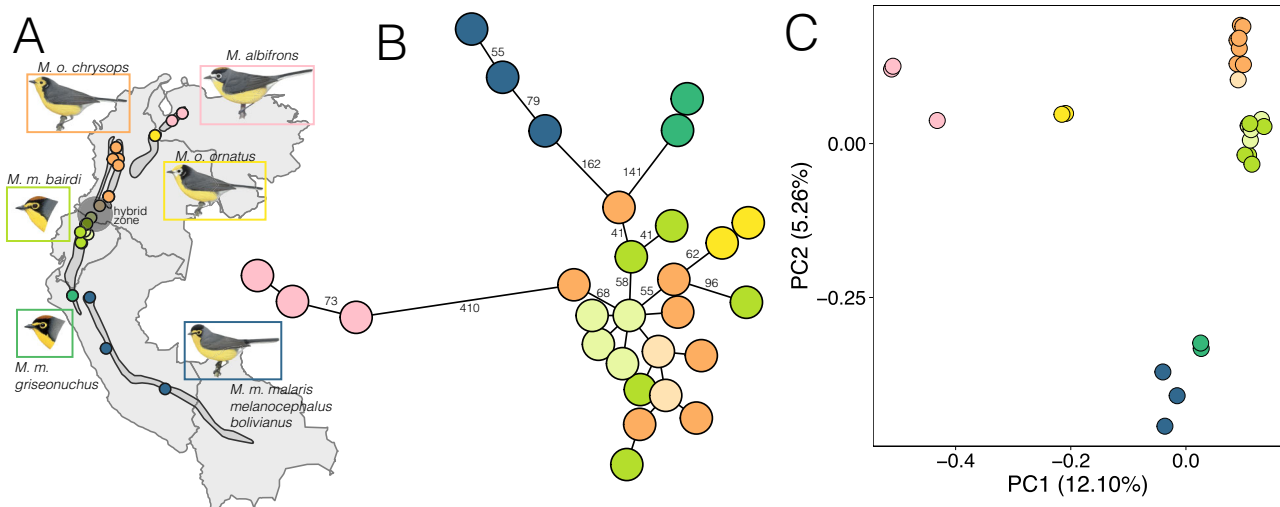


Figure 3. Genetic variation within the Tropical Andes clade (*M. albifrons*, *M. ornatus*, and *M. melanocephalus*). (A) Sampling localities colored by geographic groups. The approximate location of the hybrid zone is denoted as a gray circle, and the distribution of the three species are shown as light gray polygons with a darker border (shapefiles from NatureServe). (B) Network of mitochondrial genome haplotypes. Edge lengths are proportional to mutational steps. The specific number of mutations is shown only for edges with over 40 mutations for clarity. (C) Genotype likelihood-based PCA plot summarizing genetic variation across 12,624,354 SNPs. In both the haplotype network and the PCA plot, the colors correspond to the subspecies as in panel A. For *M. melanocephalus bairdi* and *M. ornatus chrysops*, the lighter colors correspond to individuals that were identified as admixed by Céspedes Arias et al., (2025).

ited the lowest F_{ST} observed for any pairwise comparison ($F_{ST} = 0.07$; Figure S6). In contrast, despite the low mitochondrial divergence observed, based on our sampling *M. o. ornatus* showed substantial nuclear genome-wide differentiation from both hybridizing taxa ($F_{ST} = 0.2$ for each comparison).

Genomic landscape of divergence between hybridizing *Myioborus*

Out of 102 outlier 10-kb windows between *M. m. bairdi* and *M. o. chrysops*, seven were not found in large regions of elevated divergence common to previous *Setophaga* warbler comparisons (Baiz et al., 2021). These divergent windows unique to this *Myioborus* pair fell in chromosomes 1, 1A, and 3 (Figure 4). We note that this conservative approach focuses on the strongest outlier regions, but more subtle differences could also exist and be relevant to species differences in our focal *Myioborus* taxa.

The chromosome 1 outlier window was more than 100 kb from the nearest annotated protein-coding gene in the yellow-rumped warbler genome. The highest chromosome 1A outlier, which also had the highest F_{ST} in the genome, partially overlapped the coiled-coil domain-containing 91 gene (*CCDC91*), a gene producing a protein involved in transport between the Golgi apparatus and lysosomes (Stelzer et al., 2016). The other two chromosome 1A peaks are not within 20 kb of any gene. The closest gene, approximately 30 kb away from one of these peaks, is *KITLG* (KIT ligand), a protein-coding gene involved in multiple processes, including cell survival and proliferation, hematopoiesis, gametogenesis, and melanogenesis (Stelzer et al., 2016). The chromosome 3 peak fell within the gene *KIF25*, which encodes a protein belonging to the kinesin-like protein family (Stelzer et al., 2016).

A prominent feature of the differentiation landscape between *M. o. chrysops* and *M. m. bairdi* plumage types was a region of roughly 6 Mb on the Z chromosome which contained two large blocks (~0.8 and 2.2 Mb) of elevated F_{ST} values (Figure 5A), consistent with a signal of reduced recombination. To explore this possibility, we conducted a local PCA across all chromosomes (Figure S7), and found a large deviation in the MDS1 on the Z chromosome which corresponded with these blocks of elevated F_{ST} (Figure 5B). Extended regions of elevated MDS1 suggests these regions share a particular population structure that deviates from the typical structure found on the rest of the Z chromosome. Other MDS1 outlier regions appear across the genome, but these are much smaller than the blocks observed on the Z chromosome and do not clearly correspond to the smaller F_{ST} peaks detected on other chromosomes (Figures S8 and S9). The only other large MDS1 outlier region is found on chromosome 19 (Figure S8, Table S2).

