Molecular Phylogenetics and Evolution 162 (2021) 107206

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Multiple species and deep genomic divergences despite little phenotypic differentiation in an ancient Neotropical songbird, *Tunchiornis ochraceiceps* (Sclater, 1860) (Aves: Vireonidae)

Nelson Buainain [a,*], Marina F.A. Maximiano [a], Mateus Ferreira [b], Alexandre Aleixo [c], Brant C. Faircloth [d,e], Robb T. Brumfield [d,e], Joel Cracraft [f], Camila C. Ribas [g]

[a] Instituto Nacional de Pesquisas da Amazônia (INPA), Programa de Pós-Graduação em Ecologia, Av. André Araújo, 2936, Petrópolis, Manaus, AM 69067-375, Brazil
[b] Centro de Estudos da Biodiversidade, Universidade Federal de Roraima, Av. Cap. Ene Garcez, 2413, Boa Vista, Roraima, RR 69304-000, Brazil
[c] Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland
[d] Louisiana State University, Department of Biological Sciences, Baton Rouge, LA 70803, USA
[e] Museum of Natural Science and Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA
[f] American Museum of Natural History, Department of Ornithology, New York, NY, USA
[g] Instituto Nacional de Pesquisas da Amazônia, Coordenação de Biodiversidade, Av. André Araújo, 2936, Manaus, AM 69067-375, Brazil

ARTICLE INFO

Keywords:
Biogeography
Greenlet - taxonomy
Passeri
Phylogenetics
Oscines

ABSTRACT

Several bird taxa have been recently described or elevated to full species and almost twice as many bird species than are currently recognized may exist. Defining species is one of the most basic and important issues in biological science because unknown or poorly defined species hamper subsequent studies. Here, we evaluate the species limits and evolutionary history of *Tunchiornis ochraceiceps*—a widespread forest songbird that occurs in the lowlands of Central America, Chocó and Amazonia—using an integrative approach that includes plumage coloration, morphometrics, vocalization and genomic data. The species has a relatively old crown age (~9 Ma) and comprises several lineages with little, if any, evidence of gene flow among them. We propose a taxonomic arrangement composed of four species, three with a plumage coloration diagnosis and one deeply divergent cryptic species. Most of the remaining lineages have variable but unfixed phenotypic characters despite their relatively old origin. This decoupling of genomic and phenotypic differentiation reveals a remarkable case of phenotypic conservatism, possibly due to strict habitat association. Lineages are geographically delimited by the main Amazonian rivers and the Andes, a pattern observed in studies of other understory upland forest Neotropical birds, although phylogenetic relationships and divergence times among populations are idiosyncratic.

1. Introduction

Several new Neotropical vertebrate species have been described recently (Boubli et al., 2019; Charity et al., 2016; Moraes et al., 2019; Rheindt et al., 2020) and many others have been elevated from subspecies to species level through recent taxonomic revisions (Buainain et al., 2017; Krabbe et al., 2020). Birds are one of the best-known groups of organisms in terms of their alpha taxonomy, although a recent study suggests that the current number of species may be highly underestimated, and up to two times as many bird species may exist (Barrowclough et al., 2016). Defining species and their phylogenetic relationships is one of the foundational pieces of knowledge in the biological sciences, and poorly defined or unknown species may hamper conclusions of subsequent studies that depend on accurate estimates of alpha taxonomy, including conservation planning and macroecology (Bortolus, 2008).

Current bird taxonomy in the Neotropics is largely based on historical examination of museum skins (especially by the cataloguers such as J. L. Peters, C. B. Cory and C. E. Hellmayr), whereas our understanding of phylogenetic relationships and the evolutionary mechanisms underlying them are usually based solely on molecular data (especially mitochondrial DNA, mtDNA). Nevertheless, there is a growing notion that coupling phenotypic and molecular data, including mtDNA and nuclear DNA (nuDNA), while considering the organisms' ecological

* Corresponding author.
E-mail address: nnbuainain@gmail.com (N. Buainain).

https://doi.org/10.1016/j.ympev.2021.107206
Received 9 November 2020; Received in revised form 4 May 2021; Accepted 11 May 2021
Available online 18 May 2021

characteristics (natural history) is essential to fully understand species limits and the evolutionary drivers of diversification (Cadena and Cuervo, 2010; Cadena and Zapata, 2021; Yoder et al., 2005; Zamudio et al., 2016). In fact, studies based on multiple kinds of data have often challenged current taxonomy of Neotropical birds, either by discovering new species or by re-arranging higher taxonomic categories (Cadena et al., 2020; Krabbe et al., 2020).

One example of the instability of current Neotropical avian taxonomy is the recent finding based on molecular data, of the polyphyly of the genus *Hylophilus*, which resulted in substantial taxonomic rearrangements, including creation of the monotypic genus *Tunchiornis* (Slager et al., 2014; Slager and Klicka, 2014). The Tawny-Crowned Greenlet *Tunchiornis ochraceiceps* (Sclater, 1860) is a small (11.5 cm, 11.6 g) bird species with discreet colors (mostly greenish or brownish) that occurs in lowland forests (0–1300 m) from Mexico to Ecuador, including most of Amazonia (Howard et al., 2014; Ridgely and Tudor, 2009; Wilman et al., 2014). It occurs in the interior and undergrowth—being rarely seen at the edge or canopy—of humid forest, semi-deciduous forests and adjacent second-growth woodland, although it is more common in primary forest (Antongiovanni and Metzger, 2005), where it forages for invertebrates in mixed species flocks (Howell and Webb, 1995; Ridgely and Tudor, 2009; Stiles et al., 1989).

The species is polymorphic and different authors have recognized morphological differentiation of some populations based on subtle differences in plumage coloration and morphometric characters, leading to the recognition of 10 subspecies (Fig. 1A) (Dickinson and Christidis, 2014). Although several taxa have been described as full species, most authors consider them as subspecies, following Hellmayr (1935), due to their overall morphological similarity and the fact that they are allo-replacements of each other, factors that could be interpreted as evidence against complete reproductive isolation under the Biological Species Concept (BSC) (Mayr, 1963). Since Hellmayr (1935), skin collections have grown significantly in size and the quality of the metadata associated with more recent specimens has improved (Remsen, 1995), providing a clearer basis for evaluating morphological differences among lineages and species limits. Yet, no recent morphological assessment has been performed, and vocal characters have not been systematically evaluated in *Tunchiornis* (but see (Boesman, 2016)).

Previous molecular studies have shown that *T. ochraceiceps* is an old lineage (~10 Ma) with deep intraspecific mtDNA divergences (Milá et al., 2012; Naka and Brumfield, 2018; Slager et al., 2014), suggesting that its diversity might be underestimated by the current taxonomic arrangement. Additionally, *T. ochraceiceps* has an intercontinental distribution, and its divergence time encompasses a period of intense landscape changes in the Neotropics, which is thought to have driven diversification across a wide spectrum of organisms. These changes include the uplift of the Isthmus of Panama and northern Andes, the establishment of the current Amazonian drainage system and climatic

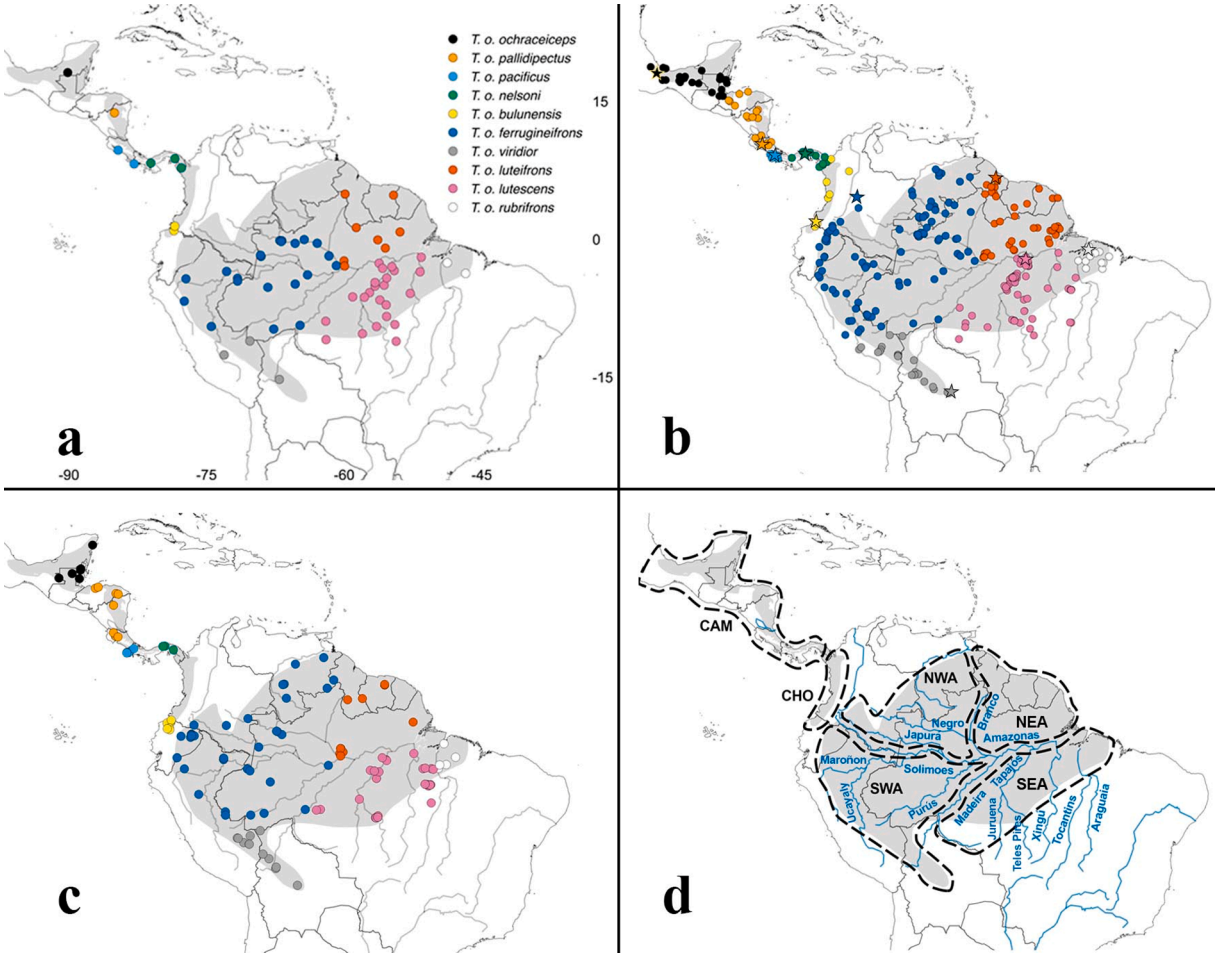

Fig. 1. Study sampling design used to assess the genetic and phenotypic variations of *Tunchiornis ochraceiceps*. (a) Samples used in genomic analyses, (b) skin specimens used in plumage coloration and morphometric analyses, stars represent the type material specimens (type locality) (c) recordings used in vocalization analyses; (d) name of main Amazonian rivers (blue) and general geographic regions (dashed lines) used as reference when describing variations in the text (d) - Central America (CAM), Choco (CHO), northeastern (NEA), northwestern (NWA), southwestern (SWA) and southern (SEA) Amazon. Samples are colored according to the current taxonomic hypothesis (subspecies). Gray area is the currently known species distribution based on BirdLife International (2019). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cycles of the Pleistocene (Haffer, 1969; Hoorn et al., 2010; Jaramillo, 2018). Hence, we expect that (1) the evolutionary history of *T. ochraceiceps* is at least partially congruent with some of these events, contributing to understanding the relationships between landscape change and biotic evolution in the Neotropics, and (2) that genetic divergence within the species will be larger and more spatially structured than phenotypic variation indicates.

Here, we assess species delimitation, phylogenetic relationships and the evolutionary history of *T. ochraceiceps*, employing an integrative approach that includes plumage coloration, morphometrics, vocalization and genomic data. We use the revision of genetic and phenotypic variation in combination with well supported phylogenetic relationships to discuss the clade's biogeographic history and the decoupled evolution of genomic and phenotypic characters, relating these patterns to habitat association and the diversification processes common to other Neotropical groups of organisms.

2. Methods

2.1. Sampling

We examined 625 skins and 152 vocal recordings (Fig. 1). For genomic analyses we obtained 67 fresh tissue and two toepad samples from historical skin specimens (Fig. 1). Samples represented all currently described taxa for the species and covered most of its currently known geographical distribution according to BirdLife International (2019) (Fig. 1). One tissue of *Pachysylvia hypoxantha* (Pelzeln, 1868) was used as an outgroup. We examined photographs of the type material and original descriptions of all taxa. A complete list of the examined material can be found in Supplementary Material 1.

2.2. Genomic data acquisition and processing

We extracted DNA using the DNeasy kit (Qiagen Inc.) and sent the extracts to RapidGenomics® (Gainsville, FL) for sequencing, using a probe set targeting 2,321 Ultra Conserved Elements (UCE) loci (Faircloth et al., 2012; Zucker et al., 2016). UCEs have been shown to provide robust phylogenetic information both at deeper and shallower divergence levels (Faircloth et al., 2012; Smith et al., 2014).

We processed the raw data from UCEs using Phyluce (Faircloth, 2016) incorporating the allele phasing steps from Andermann et al. (2018). Sequences with adapter contamination, and those of low-quality, were trimmed using illumiprocessor (Faircloth, 2013). We used Itero v.1.1. (https://itero.readthedocs.io/en/latest/purpose.html), an iterative reference-based approach, to assemble contigs of our four best quality samples according to number and length of reads. We used the 2,321 UCE probe sets as reference (Faircloth et al., 2012; Zucker et al., 2016) for the assemblies and performed 5 iterations of reference guided assembly per sample. Contigs from the different samples were aligned and edge-trimmed with MAFFT using default settings (Katoh and Standley, 2013). The concatenated alignments were imported in Geneious R7 (Kearse et al., 2012) and we manually trimmed some loci to remove remaining areas of low alignment quality. After cleaning, we generated a consensus sequence to use as reference in the alleles phasing pipeline (Andermann et al., 2018). Accordingly, clean reads were mapped to the reference using BWA 0.7.17 (Li and Durbin, 2009) and alleles were sorted and phased using SAMtools 0.1.19 (Li et al., 2009) and Picard (http://broadinstitute.github.io/picard). We then aligned and edge-trimmed the two phased alleles using MAFFT. Finally, we used the script of Andermann et al. (2019) to merge the two alleles of each individual using the IUPAC ambiguity codes for heterozygous sites. The final sequence data matrix was produced with alignments present in at least 90% of samples and contained 2,267 loci.

We applied a python script (snps_from_uce_alignments.py, available from: github.com/tobiashofmann88/snp_extraction_from_alignments/) from Andermann et al. (2019) to the phased alleles sequences to extract one biallelic Single Nucleotide Polymorphism (SNP) per locus (to reduce bias caused by linkage-disequilibrium) not allowing for missing data. This SNP dataset was used in the population structure and estimated effective migration surface analyses.

2.3. Mitochondrial DNA

We used MITObim v.1.9.1 (Mitochondrial baiting and interactive mapping) (Hahn et al., 2013) to retrieve and assemble the mitochondrial genome from our UCE raw data. The mitogenome of *Vireo olivaceous*, the closest relative to *T. ochaceiceps* with an annotated mitogenome available, was retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (Accession number: NC_024869.1) and used as the reference for MITObim assembly. Maximum iteration was set to 100. Final contigs were imported to Geneious R7 (Kearse et al., 2012), and mapped back to each of the 13 coding sequence (CDS) regions of the *V. olivaceus* mitogenome to annotate and extract those genes. The contigs of the 13 CDS genes of all samples were then aligned using the Geneious alignment tool.

2.4. Population structure

We used sNMF (Frichot et al., 2014) to infer population genomic structure and perform individual assignment to these populations based on the UCE SNP data. We evaluated the optimal number of populations (k value) from 1 to 12 (the number of described subspecies + 2) with 100 runs per k, 10,000 iterations; we employed four alpha regularization parameter values: 1, 10, 100, 1000. We selected the run with the smallest cross entropy value among all alpha and k values. Because population structure can be hierarchical (Janes et al., 2017; Pritchard et al., 2000), once we detected the optimal k, we resampled SNPs within each population initially delimited by the software, and ran sNMF again using only the samples from each of those populations separately. This was repeated until we detected no additional structure.

We used EEMS (Estimated Effective Migration Surface) (Petkova et al., 2015), a spatially explicit approach that evaluates distribution of genetic structure and detects regions with higher or lower-than-average historic gene flow than expected from an isolation-by-distance model. This approach allows visualization of geographic barriers and corridors to effective migration between populations/samples. We calculated Euclidean genetic distances between geo-referenced individuals with the function "dist.genpop" in ADEGENET2.0 (Jombart, 2008) and the method "Roger's distance" (Rogers, 1972). We used the species distribution polygon provided by BirdLife International (2019)—slightly modified to eliminate disconnected (*e.g.* islands) and other problematic areas (areas too narrow to allow connection between neighboring demes)—as a background for demes and to project the interpolated effective estimated migration surface. We used 1,000 demes, which provided reasonable resolution for the amount of points used, and grid cells that were small enough to connect demes within our polygon. We conducted three independent runs, with 15,000,000 MCMC (Markov chain Monte Carlo) generations, discarding the first 14,000,000 as burn-in, sampling every 5,000 MCMC. We performed additional runs in each major region (after confirming monophyly of populations, see Section 2.5 for the phylogenetic methods used) in a similar fashion to that used for sNMF analyses (resampling SNPs) to infer more detailed effective migration within and between major regions. Stationarity of the run was visually inferred by plotting the MCMCs log posteriors with the rEEMSplots script provided by the program. All runs were combined to generate the final results.

2.5. Phylogenetic analyses based on UCE data

Both concatenated and species tree analyses were employed to infer phylogenetic relationships, without using the prior information from the population structure results. First, we employed IQ-TREE (Nguyen et al.,

2014) to produce a concatenated tree based on maximum likelihood estimation (MLE) with the concatenated sequence data. Best-fitting substitution model was inferred in *ModelFinder* (Kalyaanamoorthy et al., 2017) and node support was assessed using two methods, Ultra-Fast Bootstrap (UFBoot) (Hoang et al., 2017) and SH-aLRT branch test (Guindon et al., 2010) with 1000 replicates each. Reliable clades have node support $\geq$ 95% for UFBoot and $\geq$ 80% for SH-aLRT (Nguyen et al., 2014). Second, we used Exabayes (Aberer et al., 2014) to produce Bayesian Inference (BI) trees with the same dataset. We performed four independent runs with 2,000,000 MCMC each, sampling every 500 generations. Stationarity and convergence of the independent runs were inferred using Tracer 1.6 (Rambaut et al., 2014). All parameters had Effective Sample Size (EES) values above 200 assuring sampling adequacy of the analyses. A consensus tree was generated combining the four runs. Finally, we used the multi-species coalescent species tree (ST) approach SVDquartets implemented in PAUP* 4.0a (build 165) (Swofford, 2002) with partitioned UCE sequences, which takes into account the history of the different genes independently. Samples were not assigned to species *a priori*. All possible quartets were evaluated. Bootstrap values were estimated using 10,000 replicates.

2.6. Time calibrated trees based on mtDNA data

We used BEAST 2.6.0 (Bouckaert et al., 2019) to estimate phylogenetic relationships and divergence times based on 13 CDS mitochondrial genes. We used PartitionFinder2 (Lanfear et al., 2017) to find the best fit substitution model for each gene, not partitioning by codon position because initial runs with data partitioned by codon failed to stabilize/converge. We used an unlinked relaxed clock model, which allows independent rates among genes and variable rates along the tree branches. For time calibration we used the substitution rate of 2.1% per myr (Weir and Schluter, 2008), using a more strict prior (normal distribution, 0.0105 mean and 0.0034 SD) for the CYTB gene and a more relaxed prior (mean 0.01 and 0.004 SD) for the other coding genes, because their mutation rates are more variable but still close to 2% (Campillo et al., 2019). A birth-death speciation model was used, because extinction may play a significant role in time scale of diversification of the studied group. We initially set a uniform prior for birth-death rates with lower and upper limits of 0 and 1. We then experimented with more relaxed priors increasing the upper limits to 10, 100 and 1000 and we also used an exponential distribution around the same values. Because we consistently estimated the same values for the birth-death rates, regardless of the experimental prior used, for the final run we set a more strict prior with normal distribution and mean 0.26 and 0.3 SD for the BirthRate and 0.94 mean and 0.1 SD for DeathRate.

We performed three independent runs with 200,000,000 MCMC and discarded 10% as burn-in. Stationarity and convergence of runs were inspected using Tracer 1.6 (Rambaut et al., 2014). All parameters had ESS values above 200. Runs were then combined with LogCombiner (Rambaut and Drummond, 2019a) and the maximum clade credibility tree was extracted with TreeAnnotator (Rambaut and Drummond, 2019b).

2.7. Plumage coloration and morphometrics

We used Smithe (1975) as a color guide for specimen plumage descriptions. Nomenclature of plumage parts followed Proctor and Lynch (1993). Because some of the characters were continuous in variation (*e. g.* amount of buff on the chest), we defined categories and classified specimens accordingly. Because classifications into these categories can be somewhat subjective, we also assessed morphological diagnoses of populations through direct comparison of a large series of specimens archived in different museums that were loaned to the American Museum of Natural History, New York. Simultaneous direct comparison of hundreds of specimens allowed better assignment to the categories defined, and better evaluation of some subtle plumage variations proposed as diagnoses, which could be otherwise difficult to infer due to the lack of precision of some colors in the color catalog. We used QGIS 2.18 (Qgis Development Team, 2016) to map plumage character and look for geographic variations and fixed characters.

We used a digital caliper (150 $\times$ 0.01 mm) to collect the following morphometric measurements (Baldwin et al., 1931): left wing (closed wing, "chord"), tail (central rectrices) and bill length (total culmen), bill height (at proximal portion of nostrils) and width (at base). All measurements were taken by NB. To remove the scale differences between variables, we standardized the data with the function "decostand" ("standardize") in the Vegan package (Oksanen et al., 2019) in the R environment (R Core Team, 2019), prior to multivariate analyses. We tested the number of clusters in the morphometric data using a model-based clustering algorithm, mclust5 (Scrucca et al., 2016) in R (R Core Team, 2019). The most likely number of clusters was determined using Bayesian Information Criteria (BIC). We then inspected the classification matrix to check if clusters were biologically meaningful (*e. g.* different sexes, taxa or geographical groups). Additionally, we performed a Principal Component Analysis (PCA) with the morphometric measurements using the function "prcomp" in R (R Core Team, 2019) to visualize structure in the dataset without prior information on groups.

We plotted measurement values using the main genomic groups (clades) for comparison. Descriptive statistics (mean m, standard deviation sd, minimum min and maximum max) are provided for each group delimited at the end of all analyses. We discarded data from immature individuals for both the coloration and morphometric analyses, although we used these individuals to re-describe the final delimited taxa. Because our question whether there were diagnosable differences (no measurement overlap) between groups, and not if they were statistically different, we did not perform variance tests.

2.8. Vocalizations

We used RavenPro1.5 (Center for Conservation Bioacoustics, 2014) to import vocal recordings and to obtain sonograms and oscillograms for qualitative and quantitative analyses of songs. We followed the nomenclature of vocal traits proposed by Catchpole and Slater (2008), in which phrases consist of a series of units that occur together in a particular pattern. These units were referred to as syllables. We evaluated the following characters: song duration, number of syllables and modulation of each syllable. We also measured five frequency characters (min 5%, max 95%, bandwidth 90%, central and max frequencies) from both the whole song and for each syllable separately. Durations of phrases and syllables were measured from oscillograms due to its higher numerical precision (Köhler et al., 2017). All spectrograms were analyzed using the Hann window type with a frequency resolution of 512 samples.

We used QGIS 2.18 (Qgis Development Team, 2016) to map qualitative characters. Quantitative characters measured from spectrogram (duration and frequency) were standardized before being used in multivariate analyses. Statistical procedures were the same used for morphometric analyses (mclust, PCA and scatter plots). Because vocalizations were variable in number of syllables, we could not quantitatively compare each syllable separately. Thus, we used the number of syllables, and frequency and time characters measured from the whole song (regardless of syllable number), which were comparable among all vocalizations.

3. Results

3.1. Population structure

Population structure analyses with sNMF initially detected six groups (Fig. 2) but two of the groups had substructure (a2 and b2). East of the Andes, clusters were delimited by large Amazonian rivers (Branco, Solimões/Amazonas, Madeira, Tapajós and Xingú), while western

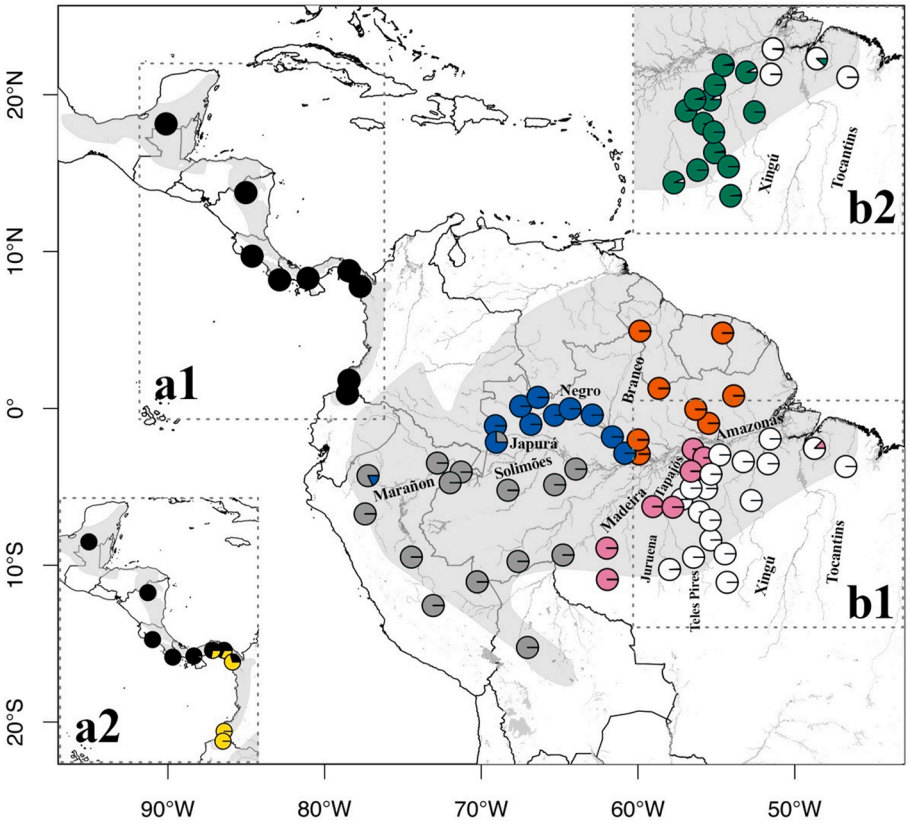

Fig. 2. Population structure in *Tunchiornis ochraceiceps* inferred with sNMF using SNPs extracted from the UCE data. The first round detected six structured populations corresponded to the different colors. Pie charts were colored proportionally to their ancestry coefficients (probability to belong to one or more group) of each sample. Subsequent analyses ran in each of the six populations detected at first, showed that two groups (a1, black and b1, white) had substructure (a2 and b2). The dataset for the first round contained 1,889 SNPs, while the dataset used in the subsequent rounds, a and b, had 902 and 2,058 SNPs respectively.

Andean clades were spatially congruent with the Central America (CAM) and Chocó (CHO) regions (see Fig. 1d for general geographic areas and rivers referenced). Admixed genotypes were found between populations: 1) from CAM and CHO in Panama (Fig. 2a); 2) from northwestern and southwestern Amazonia (NWA and SWA) at the Solimões-Japurá interfluve and at the extreme west of Amazonia in northwestern Peru (close to the Marañon-Santiago river confluence); 3) from both banks of the Tapajós River (Fig. 2b) in southeastern Amazonia (SEA); and 4) from both banks of the Xingú River, also in SEA.