These patterns indicate that Z chromosome regions of interest are characterized both by high genetic differentiation between species and by distinct population structure relative to the rest of the chromosome (Figure 5). To visualize this difference, we calculated principal components from genotype likelihoods in four blocks of 30,000 SNPs each, including an MDS outlier region and three “control” regions. In the MDS and F_{ST} outlier block, consistent with heavily reduced recombination, PC1 explained a large proportion of variation (48.4%) and separated individuals into three distinct clusters, likely corresponding to homozygotes for two alternate haplotypes and heterozygotes (Figure 5F), while control regions showed no such clustering (Figure 5C–E). While the haplotypes mostly segregated by species, the one “mismatched” individual and three of the four putative heterozygotes were from inside the hybrid zone (Figure 5G).

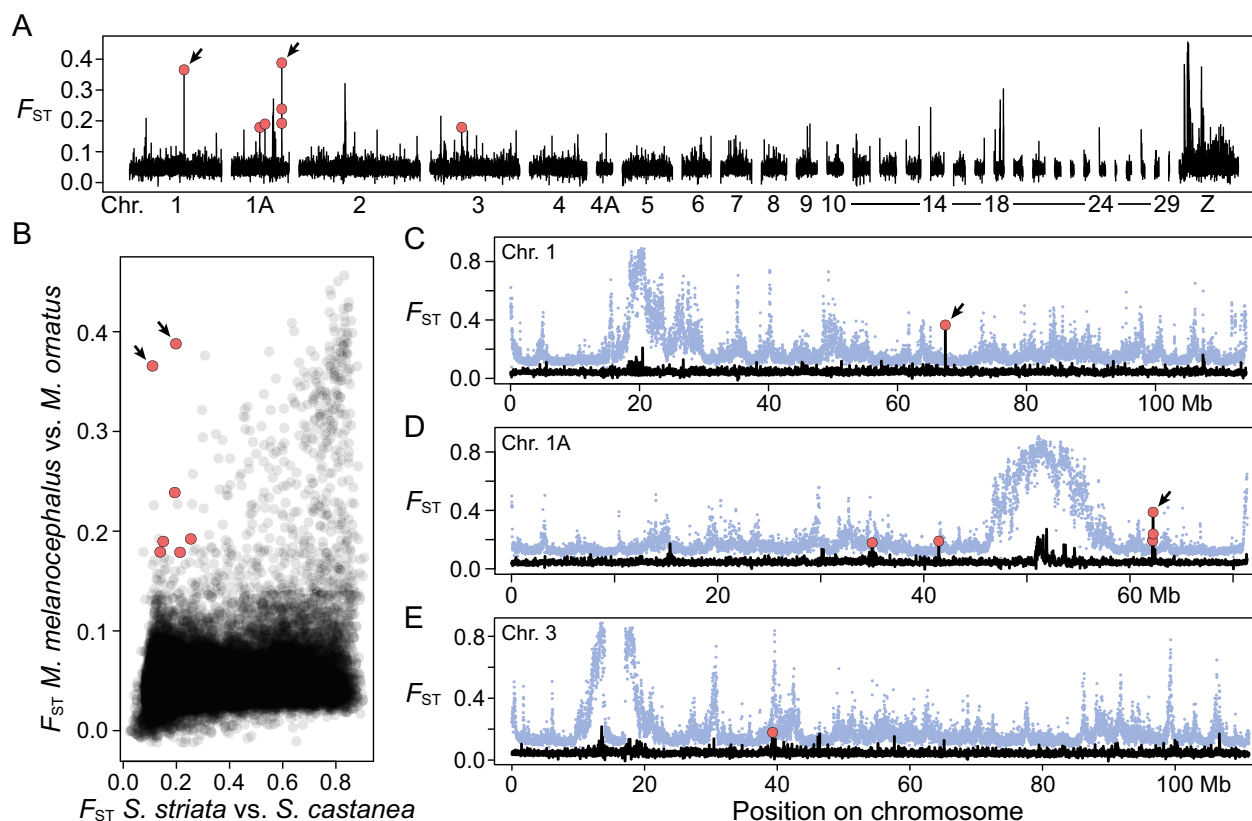


Figure 4. The genomic landscape of divergence between the hybridizing *M. ornatus chrysops* and *M. melanocephalus bairdi*. (A) F_{ST} for the whole genome plotted in 10 kbp windows. Red points indicate 99.9% outliers that did not fall within large regions of differentiation between multiple pairs of warblers in the genus *Setophaga*, identified in a previous study. (B) A comparison of F_{ST} values between *M. o. chrysops* and *M. m. bairdi* and the same 10 kbp window between *S. striata* and *S. castanea*. (C–E) Zoomed plots from (A) showing chromosomes that contain *Myioborus* F_{ST} outliers. F_{ST} between *S. striata* and *S. castanea* is plotted in blue. In all panels, the outliers discussed in the text are highlighted with arrows.