The EEMS analysis confirmed that large Amazonian rivers and the Andes are strong barriers to effective migration between most populations (Fig. 3). In contrast, high effective migration was detected between *trans*-Andean populations. We also observed a previously undetected (by sNMF) barrier related to the upper Amazonas/Marañon River confluence in northwestern Peru. The structure we detected between populations from opposite banks of the Xingú River appears to be less strong than what we observed across other rivers. When SNPs were resampled for each region separately, two regions had additional patterns revealed. First effective migration was still high in CAM but two regions with reduced migration were found between the CAM and CHO (Fig. 3, a2), a stronger (lower values of effective migration) one in eastern Panama and a less effective one in western Panama. Second, in western Amazonia, the Japura appeared to be more evidenced as a barrier, and there was a possible additional structure associated with the mid Ucayali river (Peru) (Fig. 3, b2).

3.2. Phylogenetic analyses

All analyses of the UCE dataset resulted in the same basic topology (MLE, BI and ST) with minor variations in node support and relationships among tips within the main clades (Fig. 4 and Supplementary Material 2). The phylogenetic analyses showed that *T. ochraceiceps* is composed of multiple well-supported lineages, and clade composition is almost entirely congruent with the population structure of the groups.

The first split separates the western (CAM, CHO, NWA and SWA) and eastern (NEA and SEA) lineages. The western clade is divided into trans- and *cis*-Andean lineages. The *trans*-Andean lineage is further divided in CAM and CHO (although one sample from eastern Darien, eastern Panama, in the CHO region, grouped with samples from CAM). In western Amazonia, the first split separates lineages south (SWA) and north (NWA) of the Solimões river. Within NWA, there are three lineages in the phylogenetic tree, separated by the upper Negro and Japurá rivers, although the single individual sampled at the Japurá-Solimões (light orange circle) interfluve has an admixed genotype (Fig. 2). The Japurá-Negro clade was only recovered in the BI and MLE trees (but not in the ST) and with overall low node support. Within SWA, the single sample in northwestern Peru with an admixed genotype is sister to all the remaining samples (purple and dashed circle "b" in the map, Fig. 4). Within the eastern clade, the first split separates the samples occurring north (Guyana Shield, northeastern Amazonia, NEA) and south (Brazilian Shield, southeastern Amazonia SEA) of the Amazon River. In SEA, the first split is between lineages from opposite banks of the Tapajós River, with eastern clade further subdivided by the Xingú River. The multispecies coalescent method (species tree) produced the exact same topology, but the Xingú-Tapajós clade (green) had low support (Supplementary Material 2).

The phylogenetic tree obtained from analyses of the mtDNA dataset had a similar topology to the UCE trees, but with some relevant discordances. In the mtDNA tree: 1) the *trans*-Andean lineages are more structured and slightly geographically rearranged compared to the UCE tree. Samples from eastern Panama (red dashed circle "a" in Fig. 4) form a distinct clade (red in Fig. 5), sister to samples from CHO, and not to other samples from CAM; 2) the single sample from northwestern Peru ("b" in Fig. 4, and purple in Figs. 4 and 5) is sister to the whole NWA lineage (and not to SWA), but with low support; 3) the single Solimões-Japurá sample (light orange in Figs. 4 and 5) is sister to a clade formed by all remaining SWA and NWA samples instead of sister to NWA; and 4) composition of the two clades north of the Japurá river (light and dark

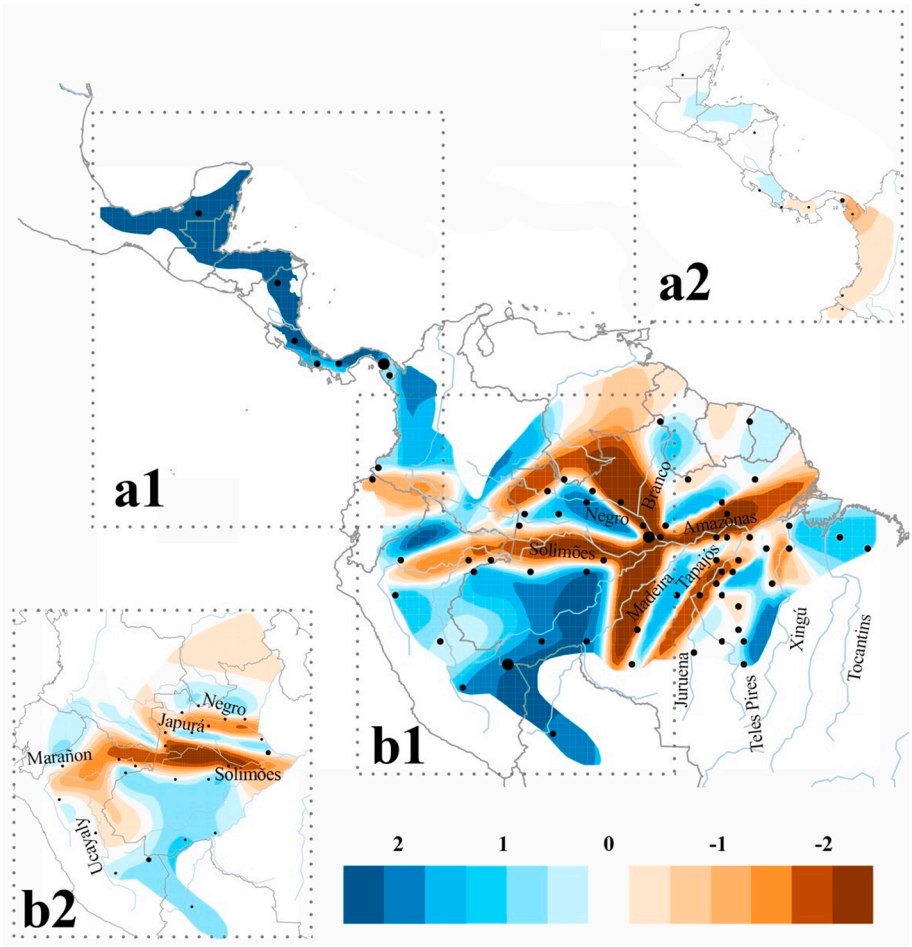

Fig. 3. Estimated Effective Migration Surface (EEMS) plots of *Tunchiornis ochraceiceps*. Colors represent effective migration rates (m) on the $\log_{10}$ scale between genomic samples. Blue color represents areas with more migration (corridors), dark orange represent areas with less migration (barriers), and white represent areas consistent with the null model (isolation by distance). Size of black circles represent number of samples in the same deme (sampling site). Light blue lines represent river courses. Dashed rectangles a2 and b2 show results of EEMS runs for each region (a1 and b1) separately, after resampling SNPs from those samples only. The dataset for the first round contained 1,889 SNPs, while the dataset used in the subsequent rounds, a and b, had 902 and 1,932 SNPs respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

blue) is slightly different from the UCE tree, although short branches within that whole lineage suggests a relatively low differentiation among them.

3.3. Molecular dating

The initial divergence time between the western and eastern lineages was estimated to be late Miocene (9 Ma, 95% HPD = 6.1–12.7 Ma). In the eastern clade, the split north and south of the Amazon River is estimated at 7.5 Ma (95% HPD = 5–10 Ma). The divergences between lineages from opposite banks of the Tapajós and Xingú rivers are much younger, occurring during the Pleistocene (2.2 Ma, 95% HPD = 1.5–3.2 Ma and 0.8 Ma, 95% HPD = 0.5–1.1 Ma, respectively). In the western clade, the split between cis- and *trans*-Andean lineages dates back to the Pliocene (4.6 Ma, 95% HPD = 3.1–6.5 Ma). The divergence between the CAM and CHO lineages is estimated at 1.8 Ma (95% HPD = 1.2–2.2 Ma) and diversification of the western Amazonian lineages (NWA and SWA) starts at 1.1 Ma (95% HPD = 0.7–1.5 Ma).

3.4. Plumage coloration

Only one plumage coloration character is geographically fixed, yet unmistakably distinguishing three different groups. All western specimens (CAM, CHO, SWA and NWA) have extended rufous (combination of color coded as 140 + 132 in Smithe (1975)) to ochraceous (24 + 38) forehead and crown (further referenced as crown) which are variable from darker to paler tones (Fig. 6a,b). Specimens in SEA have the rufous restricted to the lores, forehead and supercilium, whereas specimens in NEA have either a plain olive green (48 or 49) with no rufous/ochraceous crown, or a vestigial and discreet whitish or light yellow (157) lore, which can be more or less extended towards the eye. In general, NEA specimens have overall olivaceous (48/49) and buff (24) tones, visible both on dorsum and ventrum and whitish throat; SEA have more yellow and buff ventrum (including throat), buff also over the dorsum, which sometimes have gray (86) suffusion especially over the crown; and the CAM, CHO, SWA and NWA specimens are highly variable in coloration of both ventrum and dorsum (see below) but have predominantly gray throat. However, despite those trends these characters (except the pattern of front coloration) tend to overlap among most groups.

Other characteristics are not fixed but present noteworthy geographic variation. Information from specimen tags indicates that iris color is white/pale in western groups and brown/dark in eastern groups, although four adult specimens (inferred from gonads and skull ossification) had a different iris color than expected for their locality (Fig. 6c). Specimens from northern CAM typically have a paler crown (more ochraceous) with yellow on lore, brown (37/129) dorsum (including tertiaries and rectrices), and yellow (157) ventrum heavily buffed; *trans*-Andean specimens in the southern part of CHO, typically have a darker tone rufous crown, olive-green dorsum, tertiaries and rectrices variable in brown and green, and grayish or yellow ventrum with no buff (Figs. 6 and 7). However, specimens from the intermediate portion of their distribution (especially in the Panama Canal area) seem to be morphologically intermediate between the two forms in most characters, especially coloration of ventrum and dorsum and crown. Therefore, the geographic extremes are easily distinguishable but there is either a smooth cline or a large area with intermediate phenotypes, making it hard to delimit the geographic boundaries between both groups.

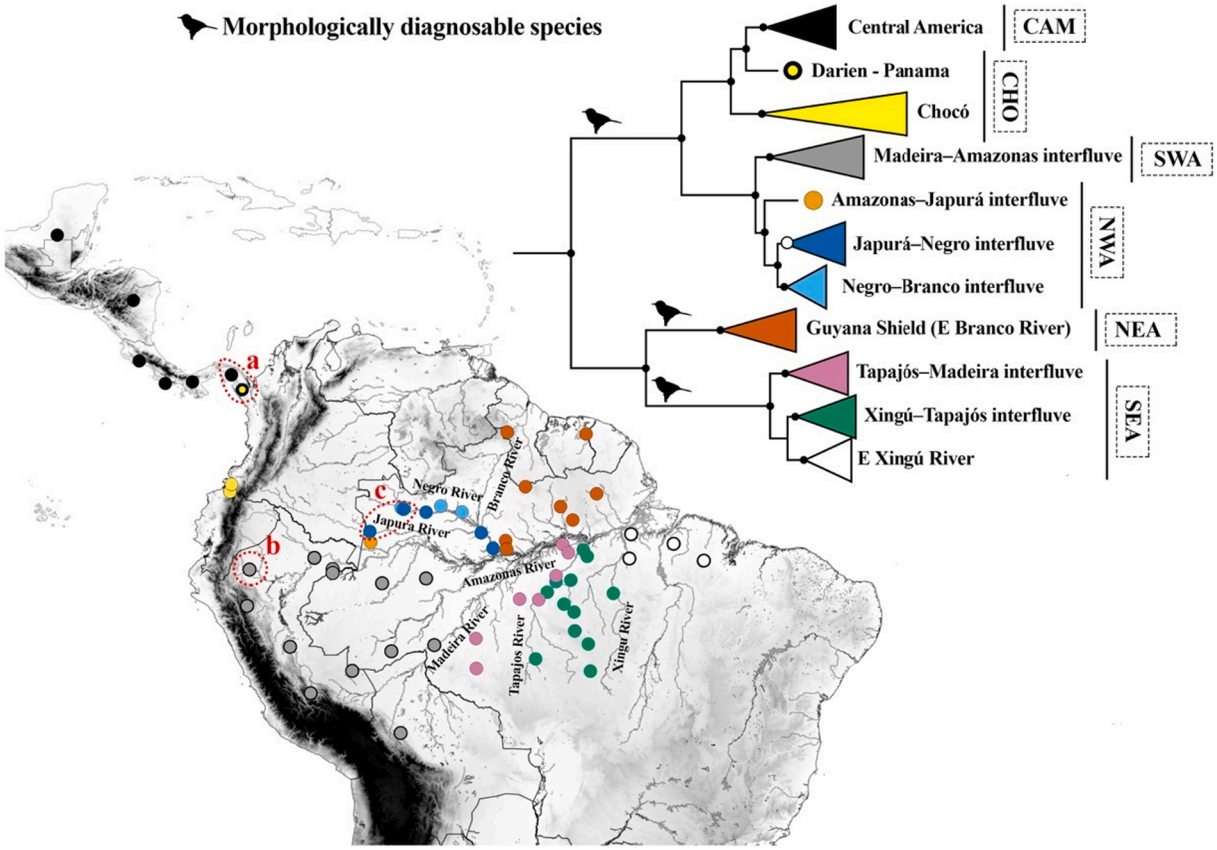

Fig. 4. Bayesian tree of *Tunchiornis ochraceiceps* inferred with ExaBayes and the 2,267 concatenated UCE dataset. Node supports with posterior ≥ 0.95 are represented as black circles and nodes with ≤ -0.95 are represented as white circles. The deepest lineages correspond to the clearly morphologically distinguishable taxa (bird silhouette over tree branch). Terminals with circles are represented by a single sample each. Red dashed areas indicate samples that grouped in distinct clades in the mitochondrial and nuclear trees (see results section). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

NWA specimens, especially in the upper Negro River and Venezuela, are overall more similar to CHO specimens, having olive-green dorsum and darker rufous crown, but with grayish and olive-green ventrum (usually more yellow on CHO). SWA specimens, especially in southern Peru and Acre, Brazil, have paler crown (ochraceous), lighter green (yellowish olive-green, 50) dorsum (including tertiaries and rectrices) and in general have more yellowish strikes on their ventrum (similar to the CHO). However, many specimens had intermediate conditions (for crown, ventrum and dorsum coloration) and in a direct comparison of a large series of specimens from SWA, NWA and CHO, it was not possible to clearly differentiate them based on any of the characters and methods used (Figs. 6 and 7).