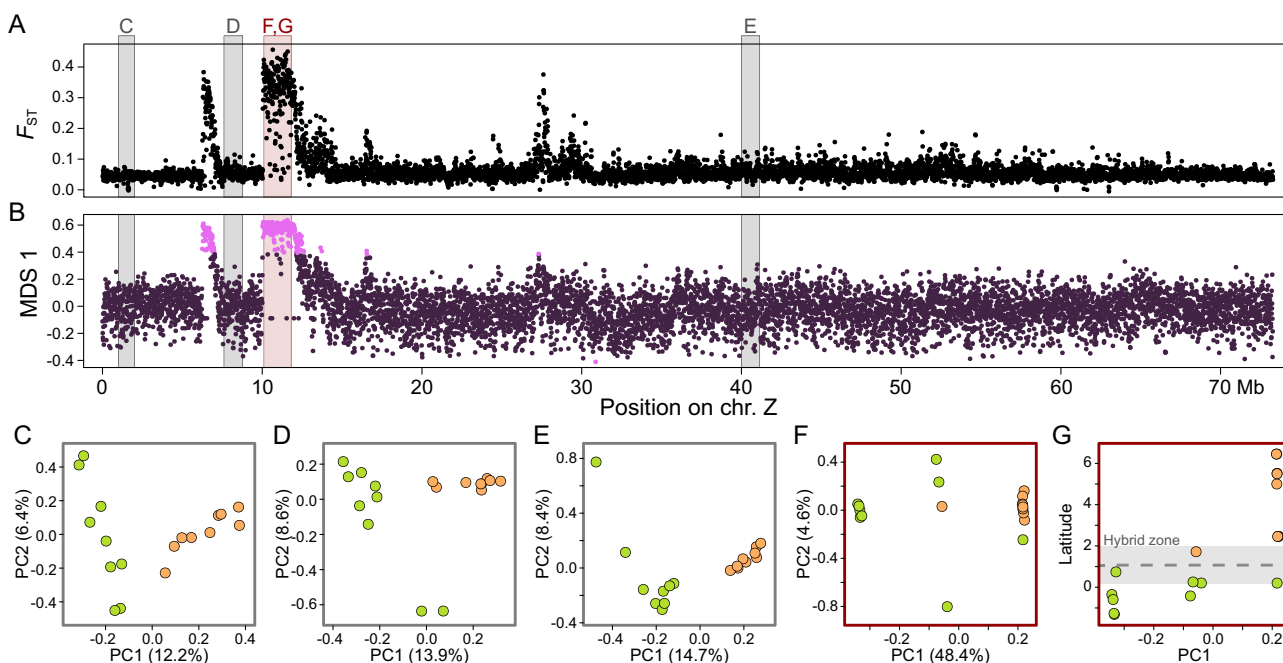


Figure 5. Signals of suppressed recombination in F_{ST} peaks on the Z chromosome. (A) F_{ST} plotted in 10-kb windows across the Z chromosome. Four blocks of 30,000 SNPs are labeled corresponding to their PCA panel below. (B) MDS dimension 1 values plotted in 10-kb windows. Outliers by median absolute deviation are in pink. (C–F) Principal component analysis plots for each highlighted region of the chromosome in (A). Orange represents *Myioborus ornatus chrysops*, and green represents *M. melanocephalus bairdi*. (G) The PC1 values from (F) plotted against latitude of sample origin. The approximate extent of the hybrid zone is shaded gray, with the approximate center in a dashed line (Céspedes Arias et al., 2021).

Discussion

We integrated phylogenetic and population-level genomic analyses to gain insights into the diversification of *Myioborus*, the youngest genus in the rapid Parulidae radiation and one characterized prominent variation head plumage patterns. Although topological incongruence is common in rapid radiations (Gwee et al., 2020), the tree topologies we inferred using UCEs (TREE-QMC and concatenated) and mitogenomes were largely concordant and agreed with previous phylogenetic hypotheses, especially at older nodes in the genus (Bennett et al., 2025; Lovette et al., 2010; Pérez-Emán, 2005; Zhao et al., 2025). Two notable common features of our tree topologies were that (1) the earliest split separated *M. pictus* (which occurs furthest north at the lowest elevation) from the rest of the genus, and (2) the next-earliest split separated *M. miniatus*, a widely distributed mid-elevation species, from an upper-montane clade composed of allospecies that replace *M. miniatus* at higher elevations along different portions of these montane systems (although with some elevational sympatry; Curson et al., 1994; Jablonski et al., 2020).

This sister relationship between *M. miniatus* and a lineage of high elevation species supports a long-established pattern in elevational zonation: species that replace each other by elevation show deeper divergence than species occurring allopatrically but occupying similar elevations, as documented in other Andean bird lineages (Benham et al., 2015; Cadena et al., 2019). High-elevation *Myioborus* species have likely diversified as a result of allopatric divergence across topographic barriers, whose effectiveness in restricting gene flow varied with cycles of upslope and downslope shifts of vegetation belts driven by glacial-interglacial climatic fluctuations (Flantua et al., 2019; Ramírez-Barahona & Eguiarte 2013).