Within the SEA, there may be a greater frequency of the predominantly yellow with less buffy ventrum morphotype west of the Tapajós River compared to specimens east of that river, but it is very clear that this character is also not constant (Fig. 7a).

Additional photographs and brief discussions on the overlap and variation on some plumage characters can be found in the Supplementary Material 3.

3.5. Morphometrics

Clustering analysis (mclust) selected VVE (ellipsoidal, equal orientation) with two components (clusters) as the best fit model for the data (Supplementary Material 4). However, no clear spatial separation between samples belonging to the two clusters can be seen in the classification graph (Supplementary Material 4). Comparing the classification matrix with subspecies, genomic group or sex matrices of samples suggests there is not easily discerned biological meaning for mclust's classifications (Supplementary Material 4). PCA shows a similar result with no structure in the data (Supplementary Material 4). Plotting the measurements values confirms that there is a high overlap between males and females from the groups delimited by genomic analyses (Supplementary Material 4), corroborating the lack of morphometric differences between populations and sexes. A table with the summary statistics (number of samples, mean, maximum and minimum values of each character) can be found in Supplementary Material 4.

3.6. Vocal analyses

The qualitative analysis shows no diagnostic vocal characters for any of the populations of *T. ochraceiceps* (Fig. 8a–d). Some variation, however, is noteworthy and a relatively reliable indicator of population assignment. Most recordings are composed of a very simple song type with a single phrase containing one whistled syllable between the frequencies of ~ 2–4 KHz (for descriptive statistics regarding song characters see Supplementary Material 5) and variable in frequency modulation (descending, flat or ascending along time) (Fig. 8b). The NEA populations east of the Branco River, predominantly produced a distinct song with two whistled syllables, between the frequencies of ~ 2.7–3.8 KHz, being the last syllable descending in modulation. However, it is clear that these birds can eventually produce one syllable vocalizations (ML 80429, NB observation during field work around Manaus, AM, Brazil) that are apparently indistinguishable from songs with one syllable from the other populations. One recording from eastern Venezuela (ML70336), initially attributed to the NWA population, has a three syllable song and most likely corresponds to the northwestern-most record of the NEA group.

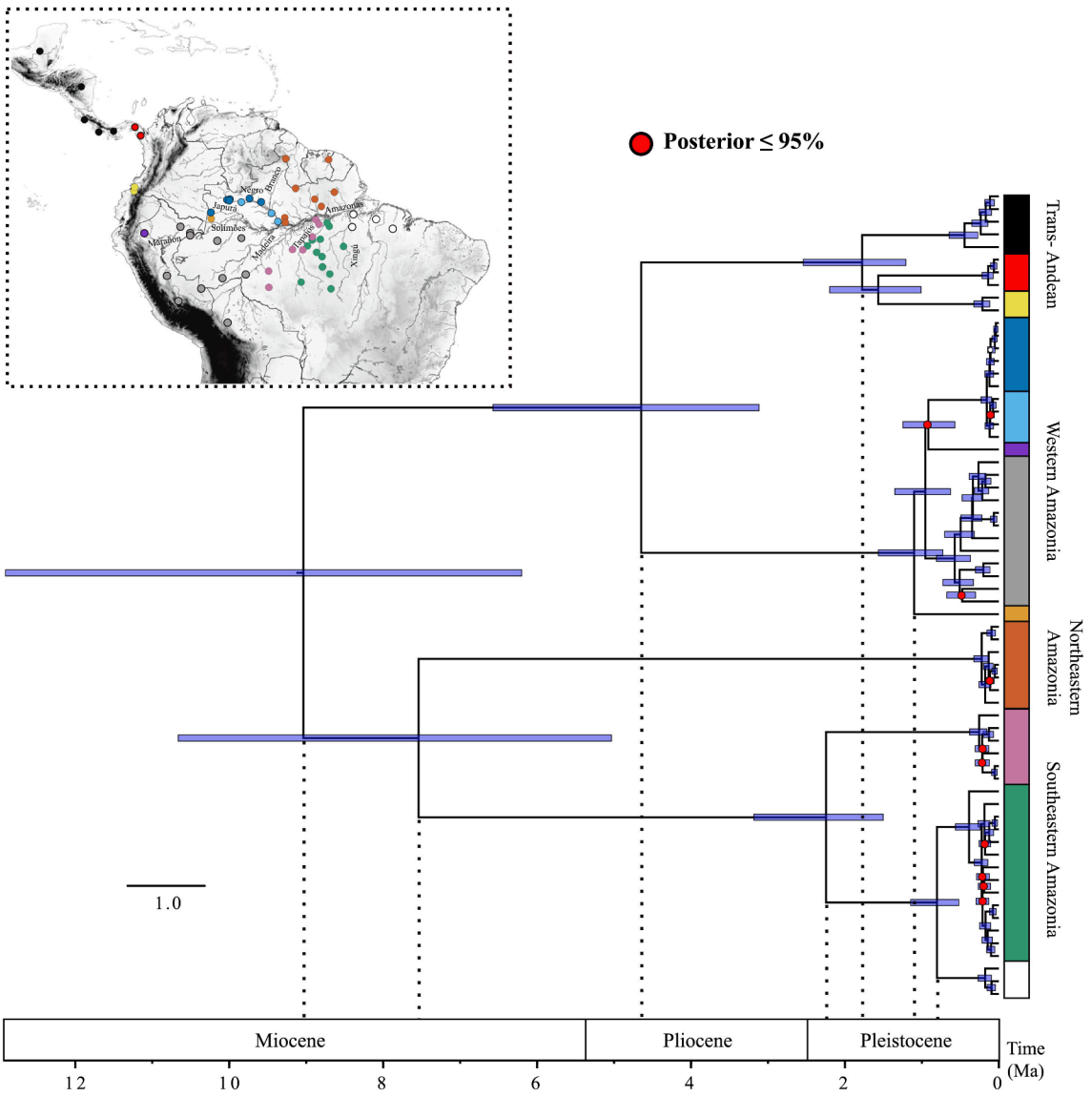

Fig. 5. Bayesian phylogenetic time tree of *Tunchiornis ochraceiceps* inferred in BEAST2 using the 13 CDS mitochondrial genes. The final result is a consensus tree generated from three independent runs combined. All nodes had posterior ≥ 0.95 except the ones colored in red. Error bars represent the 95% HDP (High Posterior Density) of node ages. Map inside dashed rectangle represent geographic position of samples used in the analyses, colored by clade in this tree. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

All recordings from the Madeira-Tapajós interfluve, in SEA, contained a distinct song with two to three syllables, between the frequencies 2.8–3.5 KHz, but with ascending second and third syllables, thus different from the NEA population in modulation. However, songs with one and two syllables were recorded in the Juruena-Teles Pires (whose confluence form the Tapajos River) interfluve. This area lies in the limit between the single and multiple syllable song populations in SEA. Thus, although some interesting difference in frequency of vocal pattern exist, it is not possible to unmistakably distinguish populations by their songs.

The mclust analysis detected two clusters: one containing the multiple-syllable songs from populations east of the Branco River and from the Madeira to Teles Pires interfluvia and one cluster containing single-syllable songs including single-syllable vocalizations from the "multiple-syllable song" populations (Supplementary Material 5). Similarly, the PCA shows differentiation with overlap between groups, which correspond to these songs "atypical" for their area (Fig. 9h). Both PCs explained together 88% (PC1 = 55%, PC2 = 34%) of the total variation in the data. The variables most related with PC1 were max, center, 5% and 95% frequency and with PC2 were duration, bandwidth 90% and number of syllables.

The plots for the quantitative vocal characters show overlap among populations in all characters (Fig. 9). The multiple syllable songs are clearly longer in duration (Fig. 9a) and have greater Bandwidth 90% (Fig. 9e) values, especially in the NEA population. The overlap in measurements between populations with multiple-syllable songs with the remaining populations can be attributed to the one-syllable songs that are detected (Fig. 9b). The *trans*-Andean populations tend to have higher frequency (higher pitch) songs (Fig. 9c–g), although there is much overlap with other populations. A table with the summary statistics (number of samples, mean, maximum and minimum values of each character) can be found in Supplementary Material 5.

4. Discussion

Our analyses indicate that *T. ochraceiceps* comprises several different lineages. Some have corresponding subtle but fixed phenotypic differentiation, whereas most do not. This phenotypic conservatism is

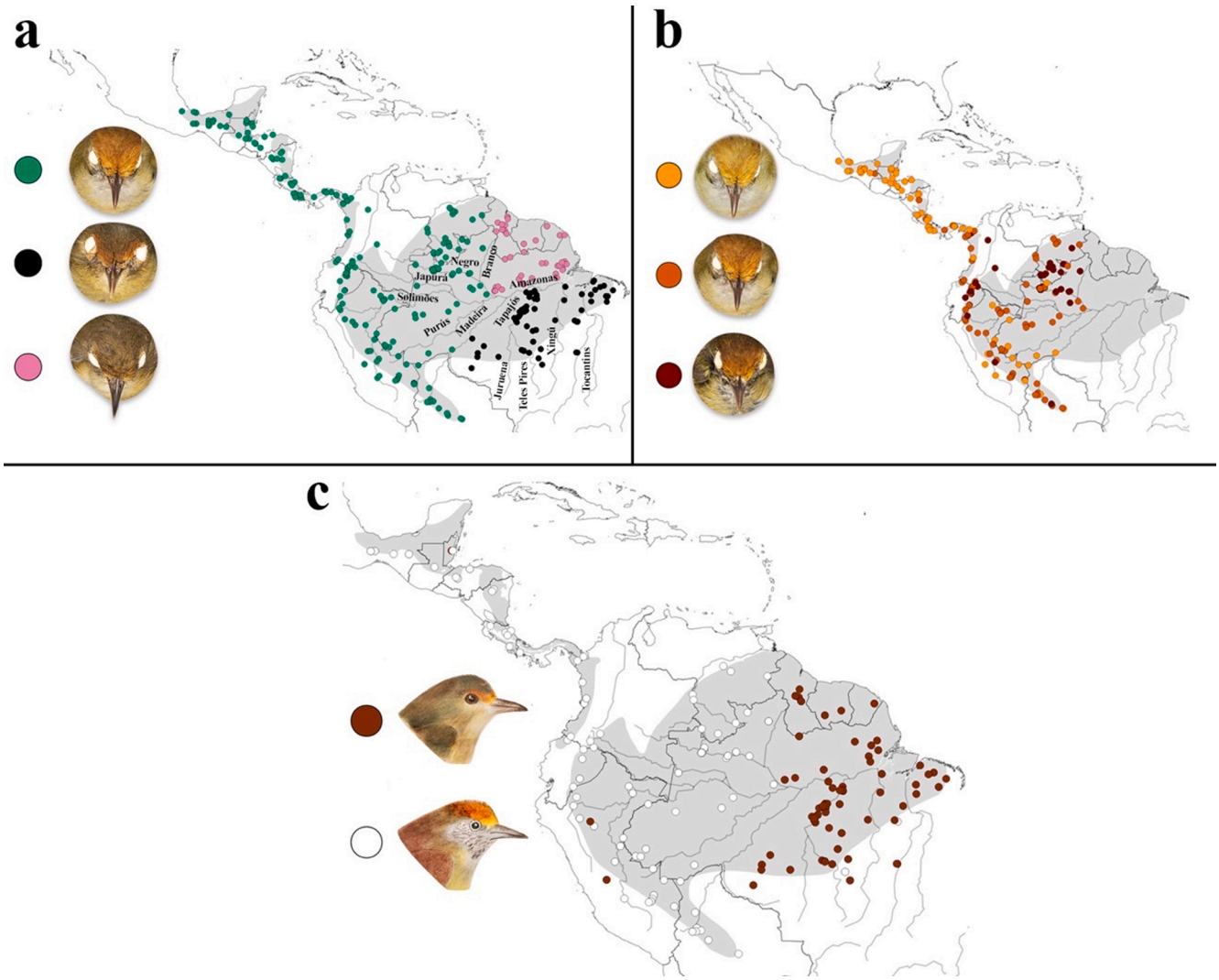

Fig. 6. Geographic variations of plumage color/pattern in *Tunchiornis ochraceiceps*. Numbers in brackets correspond to color codes in Smithe (1975). Colors in parentheses correspond to the colors of the symbols plotted in the map. (a) Extension of frontal pattern: rufous (variable in tone) and extended, forming a crown (green); rufous rufous (variable in tone) but restricted to the lores and around the eyes (black); and absent or vestigial in whitish or light yellow colors [157] (pink). (b) Color variation of the front in specimens with extended rufous/ochraceous front: paler/ochraceous [24 + 38] (light orange); intermediate [140] (medium orange); darker/rufous [140 + 132] (brown). (c) Iris color: pale [specimen's tag information, not color referenced], (white) and dark (brown). Illustration (c) by Nelson Buainain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

surprising considering the relatively old history of the complex when compared to other Neotropical birds (Silva et al., 2019). We found few but nonetheless interesting discordances between mitochondrial and genomic datasets, which suggests their evolutionary histories might have been complex in some areas. The distributions of lineages are mainly delimited by large Amazonian rivers and the Andes, a classic pattern already described for other organisms (Cracraft, 1985; Cracraft et al., 2020; Wallace, 1852), although phylogenetic relationships among them are idiosyncratic. Below, we describe our proposal of revised species limits for *Tunchiornis*, applying the International Code of Zoological Nomenclature (ICZN, 1999), and discuss some of the mechanisms that might underlie the temporal and spatial pattern of their diversification, the discordance between mtDNA and nuDNA phylogenetic trees, and the decoupling between phenotypic and genotypic patterns.

4.1. Species delimitation

The two deepest nodes in our phylogeny (Fig. 4) separate three unmistakably distinct species that can be readily differentiated by a single plumage character: the coloration pattern of the front and crown (Fig. 6a). The first split also separates the white from the brown-eyed clades (even though this character is not fully fixed, Fig. 6c) (Todd, 1929), which roughly correspond to a west-east geographic separation. Then, the eastern clade is separated into two lineages, distributed on the Guyana and Brazilian Shields north and south from the Amazon River.

The Guyana shield lineage has a relatively distinct song pattern with most songs being longer in duration and composed of two descending syllables, a characteristic found only in this group. Although this character is relatively reliable to distinguish this lineage—and this certainly has a biological meaning as evidence of a distinct evolutionary pathway—, it cannot be used as a diagnostic character because these birds also sing one syllable songs, which are not different from the songs of the remaining lineages.