Within the well supported high-elevation clade, we found some uncertainty in the position of *M. brunnicaps* and *M. torquatus* which are, respectively, the southernmost and northernmost taxa in this group (an incongruence also inferred in a family-wide study by Zhao et al., 2025). However, given differences in support values and extremely short internodes in this section of the mtDNA tree, we interpret our results as a whole as supporting the position of *M. torquatus* as sister to the rest of the upper montane taxa, as inferred in both UCE trees. This relationship, in turn, would suggest a north-to-south colonization of high elevation peaks in the Neotropical region during the diversification of these warblers, as has been inferred for several other montane birds (Cadena et al., 2007; Gutiérrez-Pinto et al., 2017; Weir et al., 2008). When considered alongside the phylogeographic findings of Pérez-Emán et al. (2010), which identified an older Mexican lineage of *M. miniatus* and relatively low mitochondrial divergence across Central and South American *M. miniatus* lineages, these results are consistent with the possibility of two colonizations of South America: one by a high-elevation lineage that diversified across Central and South American mountain ranges, and another by *M. miniatus*, which occupies mid-elevation habitats and has maintained gene flow across much of its range.

Within the upper montane group, two clades with clear geographic affinities are well supported across all analyses: a Pantepui plus coastal Venezuela group (*M. pariae*, *M. castaneocapilla*, *M. cardonai*, and *M. albifacies*) and a tropical Andes group (*M. albifrons*, *M. ornatus*, and *M.*

melanocephalus). Within this first clade, we found that the coastal Venezuela taxon is sister to the species allopatrically distributed across different tepuis, suggestive of in situ diversification in the Pantepui region, which is characterized by an old geological origin and high levels of endemism (Borges et al., 2018). Within both the Pantepui and coastal Venezuela group and the Tropical Andes group, we found topological incongruence between UCEs and mitogenomes. Several factors could cause this discordance (Toews & Brelsford, 2012), including past introgression of mitochondrial or nuclear DNA (Berbel-Filho et al., 2022; Kearns et al., 2018; Toews et al., 2013) or extensive incomplete lineage sorting (DeRaad et al., 2023). Both explanations are plausible, especially for a rapidly diversifying group.

All three of our phylogenies strongly support the existence of a tropical Andes clade formed by *M. albifrons* and the geographically variable *M. melanocephalus* and *M. ornatus*. Within this group, our results reflect two alternate relationships based on either our mtDNA or nuclear data (UCEs and genome-wide SNPs). In the mitogenome phylogeny and haplotype network, *M. albifrons* is quite distinct and sister to a clade including all subspecies of *M. ornatus* and *M. melanocephalus*. These relationships are broadly consistent with previous phylogenetic studies that used exclusively or predominantly mtDNA (Lovette et al., 2010; Pérez-Emán, 2005). In contrast, our UCE topologies indicate a sister relationship between *M. o. ornatus* and *M. albifrons*. This sister relationship is at odds with previous taxonomic delimitations (Clements et al., 2025; Cuervo & Céspedes Arias, 2023; Remsen et al., 2026) and the characterization of *M. ornatus* and *M. melanocephalus* as a species complex, but consistent with a previous warbler UCE phylogeny (Bennett et al., 2025), which used a subset of the *M. o. ornatus* and *M. albifrons* samples in the present study.

Adding further complexity, a related study focused exclusively on *M. ornatus* and *M. melanocephalus* using a geographically comprehensive ddRAD dataset (Céspedes Arias et al., 2025), found little genetic differentiation among *M. o. ornatus*, *M. o. chrysops*, and *M. m. bairdi*. While that study lacked samples for *M. albifrons*, its results support an *ornatus-bairdi-chrysops* group, which contrasts with the view of *M. o. ornatus* as a lineage outside the *M. melanocephalus-ornatus* complex, as suggested by our UCE-based phylogenies.

Given the short internodes in the tropical Andean clade, suggestive of rapid diversification, incomplete lineage sorting is a plausible explanation for the apparent mito-nuclear discordance (Degnan & Rosenberg, 2009; DeRaad et al., 2023; Meleshko et al., 2021). Another non-exclusive possibility is that historical introgression between some of these *Myioborus* lineages may also be contributing to observed differences in patterns of variation in mitochondrial and nuclear DNA. For example, our *M. o. ornatus* samples were collected very close to the Táchira Depression (Figure 1), so gene flow from *M. albifrons* is a possible explanation for the sister relationship between them produced by the UCE trees. A more extensive geographic sampling and larger sample sizes within these groups, along with targeted analyses to detect introgression in different genomic regions will be necessary to disentangle these possibilities.

All of our analyses support that the hybridizing *M. m. bairdi* and *M. o. chrysops* are sister taxa and show overall

low genetic differentiation, corroborating previous work focused on few mitochondrial markers (Céspedes Arias et al., 2021; Pérez-Emán, 2005). These young taxa are characterized by clear differences in head coloration, mirroring the genus level pattern of plumage variation. Investigating the genomic landscape of divergence between these two taxa, we found that the levels of differentiation across the genome are highly heterogeneous, mostly low but with several clear peaks of differentiation. These peaks, for the most part, fall within large regions of divergence shared with pairs of *Setophaga* warblers (Baiz et al., 2021). As Baiz et al. (2021) did, we interpret this shared differentiation across independent pairs as a function of a common genomic architecture, reduced recombination, and linked selection (Burri et al., 2015; Cruickshank & Hahn, 2014). Genomic regions of reduced recombination such as centromeres are associated with increased differentiation between diverging taxa because linkage is much greater, which amplifies the effects of divergent selection (Burri et al., 2015; Van Doren et al., 2017). In the case of very closely related taxa such as *M. m. bairdi* and *M. o. chrysops*, these peaks are fairly narrow, which may provide an opportunity to identify genes underlying linked divergent selection. For instance, there are only four genes in the center of the putative centromeric peak on chromosome 2: *SLC9A3*, *BRD9*, *COL15A1*, and *TPPP*.

We also identified several peaks that were not shared with *Setophaga*, which could represent regions underlying the head coloration differences between these young hybridizing taxa. One of these regions, located on chromosome 1A (Figure 4D), contains the gene *CCDC91*, which encodes a protein whose function is not well understood but has been linked to acrokeratoelastoidosis, a rare skin condition in humans (Zhu et al., 2024). This gene has been found in regions of high differentiation between bird species with melanin-based plumage differences, including *Lonchura munias* that differ in head and body color (Stryjewski & Sorenson, 2017) and *Motacilla* wagtails that differ in head color (Semenov et al., 2021). In the case of the wagtails, the two subspecies differ primarily in the extent of melanin in the feathers around their eyes and in their cheek patches, which are the same facial patches that differ between *chrysops* and *bairdi*. Thus, as with other melanogenesis genes that have been found to be commonly diverged in independent avian pairs (e.g., *MC1R*, *ASIP*; Baiz et al., 2021; Campagna et al., 2017, 2022; Toews et al., 2016), *CCDC91* may have been independently recruited for similar pigmentation phenotypes in both *Motacilla* and *Myioborus*. Future work with larger sample sizes, including individuals with intermediate phenotypes, will allow a more detailed exploration of the connection between *CCDC91* and specific plumage patches, as well as the identification of other genomic regions of interest, including those that may show epistatic interactions with this gene (as observed for *ASIP*–*CCDC91* in *Motacilla*).

We found that the Z-chromosome shows a higher overall differentiation relative to autosomes (0.063 vs. 0.044), a pattern prevalent among many bird species pairs (Irwin, 2018), and particularly marked across hybrid zones (Hooper et al., 2019) in which Z-linked loci often show reduced introgression (Carling & Brumfield, 2008; Ottenburghs, 2022; Taylor et al., 2014; Walsh et al., 2016). This pattern of higher differentiation relative to autosomes is also observed in the X chromosome in organisms with an XY sex deter-

mination system (Presgraves, 2018). In general, the pervasiveness of this pattern is consistent with an important role of sex chromosomes in speciation (Irwin, 2018; Presgraves, 2018). Moreover, in the presence of gene flow, these patterns could be interpreted as evidence that sex chromosomes are less prone to introgression across species boundaries. Cline analyses in hybrid zones across multiple bird taxa appear to support this idea, as Z-linked loci often show reduced introgression relative to autosomes (Carling & Brumfield, 2008; Hooper et al., 2019; Ottenburghs, 2022; Taylor et al., 2014; Walsh et al., 2016), and furthermore, some suggest a direct involvement of Z-linked loci in genetic incompatibilities (López et al., 2021; Orr, 1997). Given the existence of a hybrid zone between *M. o. chrysops* and *M. m. bairdi*, it is possible that gene flow could be contributing to the different levels of divergence in the Z-chromosome relative to autosomes.

Besides having overall higher differentiation in the *M. o. chrysops*–*M. m. bairdi* pair, the Z chromosome contains some of the most prominent divergence peaks (Figure 5). Specifically, we identified two large regions of elevated F_{ST} . Our local PCA analysis revealed that genetic structure is similar within these regions, but that this structure is markedly different from the rest of the chromosome. In particular, the presence of three discrete clusters, clearly observed in at least the largest block, is consistent with drastically reduced recombination and the segregation of alternative haplotypes, commonly associated with chromosomal inversions (Ma & Amos, 2012; Nowling et al., 2020). Whether the distinct divergence and population structure patterns we identified on the Z chromosome were caused by inversions or by another genomic feature showing significantly reduced recombination, they may play an important role in hybridization dynamics. A lack of recombination is the main characteristic of inversions responsible for their important role in restricting gene flow across hybrid zones (Hooper et al., 2019) and contributing to speciation (Hoffmann & Rieseberg, 2008). Future studies employing long-read sequencing could test for the presence of such structural variants and assess whether they play a role in restricting gene flow across the *chrysops*–*bairdi* hybrid zone. Long-read data analyses could also determine whether there are actually one or two divergent regions or whether the appearance of the latter is caused by structural variation between *Myioborus* and the reference genome species, *Setophaga coronata*.

In summary, we used whole-genome resequencing data for 11 of the 12 species in the youngest warbler genus, *Myioborus*, to gain insights into the evolutionary history of a radiation of Neotropical birds at different time scales, which in turn sets the stage for future avenues of research. Our phylogenetic results raise the question of whether introgression has played a role in driving topological discordance. Our work suggests that the two hybridizing taxa within this genus (*M. m. bairdi* and *M. o. chrysops*) have shallow divergence in both their mitochondrial and nuclear genomes, and the landscape of divergence between them led us to identify one candidate gene for their differences in melanin-based plumage patches. Thus, future work should obtain whole-genome data from hybrids with diverse plumage phenotypes to evaluate correlations between genetic variation at this locus and the presence or absence of specific patches, and to identify additional candidate genes

by leveraging the extensive variation and admixture in this hybrid zone. Lastly, we identified large blocks of elevated differentiation on the Z chromosome, which could reflect one or more chromosomal inversions, regions resistant to introgression, or other processes maintaining extended haplotypes. Future work incorporating denser genomic sampling across the hybrid zone could test for reduced introgression in these regions, while long-read sequencing could help identify the presence of structural variants such as inversions.