The Brazilian Shield lineages can be further divided into three genomic groups (separated by the Tapajós and Xingú rivers) with no fixed morphological differences but with one lineage being relatively, but not unmistakably, distinguished by vocal characters. Birds west of the Tapajós River (Madeira-Tapajós interluve) have a distinct song with two to three syllables (as opposed to one) with the three-syllable song

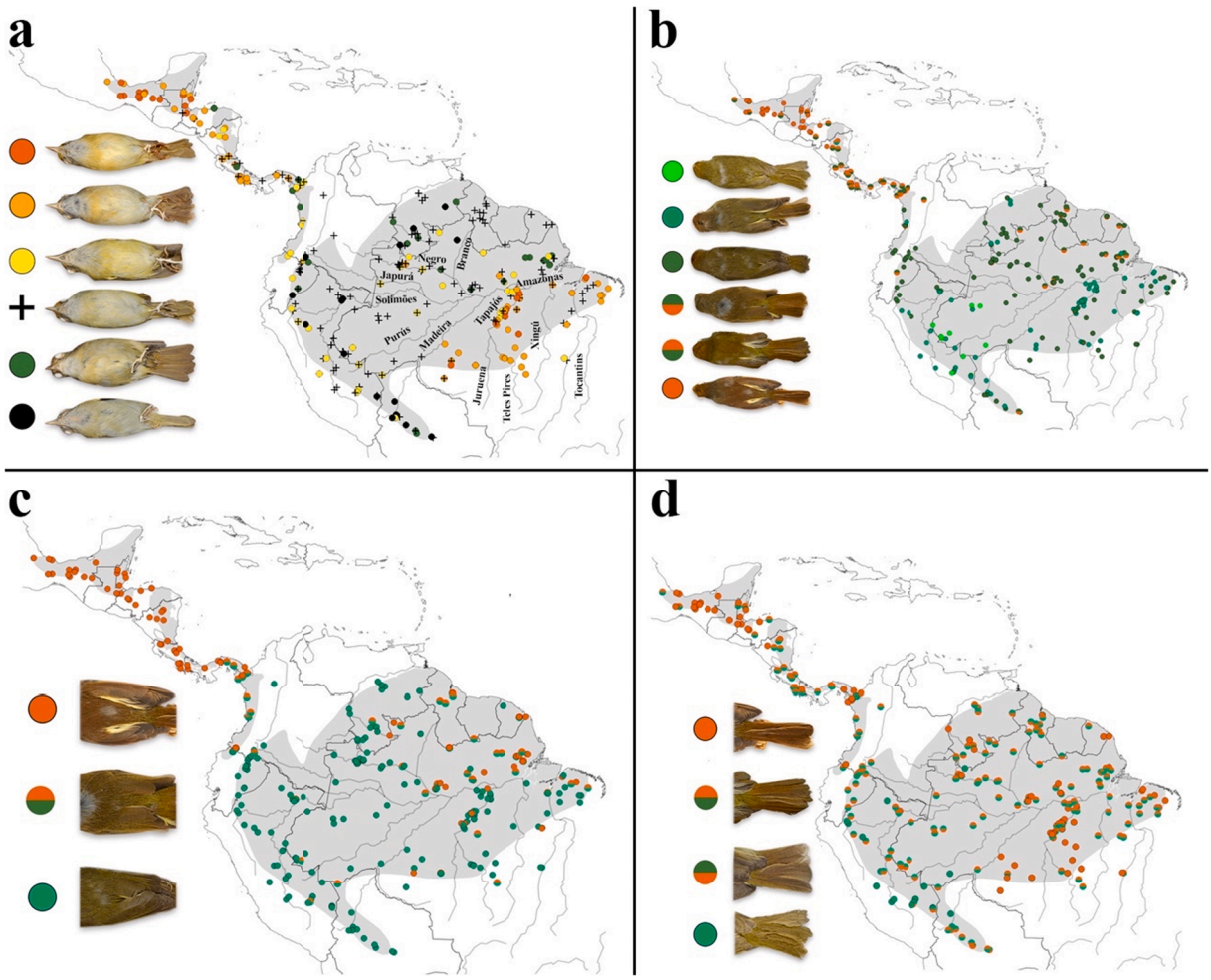

Fig. 7. Geographic variations of plumage color/pattern in *Tunchiornis ochraceiceps*. Numbers in brackets correspond to color codes in Smithe (1975). Colors in parentheses correspond to the colors of the symbols plotted in the map. (a) Color pattern in ventrum (except throat): yellow [157] and gray [86] strongly tinged buff [24] (dark orange); yellow and gray somewhat tinged buff (light orange); gray strongly tinged yellow (yellow); gray with some yellow streaks (cross); gray tinged with buff and olive-green [48 or 49] (green); gray tinged olive-green (black). (b) Color of ventrum: yellowish olive-green [50] (light green); intermediate between yellowish olive-green and olive-green (medium green); olive-green (dark green); predominantly olive-green with brown [37 or 129] (green over orange); predominantly brown with olive-green (orange over green); brown (orange). (c) Color of suffusion over sepia tertiaries: brown (orange); mixture of brown and green [48, 49 or 50] (orange over green) and green (green). (d) Color of rectrices: brown (orange); predominantly brown with green suffusion (orange over green); predominantly green with brown suffusion (green over orange); green (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ascending in frequency, and thus different from the closely related lineage east of the Tapajós, as well as different from all other lineages. However, songs with one and two syllables were found at the Juruena-Teles Pires interfluve, at the limits of their distributions in Southeastern Amazonia. Only the genotype associated with the single-syllable song pattern (clade east of the Tapajós) was found in that region. Thus, it is possible that either the eastern populations have more song plasticity in that area, or that both lineages co-occur there, possibly hybridizing, although no evidence of admixture was found in those samples. The co-occurrence hypothesis may be more plausible given the constancy of song pattern in the remaining parts of the eastern lineage distribution and because the area in question has been shown to be a contact zone for other bird taxa (Weir et al., 2015). However, our vocal sampling in the area is not very extensive so the observed patterns have to be interpreted with caution considering that (i) the multiple-syllable population on the Guyana Shield can also occasionally produce single-syllable songs; (ii) the possible formation of dialects (*i. e.* nearby birds tend to sing more similar song due to a strong learning component); and (iii) large repertoires. Both dialects and large repertories are common phenomena in songbirds (suborder Passeri) (Catchpole and Slater, 2008).

In addition to the complex scenario of phenotypic variation, we found admixed ancestry among the three groups in SEA (Fig. 2), and the multi-species coalescent tree did not fully support the Tapajós-Xingú clade. Thus, we cannot rule out the possible role of gene flow or incomplete lineage sorting among those three lineages. Despite some evidence supporting differentiation, overall evidence for recognition of more than one species in SEA is dubious. Further genomic and vocal sampling in the Juruena-Teles Pires region is necessary to clarify the species limits in the area. Based on current evidence, the lineage at the Madeira-Tapajós interfluve can be considered a distinct subspecies, since it is monophyletic and relatively differentiated by song.

Within the western, white-eyed clade, the geographic extremes of the *trans*-Andean populations are distinct in morphology (but not vocally), but samples from Costa Rica to the northwestern limits of Colombia are clearly intermediate. Genomic data suggests these intermediate individuals also have admixed ancestry in the genomic data and some are assigned to different clades in the mtDNA and nuDNA datasets. The EEMS analyses showed extensive effective migration in the CAM with more limited migration between the CAM and CHO populations in the Panama region. Overall phenotypic and genotypic results indicate

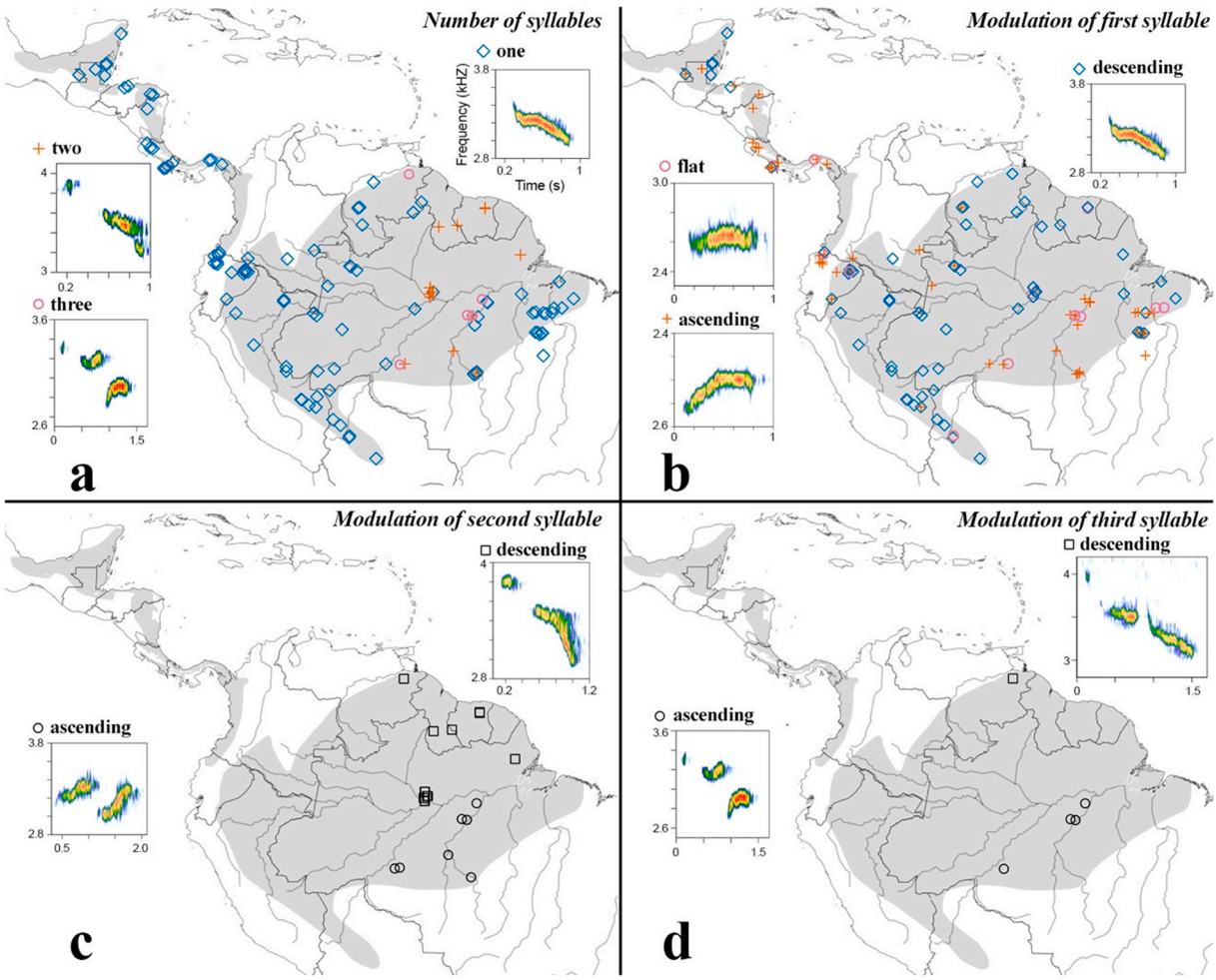

Fig. 8. Geographic variation of descriptive vocal character in *Tunchiornis ochraceiceps*: (a) number of syllables; and modulations of (b) first, (c) second and (d) third syllables. Sonograms inside windows show a visual representation of each character state determined for each character.

differentiation with introgression between those two genomic lineages. In addition, the *cis*-Andean western Amazonian lineages cannot be morphologically or vocally distinguished from birds in the Chocó region. So, although there is a tendency for morphological differentiation, there is no clear phenotypic diagnosis between the lineages in CAM, CHO, SWA and NWA. Nevertheless, the cis- and *trans*-Andean lineages are currently geographically isolated, form different clades, have no admixed genotypes, and no effective migration between them, which suggests no geneflow at present or in the recent past. These two lineages split over 4 Ma, an astonishingly long time considering their phenotypic similarity. This divergence time is far older than many other Neotropical birds currently considered different species (or even genera) (Barker et al., 2015; Imfeld et al., 2020; Silva et al., 2019). Although we did not include genetic samples from northern Peru and western Colombia, the closest area between the cis and trans Andean lineages, introgression between them is unlikely considering the divergence time and the magnitude of the geographic barrier separating them. Even though specimens from that area usually have the phenotype common in other NWA specimens, future survey is desirable to determine the genomic assignment of those populations . Thus, we suggest that the cis- and *trans*-Andean lineages are cryptic species, despite the apparent lack of diagnosability. The similarity in plumage pattern is probably due to retention of ancestral polymorphism, and it would be unreasonable to disregard all the evolutionary evidence provided by molecular data pointing to the deep differentiation and geographic isolation between these two lineages.

Within western Amazonia, samples were divided into at least two major genomic clades (north and south of the Solimões River) possibly with further and shallower substructure that is more evident in the mtDNA. Although some individuals from southwestern Amazonia tend to have a more citrine dorsal plumage and a paler rufous crown, and northwestern specimens tend to have grayer ventrum with olivaceous feathers and a darker rufous crown, identical phenotypes can be found in both northwestern and southwestern Amazon. No vocal differentiation was found among them and admixed genotypes were found among the two areas. Therefore, these variations are best characterized as structured populations of the same species and do not warrant being designated as separate taxonomic units.

4.2. Nomenclatural applications

We suggest a taxonomic arrangement with four species, two of which include two subspecies each, for a total of six taxa (Fig. 10) and the names that should be applied to them are: 1) *T. ochraceiceps* (Type locality Playa Vicente, Oaxaca, Mexico, Type material BM1886-9-15-315 in British Museum) from the *trans*-Andean region; 2) *T. ferrugineifrons* (Sclater, 1862) (Type locality "Bogota", Colombia, Type material BM86-9-15-317 in British Museum) a cryptic species from western Amazonia occurring between the Madeira and Branco rivers; 3) *T. luteifrons* (Sclater, 1881) from the Guyana Shield east of the Branco River (Type locality "Bartica Grove, British Guyana", Type material not examined, in British Museum); and 4) *T. rubrifrons* (Sclater & Salvin, 1867) from the Brazilian Shield, east from the Madeira River (Type locality "River Amazon, Para", Type material BM1886-9-15-316 in British Museum).

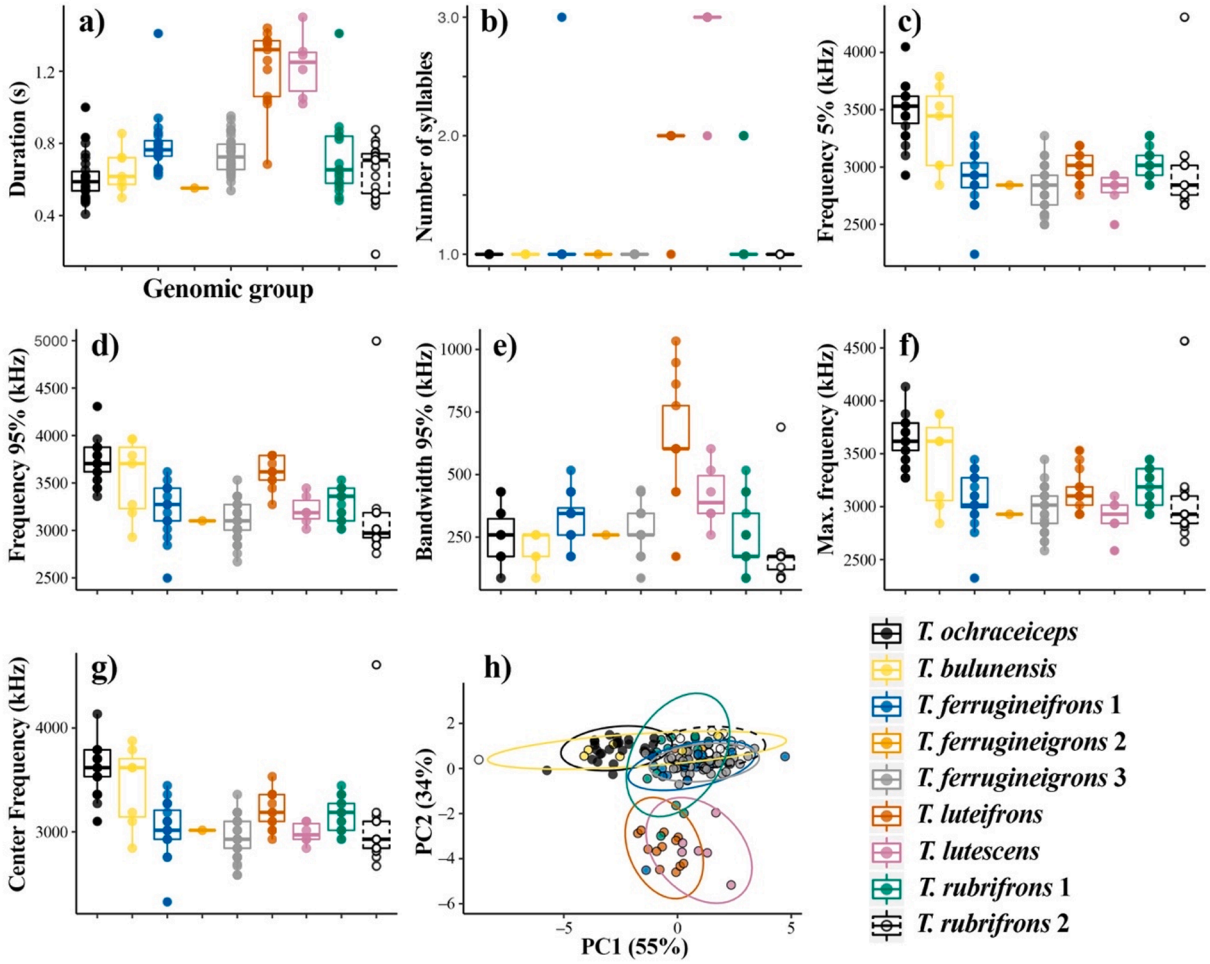

Fig. 9. Scatter plot and Principal Component analysis quantitative vocal characters of *Tunchiornis ochraceiceps* populations classified by genomic groups. Circles, boxplots and ellipses are colored according to population assignments. Ellipses represent the 95% confidence level.

The Madeira-Tapajós lineage may be attributed to a different subspecies, *T. rubrifrons lutescens* (Snethlage, 1914) (Type locality "Boim, Rio Tapajoz, Para, Brazil", type material MPEG 8552 in Museu Goeldi). Although our genomic sampling in CAM is limited, the phenotypic and genomic results suggest that the populations (including the type material) carrying the subspecific names *T. o. pallidipectus* (Ridgway, 1903) and *T. o. pacificus* (Parkes, 1991) are not distinguishable from specimens of *T. o. ochraceiceps*. *Tunchiornis o. nelsoni* (Todd, 1929) is the morphological and geographic intermediate (as already observed by Todd (1929)), and a genomically-admixed form between the two *trans*-Andean parental forms *T. o. ochraceiceps* and *T. o. bulunensis* (Hartert, 1902), although mtDNA and EEMS results suggest that the evolutionary history in the area is more complex (see mtDNA and nuDNA discordance section). Thus, we suggest that all taxa in CAM should be considered junior synonyms of *T. ochraceiceps*, while *Tunchiornis o. bulunensis*, from the CHO region, may be considered a different subspecies (Type locality "Bulún, Esmeraldas, Ecuador", misspelling of Pulún, type material AMNH 505,298 in American Museum of Natural History). The subspecies *T. o. viridior*, associated with the paler plumages of southwestern Amazonia in southern Peru and northern Bolivia is not a valid taxon and should be synonymized with *T. ferrugineifrons*.

In summary, our suggested taxonomic arrangement consists of the following six taxa: *T. o. ochraceiceps*, *T. o. bulunensis*, *T. ferrugineifrons*, *T. luteifrons*, *T. r. rubrifrons* and *T. r. lutescens* (Fig. 10). All names applied are in agreement with their original description, type material, and within their type locality range. A further discussion on type specimens and their localities, the descriptions of each taxon, geographic distribution, and the lists of type material and synonyms can be found in Supplementary Material 6.

4.3. mtDNA and nuDNA discordance

West of the Andes, nuDNA shows admixed genotypes between two parental populations with limited effective migration in Panama (Figs. 2 and 3), but mtDNA revealed a third mitochondrial lineage exclusive to the eastern Panamanian region and sister to the CHO lineage instead of to the remaining CAM samples. The associated divergence is relatively old (~2 Ma). This suggests that gene flow with the CAM lineage was once limited or absent, allowing mitochondrial differentiation, but that secondary contact may have caused admixture of genotypes before complete isolation, consequently erasing a part of the evolutionary history from the genomic DNA. Thus, *trans*-Andean diversification is probably not due to primary differentiation as suggested by a possible clinal variation in plumage and may be more complex than a single secondary contact event between the CAM and CHO populations. More investigation is required to elucidate the diversification processes in that area, which is known to be evolutionarily complex (Brumfield and Braun, 2001; Brumfield et al., 2007).

In western Amazonia, besides the distinct placement of the admixed samples from the Japurá-Solimões interfluve (orange in Figs. 4 and 5) and northwestern Peru (purple in Figs. 4 and 5) in the mtDNA and nuDNA phylogenetic trees, they both represent relatively old mtDNA lineages. Additional structure in western Amazonia is also suggested by EEMS (Fig. 3, b2) in these two regions and possibly in the mid Ucayali

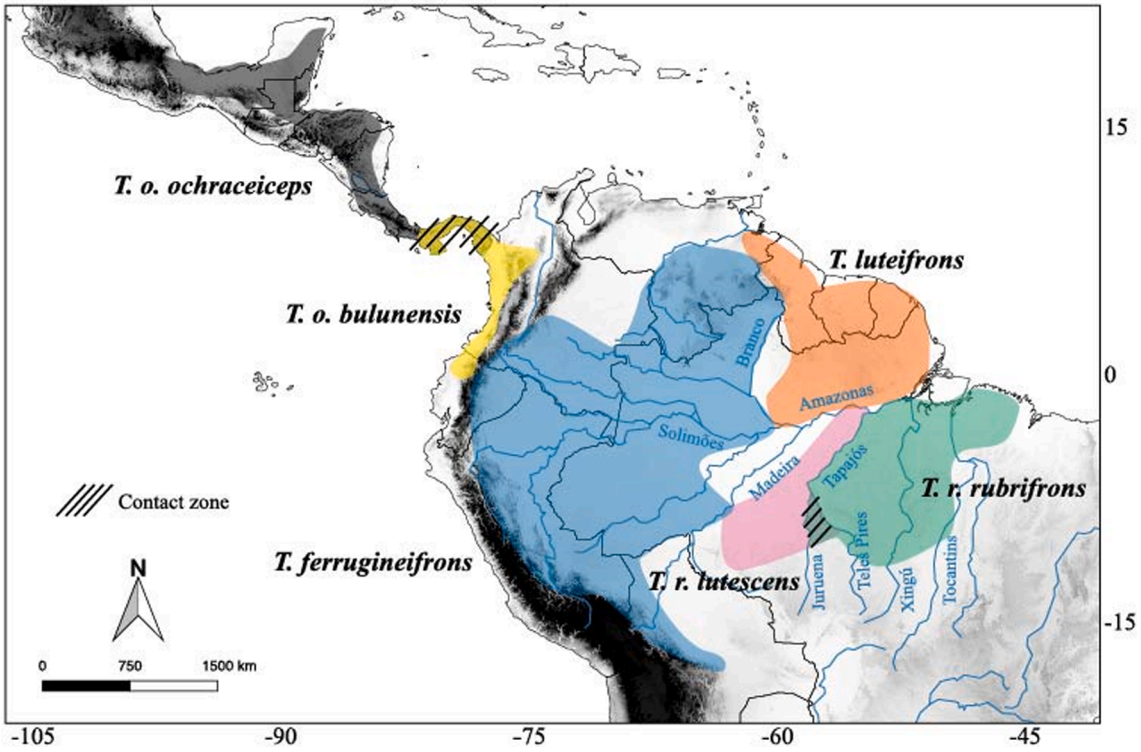

Fig. 10. Geographic distribution of the taxa delimited in the *Tunchiornis* genus after our taxonomic decision based on genomic, morphologic and vocal data. The distribution polygons were drawn around all samples used in this study (see Fig. 1). Possible contact zones between taxa are shown in black diagonal bars.

River. This possibility is not surprising given that geographically restricted lineages in western Amazonia were also found in other upland forest birds (Berv et al., 2019; Cheviron et al., 2005; Ferreira et al., 2017). However, further sampling is necessary to evaluate if: 1) these lineages are actually distinct, especially in nuDNA, and their admixed genotypes obtained in sNMF are misleading due to small sample sizes; and/or 2) isolation with incomplete speciation or introgression occurred in some parts of western Amazonia. Evidence for introgression between lineages from different Amazonian interfluvia is becoming more frequent with the use of genomic data and might be more common than previously thought (Buainain et al., 2020; Dias et al., 2018; Ferreira et al., 2018). In any case, overall evidence points to a complex and dynamic diversification in western Amazonia during the Pleistocene.

4.4. Contribution to the historical biogeography of northern south American forests

The revised taxonomy and robust phylogenetic relationships presented here allow the analysis of one of the few diversification patterns available for lowland northern South American oscines. Although time estimates are based on mtDNA evolutionary rates with large confidence intervals, the pattern obtained suggests that in contrast to most oscines, which colonized South America after the final closure of the Panama Isthmus (usually in the last 4 myr) (Barker et al., 2015; Smith and Klicka, 2010), *Tunchiornis* has been diversifying in South America for a relatively longer period (9 Ma). This time frame is old even when compared with most other modern Amazonian terrestrial vertebrates, many of which originated in the last 5 myr (Cracraft et al., 2020).

Although lineages of *T. ochraceiceps* are delimited by large Amazonian rivers, a pattern commonly found in many other understory birds associated with upland forests (Cracraft, 1985; Cracraft et al., 2020), the spatial and temporal patterns we found are not very common in other studies. The oldest divergence within *Tunchiornis* separates clades that are currently delimited by the Branco and Madeira rivers, in an east-west pattern. Usually, the first divergence events observed in groups with similar distribution are either between cis- and *trans*-Andean lineages, or between taxa north and south of the Amazon River, and at more recent times (Bates et al., 1998; Silva et al., 2019). It is possible that the initial divergence of *Tunchiornis* can be attributed to an ancestral distribution restricted to upland forests at the base of the Andes, and at the Brazilian and Guyana shields, that were isolated by an unsuitable region largely dominated by aquatic, flooded and grassy habitats, corresponding to the Solimões sedimentary basin (Bicudo et al., 2019). A recent colonization of the sedimentary basin is also suggested by the long branches leading to western Amazonian lineages, whose diversification started only in the last 1.1 myr. Recent diversification in western Amazonia has been previously recovered for other groups and has been attributed to a recent expansion of upland forest into the sedimentary basin (Bicudo et al., 2019; Pupim et al., 2019; Ribas et al., 2018).

The establishment of the modern Amazonian drainage system, as a result of the final uplift of the Andes, is considered one of the most important geological event for the assembly of the current Amazonian biota. This process may have caused isolation and vicariance among previously widespread populations (Hoorn et al., 2010; Ribas et al., 2012). However, the timing of that event is debatable and ranges from 10 to 2.5 Ma (Campbell et al., 2006; Hoorn et al., 2010; Latrubesse et al., 2010). Although the Miocene model (10–7 Ma), based on the arrival of Andean sourced sediments at the Amazon fan, is one of the most referenced, finer sampling in bird and primate phylogeographic studies have failed to corroborate this date, as divergence times are usually younger (Plio and Pleistocene) (Boubli et al., 2015; Byrne et al., 2018; Ferreira et al., 2017; Ribas et al., 2012; Silva et al., 2019). The divergence time we find between the SEA and NEA lineages in eastern Amazonia (7.5 Ma) might be the first case of bird taxa being congruent with the establishment of a *trans*-continental configuration of the Solimões-Amazonas course proposed by the Miocene model. But interestingly, the north-south divergence obtained for western Amazonian lineages (1.1 myr) was not synchronous with the same event, suggesting that different processes may have acted on upper and lower Amazonas-Solimões. A more direct vicariant impact of the final uplift of northern and central

Andes is suggested by the divergence among the *Tunchiornis* lineages currently distributed on opposite sides of the Andes at 4.5 Ma. This time period is congruent with geological estimates for that event at 5–2.5 Ma (Gregory-Wodzicki, 2000; Hoorn et al., 2010), and with divergence times found for other taxa with similar distribution (Brumfield and Edwards, 2007; Weir and Price, 2011).