Supplementary material

Supplementary material is available online at [Evolution](#).

Data availability

Raw sequence data is available from NCBI (BioProjects PRJNA630247 and PRJNA1423728). All other data and code necessary to reproduce the analyses from this study are available from Dryad (DOI: 10.5061/dryad.j3tx95xt3).

Author contributions

L.N.C.A., K.F.P.B., L.C., and D.P.L.T. conceived the study; L.N.C.A. and A.M.C. conducted fieldwork to collect *M. m. bairdi* and *M. o. chrysops* samples, with logistical support from C.D.C. and E.B. A.W., K.F.P.B., and L.N.C.A. conducted lab work. I.J.L. and L.C. supported L.N.C.A.'s work at the Cornell Lab of Ornithology. K.F.P.B. conducted the bioinformatic processing of raw data and most data analyses. L.N.C.A. conducted the lostruct analyses. K.F.P.B., L.N.C.A., and D.P.L.T. produced figures. L.N.C.A. led the writing of the original draft with major contributions from K.F.P.B. and D.P.L.T., and additional input from all other authors. All authors contributed to the interpretation of results and to reviewing and editing the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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References

- Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C. A., Buggs, R., Butlin, R. K., Dieckmann, U., Erroukhanoff, F., Grill, A., Cahan, S. H., Hermansen, J. S., Hewitt, G., Hudson, A. G., Jiggins, C., ... Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology*, 26, 229–246. <https://doi.org/10.1111/j.1420-9101.2012.02599.x>
- Aguillon, S. M., Walsh, J., & Lovette, I. J. (2021). Extensive hybridization reveals multiple coloration genes underlying a complex plumage phenotype. *Proceedings of the Royal Society B: Biological Sciences*, 288, 2020–1805. <https://doi.org/10.1098/rspb.2020.1805>
- Baiz, M. D., Wood, A. W., Brelsford, A., Lovette, I. J., & Toews, D. P. L. (2021). Pigmentation genes show evidence of repeated divergence and multiple bouts of introgression in *Setophaga* warblers. *Current Biology*, 31, 643–649.e3. <https://doi.org/10.1016/j.cub.2020.10.094>
- Benham, P. M., Cuervo, M., McGuire, J. A., & Witt, C. C. (2015). Biogeography of the Andean metal tail hummingbirds: Contrasting evolutionary histories of tree line and habitat-generalist clades. *Journal of Biogeography*, 42, 763–777. <https://doi.org/10.1111/jbi.12452>
- Bennett, K. F. P., Wood, A. W., Baiz, M. D., Phung, L., Lovette, I. J., & Toews, D. P. L. (2025). A colorful legacy of hybridization in wood-warblers includes frequent sharing of carotenoid genes among species and genera. *PLoS Biology*, 23, 1–19. <https://doi.org/10.1371/journal.pbio.3003501>
- Berbel-Filho, W. M., Pacheco, G., Tatarenkov, A., Lira, M. G., Garcia de Leaniz, C., Rodríguez López, C. M., Lima, S. M. Q., & Consuegra, S. (2022). Phylogenomics reveals extensive introgression and a case of mito-nuclear discordance in the killifish genus *Kryptolebias*. *Molecular Phylogenetics and Evolution*, 177, 107617. <https://doi.org/10.1016/j.ympev.2022.107617>
- Borges, S. H., Santos, M. P. D., Moreira, M., Baccaro, F., Capurro, J. M. G., & Ribas, C. (2018). Dissecting bird diversity in the Pantepui area of endemism, northern South America. *Journal of Ornithology*, 159, 1073–1086. <https://doi.org/10.1007/s10336-018-1576-6>
- Broad Institute (2019). Picard Toolkit. GitHub Repository. <https://github.com/broadinstitute/picard>
- Burri, R. (2017). Interpreting differentiation landscapes in the light of long-term linked selection. *Evolution Letters*, 1(3), 118–131. <https://doi.org/10.1002/evl3.14>
- Burri, R., Nater, A., Kawakami, T., Mugal, C. F., Olason, P. I., Smeds, L., Suh, A., Dutoit, L., Bureš, S., Garamszegi, L. Z., Hogner, S., Moreno, J., Qvarnström, A., Ružić, M., Sæther, S. A., Sætre, G. P., Török, J., & Ellegren, H. (2015). Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation

- Ravinet, M., Faria, R., Butlin, R. K., Galindo, J., Bierne, N., Rafajlović, M., Noor, M. A. F., Mehlig, B., & Westram, A. M. (2017). Interpreting the genomic landscape of speciation: a road map for finding barriers to gene flow. *Journal of Evolutionary Biology*, 30, 1450–1477. <https://doi.org/10.1111/jeb.13047>
- Recuerda, M., Kraemer, S., Rosoni, J. R. R., Repenning, M., Browne, M., Cataudela, J. F., Di Giacomo, A. S., Kopuchian, C., & Campagna, L. (2025). A pangenomic approach reveals the sources of genetic variation fueling the rapid radiation of Capuchino Seed eaters. *Evolution; International Journal of Organic Evolution*, 79, 2739–2755. <https://doi.org/10.1093/evolut/qpaf188>
- Remsen, J. V., Cadena, C., Jaramillo, A., Nore, M., Pacheco, J., Robbins, M., Schulenberg, T., Stiles, F., Stotz, K. J., & Zimmer, D. F. (2026). *A classification of the bird species of South America*. American Ornithologists' Union.
- Revell, L. J. (2024). phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and other things). *PeerJ*, 12, e16505. <https://doi.org/10.7717/peerj.16505>
- Rheindt, F. E., & Edwards, S. V. (2011). Genetic introgression: An integral but neglected component of speciation in birds. *The Auk*, 128, 620–632. <https://doi.org/10.1525/auk.2011.128.4.620>
- Rohwer, S., & Wood, C. (1998). Three hybrid zones between Hermit and Townsend's warblers in Washington and Oregon. *The Auk*, 115, 284–310. <https://doi.org/10.2307/4089188>
- Rosamond, K. M., Kent, C. M., Hunter, S. C., & Sherry, T. W. (2020). Morphological traits influence prey selection by coexisting species of New World warblers (Parulidae). *Journal of Field Ornithology*, 91, 393–408. <https://doi.org/10.1111/jof.12352>
- Sambrook, J. (1987). *Molecular cloning: a laboratory manual*. Cold Harbor Spring Laboratory.
- Schubert, M., Lindgreen, S., & Orlando, L. (2016). AdapterRemoval v2: Rapid adapter trimming, identification, and read merging. *BMC Research Notes*, 9, 1–7. <https://doi.org/10.1186/s13104-016-1900-2>
- Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology & Evolution*, 19, 198–207. <https://doi.org/10.1016/j.tree.2004.01.003>
- Seehausen, O. (2006). African cichlid fish : a model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences*, 273(1597), 1987–1998. <https://doi.org/10.1098/rspb.2006.3539>
- Semenov, G. A., Linck, E., Enbody, E. D., Harris, R. B., Khaydarov, D. R., Alström, P., Andersson, L., & Taylor, S. A. (2021). Asymmetric introgression reveals the genetic architecture of a plumage trait. *Nature Communications*, 12, 1–9. <https://doi.org/10.1038/s41467-021-21340-y>
- Short, L. L., & Robbins, C. S. (1967). An intergeneric hybrid wood warbler (*Seiurus* × *Dendroica*). *The Auk*, 84, 534–543. <https://doi.org/10.2307/4083334>
- Shutler, D., & Weatherhead, P. J. (1990). Targets of sexual selection: song and plumage of wood warblers. *Evolution (N. Y.)*, 44, 1967–1977.
- Singhal, S., Derryberry, G. E., Bravo, G. A., Derryberry, E. P., Brumfield, R. T., & Harvey, M. G. (2021). The dynamics of introgression across an avian radiation. *Evolution Letters*, 5, 568–581. <https://doi.org/10.1002/evl3.256>
- Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Iny Stein, T., Nudel, R., Lieder, I., Mazon, Y., Kaplan, S., Dahan, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M., & Lancet, D. (2016). The GeneCards suite: From gene data mining to disease genome sequence analyses. *Current Protocols in Bioinformatics*, 2016, 1.30.1–1.30.33.
- Strykowski, K. F., & Sorenson, M. D. (2017). Mosaic genome evolution in a recent and rapid avian radiation. *Nature Ecology & Evolution*, 1, 1912–1922. <https://doi.org/10.1038/s41559-017-0364-7>
- Svardal, H., Quah, F. X., Malinsky, M., Ngatunga, B. P., Miska, E. A., Salzburger, W., Genner, M. J., Turner, G. F., & Durbin, R. (2020). Ancestral hybridization facilitated species diversification in the lake malawi cichlid fish adaptive radiation. *Molecular Biology and Evolution*, 37, 1100–1113. <https://doi.org/10.1093/molbev/msz294>
- Taylor, S. A., & Larson, E. L. (2019). Insights from genomes into the evolutionary importance and prevalence of hybridization in nature. *Nature Ecology & Evolution*, 3, 170–177. Springer US. <https://doi.org/10.1038/s41559-018-0777-y>
- Taylor, S. A., White, T. A., Hochachka, W. M., Ferretti, V., Curry, R. L., & Lovette, I. (2014). Climate-mediated movement of an avian hybrid zone. *Current Biology*, 24, 671–676. Elsevier Ltd. <https://doi.org/10.1016/j.cub.2014.01.069>
- Toews, D. P. L., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, 21, 3907–3930. <https://doi.org/10.1111/j.1365-294X.12.05664.x>
- Toews, D. P. L., Brelsford, A., & Irwin, D. E. (2011). Hybridization between Townsend's *Dendroica townsendi* and black-throated green warblers *D. virens* in an avian suture zone. *Journal of Avian Biology*, 42, 434–446. <https://doi.org/10.1111/j.1600-048X.2011.05360.x>
- Toews, D. P. L., Kramer, G. R., Jones, A. W., Brennan, C. L., Cloud, B. E., Andersen, D. E., Lovette, I. J., & Streby, H. (2020). Genomic identification of intergeneric hybrids in New World wood-warblers (Aves: Parulidae). *Biological Journal of the Linnean Society*, 131(1), 183–191. <https://doi.org/10.1093/biolinnean/blaa085>
- Toews, D. P. L., Mandic, M., Richards, J. G., & Irwin, D. E. (2013). Migration, mitochondria, and the yellow-rumped warbler. *Evolution*, 68(1), 241–255. <https://doi.org/10.1111/evo.12260>
- Toews, D. P. L., Taylor, S. A., Vallender, R., Brelsford, A., Butcher, B., Messer, P. W., & Lovette, I. J. (2016). Plumage genes and little else distinguish the genomes of hybridizing warblers. *Current Biology*, 26, 2313–2318. <https://doi.org/10.1016/j.cub.2016.06.034>
- Tokita, M., Yano, W., James, H. F., & Abzhanov, A. (2017). Cranial shape evolution in adaptive radiations of birds: Comparative morphometrics of Darwin's finches and Hawaiian honeycreepers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372, 20150481. <https://doi.org/10.1098/rstb.2015.0481>
- Tsai, W. L. E., Escalona, M., Garrett, K. L., Terrill, R. S., Sahasrabudhe, R., Nguyen, O., Beraut, E., Seligmann, W., Fairbairn, C. W., Harri-gan, R. J., McCormack, J. E., Alfaro, M. E., Smith, T. B., & Bay, R. A. (2024). A highly contiguous genome assembly for the yellow Warbler (*Setophaga petechia*). *Journal of Heredity*, 115, 317–325. <https://doi.org/10.1093/jhered/esae008>
- Vallender, R., Gagnon, J., Lovette, I., The, W. Journal, S., & Jun, N. (2016). An intergeneric wood-warbler hybrid (*Mniotilta varia* × *Dendroica coronata*) and use of multilocus DNA analyses to diagnose avian hybrid. *The Wilson Journal of Ornithology*, 121, 298–305.
- Vallender, R., Robertson, R. J., Friesen, V. L., & Lovette, I. J. (2007). Complex hybridization dynamics between golden-winged and blue-winged warblers (*Vermivora chrysoptera* and *Vermivora pinus*) revealed by AFLP, microsatellite, intron and mtDNA markers. *Molecular Ecology*, 16, 2017–2029. <https://doi.org/10.1111/j.1365-294X.2007.03282.x>
- Van Doren, B. M., Campagna, L., Helm, B., Illera, J. C., Lovette, I. J., & Liedvogel, M. (2017). Correlated patterns of genetic diversity and differentiation across an avian family. *Molecular Ecology*, 26, 3982–3997. <https://doi.org/10.1111/mec.14083>
- Walsh, J., Shriver, W. G., Olsen, B. J., & Kovach, A. I. (2016). Differential introgression and the maintenance of species boundaries in an advanced generation avian hybrid zone. *BMC Evolutionary Biology*, 16, 1–18.
- Weir, J. T., Bermingham, E., Miller, M. J., Klicka, J., & González, M. A. (2008). Phylogeography of a morphologically diverse Neotropical montane species, the Common Bush-Tanager (*Chlorospingus ophthalmicus*). *Molecular Phylogenetics and Evolution*, 47, 650–664. <https://doi.org/10.1016/j.ympev.2008.02.004>
- Winger, B. M., Lovette, I. J., & Winkler, D. W. (2012). Ancestry and evolution of seasonal migration in the Parulidae. *Proceedings of the*

- Royal Society B: Biological Sciences*, 279, 610–618. <https://doi.org/10.1098/rspb.2011.1045>
- Zamudio-Beltrán, L., Bossu, C. M., Bueno-Hernandez, A. A., Dunn, P. O., Sly, N. D., Rayne, C., Anderson, E. C., Hernandez-Baños, B. E., & Ruegg, K. C. (2024). Parallel and convergent evolution in genes underlying seasonal migration. *Evolution Letters*, 9(2), 1–20. <https://doi.org/10.1093/evlett/qrae064>
- Zhao, M., Kurtis, S. M., White, N. D., Moncrieff, A. E., Leite, R. N., Brumfield, R. T., Braun, E. L., & Kimball, R. T. (2023). Exploring conflicts in whole genome phylogenetics: A case study within manakins (Aves: Pipridae). *Systematic Biology*, 72, 161–178. <https://doi.org/10.1093/sysbio/syac062>
- Zhao, M., Oswald, J. A., Allen, J. M., Owens, H. L., Hosner, P. A., Guralnick, R. P., Braun, E. L., & Kimball, R. T. (2025). A phylogenomic tree of wood-warblers (Aves : Parulidae): Dealing with good, bad, and ugly samples. *Molecular Phylogenetics and Evolution*, 202, 108235. <https://doi.org/10.1016/j.ympev.2024.108235>
- Zhu, Y., Bai, Y., Yan, W., Li, M., Wu, F., Xu, M., Wu, N., Ge, H. S., & Liu, Y. (2024). A mutation in CCDC91, *Homo sapiens* coiled-coil domain containing 91 protein, cause autosomal-dominant acrokeratoidosis. *European Journal of Human Genetics*, 32, 647–655. <https://doi.org/10.1038/s41431-024-01573-3>

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