Several *Tunchiornis* lineages originated more recently, during the Pleistocene (last 2.6 Ma). Increasing evidence suggests differential impacts of climate variation and sedimentation patterns on the availability and connectedness among upland forest environments in Amazonia during this period (Buainain et al., 2020; Cheng et al., 2013; Pupim et al., 2019; Silva et al., 2019; Wang et al., 2017). The improved taxonomic and phylogenetic information presented here makes *Tunchiornis* an interesting model to further test biogeographic hypotheses in the region.

4.5. Phenotypic conservatism

It is remarkable how morphologically and vocally similar the different lineages are despite the large divergence times among them. Even the most divergent lineages have notably conserved and/ or unfixed phenotypic characters (plumage, morphometric and vocalization). The overall song variation is also low considering the striking vocal capacity in developing dialects and extensive repertoires that oscine birds have, as well as the vocal variation found in closely related species (Catchpole and Slater, 2008; Raposo et al., 1998). This decoupling between genomic and phenotypic differentiation suggests that despite deep divergence, little evolutionary pressure (social and/or ecological) may have existed to develop phenotypic differentiation, thus pointing to stabilizing, rather than directional selection over those traits. A similar scenario was observed for other Amazonian vertebrates (Kaefer et al., 2012; Pulido-Santacruz et al., 2018) suggesting that this might be a common phenomenon. Despite its wide distribution, *Tunchiornis* is primarily associated with upland rain forests (*Terra Firme*), being more abundant in the interior of primary forest than edges and second-growth (Antongiovanni and Metzger, 2005). It is possible that a more strict habitat association coupled with unsuccessful colonization of new or sub-optimal habitat, resulted in slower diversification rates by not imposing selective pressure for new phenotypic traits. Although extensive habitat heterogeneity can be found in Amazonia, even within upland forests (Tuomisto et al., 2019), *Tunchiornis* might track similar habitat conditions in different parts of the heterogeneous forest, or perhaps the magnitude of habitat variation is not relevant enough to drive phenotypic selection in the group.

Our results are congruent with other studies on Amazonian birds, which show that speciation in the tropics can occur with slow evolutionary rates in phenotypic traits responsible for pre-zygotic isolation (mating recognition) and slow accumulation of genomic differences with no directional selection. This accumulation eventually results in postzygotically isolated, but phenotypically similar species and this speciation mode might be common in tropical regions, especially in Amazonian upland forests (Cronemberger et al., 2020; Pulido-Santacruz et al., 2018).

Interestingly, in Central America, these birds also occupy deciduous forests (Stiles et al., 1989), and despite the Chocó-Central America split being relatively recent within the group, Mesoamerican birds have one of the most distinct plumage within the genus, being mostly brown on the dorsum—thus lighter in tone—instead of olive-green. This is congruent with the tendency of some birds and mammals from more humid and darker habitats, such as rainforests, to have darker coloration than those from drier and more open environments, which have been associated with more rufous tones, the so called Gloger's rule (Kamilar and Bradley, 2011; Marcondes and Brumfield, 2019; Marcondes et al., 2020; Miller et al., 2019; Zink and Remsen Jr., 1986). The distinct coloration may provide better crypsis and better chances of survival in their respective habitats, or heavier pigmentation may confer protection against feather degradation by bacteria, which are more abundant in humid environments (Burtt and Ichida, 2004; Goldstein et al., 2004).

The evolution of phenotypes can be complex, especially in evolutionarily complex environments such as the Neotropics. In this sense, different evolutionary forces (*e. g.* genetic drift, natural and social selection) may act distinctly over different phenotypic characters (Cadena and Zapata, 2021; Zamudio et al., 2016). This may result in asynchronous variations where some characters have little variation among populations (*e. g.* morphometrics), others may be variable, but not necessarily indicative of phylogenetic relationships (*e. g.* some plumage characters studied here) and some may be fixed among populations but as a result of different drivers. Further studies might help to elucidate if the ecological niche of *Tunchiornis* is restricted, if niche restriction can be associated with phenotypic conservatism, and if distinct evolutionary processes are driving the evolution of the different phenotypic characters like plumage coloration and vocalization.

5. Conclusions

By improving our knowledge about the systematics and distribution of *Tunchiornis*, we set a clearer scenario for future studies on the species and broader biological studies that rely on well-defined evolutionary units. Coupling genomic and phenotypic approaches while considering the intrinsic natural history aspects of each organism is essential for understanding the evolutionary forces acting on phenotypic characters and diversification of organisms. This allows us to understand why different lineages evolve at different paces and modes, and consequently propose more realistic species limits. *Tunchiornis* is a remarkable example of an ancient taxon with underestimated diversity due to decoupling between conserved phenotypic characters and deep molecular divergence, possibly due to stabilizing selection related to habitat specificity and/or absence of social selection. In contrast to other oscines, diversification within the genus has been occurring in South America for a relatively long time period, and its unique evolutionary history provides important information for comparative biogeographic studies.

CRediT authorship contribution statement

Nelson Buainain: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Funding acquisition. **Marina F.A. Maximiano:** Formal analysis, Writing - review & editing. **Mateus Ferreira:** Methodology, Software, Formal analysis, Data curation, Writing - review & editing. **Alexandre Aleixo:** Conceptualization, Writing - review & editing. **Brant C. Faircloth:** Methodology, Software, Resources, Writing - review & editing. **Robb T. Brumfield:** Resources, Writing - review & editing. **Joel Cracraft:** Funding acquisition, Writing - review & editing, Project administration. **Camila C. Ribas:** Conceptualization, Methodology, Resources, Supervision, Project administration, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank collectors of specimen and vocalization data for their constant hard field work, curators and staff of the collections visited to obtain the skin specimens' data and which provided tissue loans: Fatima Lima and Antonita Santana (MPEG); Paul Sweet and Thomas Trombone (AMNH); Nate Rice and Jason Weckstein (ANSP); Steve Rogers (CM); Ben Marks and John Bates (FMNH); Brian Schmidt (USNM); Marlene

Freitas (INPA); Fred Sheldon and Donna Dittmann (LSUMNS); Robb Moyle and Mark Robbins (KU); Sharon Birks (UWBM). We also thank Marcos Raposo (MNRJ) for providing photographs of type specimens and Renata Stopiglia for guidance on nomenclatural application.

Funding

CAPES (Coordenação de Aperfeiçoamento Pessoal de Pessoal de Nível Superior), FAPEAM (Fundação de Amparo à Pesquisa do Estado do Amazonas, process nº: 062.00104/2020), and CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnológico) funded NB; skin specimen examination was funded by the Collection Study Grant conceived by the AMNH and the Ernst Mayr Travel Grant conceived by MCZ, Harvard. Project was financially supported by the grant Dimensions US-Biota-São Paulo: Assembly and evolution of the Amazon biota and its environment: an integrated approach, co-funded by the US National Science Foundation (NSF DEB 1241056, and NSF DEB 1241066 to J.C.), and the Fundação de Amparo à Pesquisas do Estado de São Paulo (FAPESP grant #2012/50260-6). Portions of this research were conducted with high performance computing resources provided by Louisiana State University (http://www.hpc.lsu.edu) and by the National Laboratory for Scientific Computing (LNCC/MCTI, Brazil).

Data availability

All DNA sequence data produced are available at NCBI Sequence Read Archive (SRA) under the BioProject PRJNA675640. All phylogenetic tree files are available at the Supplementary Material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107206.

References

Aberer, A.J., Kobert, K., Stamatakis, A., 2014. ExaBayes: massively parallel bayesian tree inference for the whole-genome era. Mol. Biol. Evol. 31, 2553–2556.
Andermann, T., Cano, Á., Zizka, A., Bacon, C.D., Antonelli, A., 2018. SECAPR - A bioinformatics pipeline for the rapid and user-friendly processing of Illumina sequences, from raw reads to alignments. PeerJ Preprints 6.
Andermann, T., Fernandes, A.M., Olsson, U., Töpel, M., Pfeil, B., Oxelman, B., Aleixo, A., Faircloth, B.C., Antonelli, A., 2019. Allele phasing greatly improves the phylogenetic utility of Ultraconserved Elements. Syst. Biol. 68, 32–46.
Antongiovanni, M., Metzger, J.P., 2005. Influence of matrix habitats on the occurrence of insectivorous bird species in Amazonian forest fragments. Biol. Conserv. 122, 441–451.
Baldwin, S.P., Oberholser, H.C., Worley, L.G., 1931. Measurements of birds. Scientific Publications of the Cleveland Museum of Natural History, Cleveland, Ohio.
Barker, F.K., Burns, K.J., Klicka, J., Lanyon, S.M., Lovette, I.J., 2015. New insights into New World biogeography: An integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers, and allies. Auk 132 (333–348), 316.
Barrowclough, G.F., Cracraft, J., Klicka, J., Zink, R.M., 2016. How Many Kinds of Birds Are There and Why Does It Matter? PLoS ONE 11, e0166307.
Bates, J.M., Hackett, S.J., Cracraft, J., 1998. Area-relationship in the Neotropical lowlands: An hypothesis based on raw distributions of Passerine birds. J. Biogeogr. 25, 783–793.
Berv, J.S., Campagna, L., Feo, T.J., Castro-Astor, I., Ribas, C.C., Prum, R.O., Lovette, I.J., 2019. Genomic phylogeography of the White Crowned Manakin Pseudopipra pipra (Aves: Pipridae) illuminates a continental-scale radiation out of the Andes. bioRxiv, 713081.
Bicudo, T.C., Sacek, V., de Almeida, R.P., Bates, J.M., Ribas, C.C., 2019. Andean Tectonics and Mantle Dynamics as a Pervasive Influence on Amazonian Ecosystem. Sci. Rep. 9, 16879.
BirdLife International, 2019. IUCN Red List for birds.
Boesman, P., 2016. Notes on the vocalizations of Tawny-crowned Greenlet (Hylophilus ochraceiceps). HBW Alive Ornithological Note 168. Handbook of the Birds of the World Alive. Lynx Edicion, Barcelona.
Bortolus, A., 2008. Error cascades in the biological sciences: The unwanted consequences of using bad taxonomy in ecology. Ambio 37, 114–118.
Boubli, J.P., Byrne, H., da Silva, M.N.F., Silva-Júnior, J., Costa Araújo, R., Bertuol, F., Gonçalves, J., de Melo, F.R., Rylands, A.B., Mittermeier, R.A., Silva, F.E., Nash, S.D., Canale, G., Alencar, R.d.M., Rossi, R.V., Carneiro, J., Sampaio, I., Farias, I.P., Schneider, H., Hrbek, T., 2019. On a new species of titi monkey (Primates: Plecturocebus Byrne et al., 2016), from Alta Floresta, southern Amazon, Brazil. Mol. Phylogen. Evol. 132, 117–137.
Boubli, Jean P., Ribas, Camila, Lynch Alfaro, Jessica W., Alfaro, Michael E., da Silva, Maria Nazareth F., Pinho, Gabriela M., Farias, Izeni P., 2015. Spatial and temporal patterns of diversification on the Amazon: A test of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in Brazil. Mol. Phylogenet. Evol. 82, 400–412. https://doi.org/10.1016/j.ympev.2014.09.005.
Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F.K., Müller, N.F., Ogilvie, H.A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., Suchard, M.A., Wu, C.-H., Xie, D., Zhang, C., Stadler, T., Drummond, A.J., 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Comp. Biol. 15, e1006650.
Brumfield, R.T., Braun, M.J., 2001. Phylogenetic relationships in bearded manakins (Pipridae:Manacus) indicate that male plumage color is a misleading taxonomic marker. Condor 103 (248–258), 211.
Brumfield, R.T., Edwards, S.V., 2007. Evolution into and out of the Andes: a Bayesian analysis of historical diversification in Thamnophilus antshrikes. Evolution 61, 346–367.
Brumfield, R.T., Jernigan, R.W., McDonald, D.B., Braun, M.J., 2007. Evolutionary implications of divergent clines in an avian (*Manacus*:Aves) hybrid zone. Evolution 55, 2070–2087.
Buainain, N., Canton, R., Zuquim, G., Tuomisto, H., Hrbek, T., Sato, H., Ribas, C.C., 2020. Paleoclimatic evolution as the main driver of current genomic diversity in the widespread and polymorphic Neotropical songbird *Arremon taciturnus*. Mol Ecol.
Buainain, N., de Assis, C.P., Raposo, M.A., 2017. Geographic variation and taxonomy of the *Arremon taciturnus* (Hermann, 1783) species complex (Aves: Passerellidae). J. Ornithol. 158, 631–650.
Burtt Jr., E.H., Ichida, J.M., 2004. Gloger's Rule, Feather-Degrading Bacteria, and Color Variation Among Song Sparrows. Condor 106, 681–686.
Byrne, H., Alfaro, J.W.L., Sampaio, I., Farias, I.P., Scheneider, H., Hrbek, T., Boubli, J.P., 2018. Titi monkeys biogeography: Parallel Pleistocene spread by *Plecturocebus* and *Cheracebus* into a post-Pebas Western Amazon. Zool. Scr. 47, 499–517.
Cadena, C.D., Cuervo, A.M., 2010. Molecules, ecology, morphology, and songs in concert: how many species is *Arremon torquatus* (Aves: Emberizidae)? Biol. J. Linn. Soc. 99, 152–176.
Cadena, C.D., Cuervo, A.M., Céspedes, L.N., Bravo, G.A., Krabbe, N., Schulenberg, T.S., Derryberry, G.E., Silveira, L.F., Derryberry, E.P., Brumfield, R.T., Fjeldså, J., 2020. Systematics, biogeography, and diversification of Scytalopus tapaculos (Rhinocryptidae), an enigmatic radiation of Neotropical montane birds. Auk.
Cadena, C.D., Zapata, F., 2021. The genomic revolution and species delimitation in birds (and other organisms): Why phenotypes should not be overlooked. Ornithology 138, 1–18.
Campbell, K.E., Frailey, C.D., Romero-Pittman, L., 2006. The Pan-Amazonian Ucayali Peneplain, late Neogene sedimentation in Amazonia, and the birth of the modern Amazon River system. Palaeogeogr. Palaeoclimatol. Palaeoecol. 239, 166–219.
Campillo, L.C., Burns, K.C., Moyle, R.G., Manthey, J.D., 2019. Mitochondrial genomes of the bird genus *Piranga*: rates of sequence evolution, and discordance between mitochondrial and nuclear markers. Mitochondrial DNA Part B 4, 2566–2569.
Catchpole, C.K., Slater, P.J.B., 2008. Bird Song: Biological Themes and Variations. Cambridge University Press, New York.
Center for Conservation Bioacoustics, 2014. Raven Pro: Interactive Sound Analysis Software (Version 1.5) [Computer software]. The Cornell Lab of Ornithology, Ithaca, NY.
Charity, S., Dudley, N., Oliveira, D., Stolton, S., 2016. Living amazon report 2016: A regional approach to conservation in the Amazon. WWF Living Amazon Initiative, Brasília and Quito.
Cheng, H., Sinha, A., Cruz, F.W., Wang, X., Edwards, R.L., d'Horta, F.M., Ribas, C.C., Vuille, M., Stott, L.D., Auler, A.S., 2013. Climate change patterns in Amazonia and biodiversity. Nat. Commun. 4, 1411.
Cheviron, Z.A., Hackett, S.J., Capparella, A.P., 2005. Complex evolutionary history of a Neotropical lowland forest bird (*Lepidothrix coronata*) and its implications for historical hypotheses of the origin of Neotropical avian diversity. Mol. Phylogenet. Evol. 36, 338–357.
Cracraft, J., 1985. Historical biogeography and patterns of differentiation within the South American Avifauna: Areas of Endemism. Ornithol. Monog. 36, 49–84.
Cracraft, J., Camila Ribas, C., d'Horta, F.M., Bates, J., Almeida, R.P., Aleixo, A., Boubli, J. P., Campbell, K.E., Cruz, F.W., Ferreira, M., Fritz, S.C., Grohmann, C.H., Latrubesse, E.M., Lohmann, L.G., Musher, L.J., Nogueira, A., Sawakuchi, A.O., Baker, P., 2020. The Origin and Evolution of Amazonian Species Diversity. In: Rull, V., Carnaval, A.C. (Eds.), Neotropical Diversification: Patterns and Processes. Springer International Publishing, Cham, pp. 225–244.
Cronemberger, Á.A., Aleixo, A., Mikkelsen, E.K., Weir, J.T., 2020. Postzygotic isolation drives genomic speciation between highly cryptic Hypocnemis antbirds from Amazonia. n/a Evolution.
Dias, C., Lima, K.A., Araripe, J., Aleixo, A., Vallinoto, M., Sampaio, I., Schneider, A., Rêgo, P.S., 2018. Mitochondrial introgression obscures phylogenetic relationships among manakins of the genus *Lepidothrix* (Aves: Pipridae). Mol. Phylogenet. Evol. 126, 314–320.
Dickinson, E.C., Christidis, L., 2014. The Howard and Moore complete checklist of the birds of the World. Aves Press, Passerines.
Faircloth, B.C., 2013. Illumiprocessor: a trimmomatic wrapper for parallel adapater and quality trimming. https://doi.org/10.6079/J9ILL.
Faircloth, B.C., 2016. PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics 32, 786–788.

Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T., Glenn, T.C., 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Syst. Biol. 61, 717–726.
Ferreira, M., Aleixo, A., Ribas, C.C., Santos, M.P.D., 2017. Biogeography of the Neotropical genus *Malacoptila* (Aves: Bucconidae): the influence of the Andean orogeny, Amazonian drainage evolution and palaeoclimate. J. Biogeogr. 44, 748–759.
Ferreira, M., Fernandes, A.M., Aleixo, A., Antonelli, A., Olsson, U., Bates, J.M., Cracraft, J., Ribas, C.C., 2018. Evidence for mtDNA capture in the jacamar *Galbula leucogastra / chalcothorax* species-complex and insights on the evolution of white-sand ecosystems in the Amazon basin. Mol. Phylogenet. Evol. 129, 149–157.
Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., François, O., 2014. Fast and efficient estimation of individual ancestry coefficients. Genetics 196, 973–983.
Goldstein, G., Kelly, R.F., Beth Ann, B., Samia, M., Jann, M.I., Burtt, E.H., 2004. Bacterial Degradation of Black and White Feathers. Auk 121, 656–659.
Gregory-Wodzicki, K.M., 2000. Uplift history of the Central and Northern Andes: A review. Geol. Soc. Am. Bull. 112, 1091–1105.
Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Syst. Biol. 59, 307–321.
Haffer, J., 1969. Speciation in amazonian forest birds. Science 165, 131–137.
Hahn, C., Bachmann, L., Chevreux, B., 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. Nucl. Acids Res. 41, e129.
Hellmayr, C.E., 1935. Catalogue of birds of the Americas and the adjacent islands in Field Museum of Natural History and including all species and subspecies known to occur in North America, Mexico, Central America, South America, the West Indies, and islands of the Caribbean Sea, the Galapagos Archipelago, and other islands which may properly be included on account of their faunal affinities. Field Museum of Natural History, Chicago.
Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2017. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35, 518–522.
Hoorn, C., Wesselingh, F.P., ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartin, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Sarkinen, T., Antonelli, A., 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science 330, 927–931.
Howard, R., Dickinson, E.C., Moore, A., Christidis, L., Remsen, J.V., 2014. The Howard and Moore Complete Checklist of the Birds of the World. Aves Press, Passerines.
Howell, S.N.G., Webb, S., 1995. A guide to the birds of Mexico and northern Central America. Oxford University Press, Oxford, New York.
ICZN, 1999. International code of zoological nomenclature. The International Trust For Zoological Nomenclature, London.
Imfeld, T.S., Barker, F.K., Brumfield, R.T., 2020. Mitochondrial genomes and thousands of ultraconserved elements resolve the taxonomy and historical biogeography of the Euphonia and Chlorophonia finches (Passeriformes: Fringillidae). Auk.
Janes, J.K., Miller, J.M., Dupuis, J.R., Malenfant, R.M., Gorrell, J.C., Cullingham, C.I., Andrew, R.L., 2017. The K = 2 conundrum. Mol. Ecol. 26, 3594–3602.
Jaramillo, C., 2018. Evolution of the Isthmus of Panama: biological, paleoceanographic and paleoclimatological implications. Mount. Clim. Biodivers. 323–338.
Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405.
Kaefer, I.L., Tsuji-Nishikido, B.M., Mota, E.P., Farias, I.P., Lima, A.P., 2012. The Early Stages of Speciation in Amazonian Forest Frogs: Phenotypic Conservatism Despite Strong Genetic Structure. Evol. Biol. 40, 228–245.
Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589.
Kamilar, J.M., Bradley, B.J., 2011. Interspecific variation in primate coat colour supports Gloger's rule. J. Biogeogr. 38, 2270–2277.
Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol. Biol. Evol. 30, 772–780.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
Köhler, J., Jansen, M., Rodríguez, A., Kok, P.J.R., Toledo, L.F., Emmrich, M., Glaw, F., Haddad, C.F.B., Rödel, M.-O., Vences, M., 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. Zootaxa 4251 (1), 1–124.
Krabbe, N.K., Schulenberg, T.S., Hosner, P.A., Rosenberg, K.V., Davis, T.J., Rosenberg, G. H., Lane, D.F., Andersen, M.J., Robbins, M.B., Cadena, C.D., Valqui, T., Salter, J.F., Spencer, A.J., Angulo, F., Fjeldså, J., 2020. Untangling cryptic diversity in the High Andes: Revision of the Scytalopus [magellanicus] complex (Rhinocryptidae) in Peru reveals three new species. Auk 137.
Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773.
Latrubesse, E.M., Cozzuol, M., Silva-Caminha, S.A.F., Rigsby, C.A., Absy, M.L., Jaramillo, C., 2010. The Late Miocene paleogeography of the Amazon Basin and the evolution of the Amazon River system. Earth-Sci. Rev. 99, 99–124.
Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., Subgroup, G.P.D.P., 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079.
Marcondes, R.S., Brumfield, R.T., 2019. Fifty shades of brown: Macroevolution of plumage brightness in the Furnariida, a large clade of drab Neotropical passerines. Evolution 73, 704–719.
Marcondes, R.S., Stryjewski, K.F., Brumfield, R.T., 2020. Testing the simple and complex versions of Gloger's rule in the Variable Antshrike (Thamnophilus caerulescens, Thamnophilidae). Auk 137, 1–13.
Mayr, E., 1963. Animal species and evolution. Harvard Univerisity Press, Cambridge.
Milá, B., Tavares, E.S., Muñoz Saldaña, A., Karubian, J., Smith, T.B., Baker, A.J., 2012. A Trans-Amazonian Screening of mtDNA Reveals Deep Intraspecific Divergence in Forest Birds and Suggests a Vast Underestimation of Species Diversity. PLoS ONE 7, e40541.
Miller, E.T., Leighton, G.M., Freeman, B.G., Lees, A.C., Ligon, R.A., 2019. Ecological and geographical overlap drive plumage evolution and mimicry in woodpeckers. Nat. Commun. 10, 1602.
Moraes, L.J.C.D.L., Pavan, D., Lima, A.P., 2019. A new nurse frog of Allobates masniger-nidicola complex (Anura, Aromobatidae) from the east bank of Tapajós River, eastern Amazonia. Zootaxa 4648, 401–434.
Naka, L.N., Brumfield, R.T., 2018. The dual role of Amazonian rivers in the generation and maintenance of avian diversity. Sci. Adv. 4, eaar8575.
Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2014. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol. Biol. Evol. 32, 268–274.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package. R package version 2.5-5.
Petkova, D., Novembre, J., Stephens, M., 2015. Visualizing spatial population structure with estimated effective migration surfaces. Nat. Genet. 48, 94–100.
Pritchard, J.K., Stephens, M., Dononelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Proctor, N.S., Lynch, P.J., 1993. Manual of ornithology : avian structure & function. Yale University Press, New Haven.
Pulido-Santacruz, P., Aleixo, A., Weir, J.T., 2018. Morphologically cryptic Amazonian bird species pairs exhibit strong postzygotic reproductive isolation. Proc. R. Soc. B 285, 20172081.
Pupim, F.N., Sawakuchi, A.O., Almeida, R.P., Ribas, C.C., Kern, A.K., Hartmann, G.A., Chiessi, C.M., Tamura, L.N., Mineli, T.D., Savian, J.F., Grohmann, C.H., Bertassoli Jr., D.J., Stern, A.G., Cruz, F.W., Cracraft, J., 2019. Chronology of Terra Firme formation in Amazonian lowlands reveals a dynamic Quaternary landscape. Quat. Sci. Rev. 210, 154–163.
Qgis Development Team, 2016. Open Source Geospatial Foundation Project.
R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Rambaut, A., Drummond, A.J., 2019a. LogCombiner v2.6.1.
Rambaut, A., Drummond, A.J., 2019b. TreeAnnotator v2.6.0.
Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6.
Raposo, M.A., Parrini, R., Napoli, M., 1998. Taxonomia, morfometria e bioacústica do grupo específico Hylophilus poicilotis / H. amaurocephalus (Aves, Vireonidae). Ararajuba 6, 87–109.
Remsen, J.V., 1995. The importance of continued collecting of bird specimens to ornithology and bird conservation. Bird Conserv. Int. 5, 146–180.
Rheindt, F.E., Prawiradilaga, D.M., Ashari, H., Suparno, Gwee, C.Y., Lee, G.W.X., Wu, M. Y., Ng, N.S.R., 2020. A lost world in Wallacea: Description of a montane archipelagic avifauna. Science 367, 167–170.
Ribas, C.C., Aleixo, A., Gubili, C., d'Horta, F.M., Brumfield, R.T., Cracraft, J., 2018. Biogeography and diversification of *Rhegmatorhina* (Aves: Thamnophilidae): Implications for the evolution of Amazonian landscapes during the Quaternary. J. Biogeogr. 45, 917–928.
Ribas, C.C., Aleixo, A., Nogueira, A.C., Miyaki, C.Y., Cracraft, J., 2012. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. Proc. Biol. Sci. 279, 681–689.
Ridgely, R.S., Tudor, G., 2009. Birds of South America: Passerines. Christopher Helm.
Rogers, J.S., 1972. Measures of genetic similarity and genetic distances. Studies in Genetics. Univ. Texas Publ. 7213, 145–153.
Scrucca, L., Fop, M., Murphy, T.B., Raftery, A.E., 2016. mclust 5: clustering, classification and density estimation using Gaussian finite mixture models. R J. 8, 205–233.
Silva, S.M., Peterson, A.T., Carneiro, L.S., Burlamaqui, T.C.T., Ribas, C.C., Sousa-Neves, T., Miranda, L.S., Fernandes, A.M., d'Horta, F.M., Araújo-Silva, L.E., Batista, R., Bandeira, C.H.M.M., Dantas, S.M., Ferreira, M., Martins, D.M., Oliveira, J., Rocha, T.C., Sardelli, C.H., Thom, G., Rêgo, P.S., Santos, M.P.D., Sequeira, F., Vallinoto, M., Aleixo, A., 2019. A dynamic continental moisture gradient drove Amazonian bird diversification. Sci. Adv. 5, eaat5752.
Slager, D.L., Battey, C.J., Bryson Jr., R.W., Voelker, G., Klicka, J., 2014. A multilocus phylogeny of a major New World avian radiation: the Vireonidae. Mol. Phylogenet. Evol. 80, 95–104.
Slager, D.L., Klicka, J., 2014. Polyphyly of Hylophilus and a new genus for the Tawny-crowned Greenlet (Aves: Passeriformes: Vireonidae). 3884, 3.
Smith, B.T., Harvey, M.G., Faircloth, B.C., Glenn, T.C., Brumfield, R.T., 2014. Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. Syst. Biol. 63, 83–95.
Smith, B.T., Klicka, J., 2010. The profound influence of the Late Pliocene Panamanian uplift on the exchange, diversification, and distribution of New World birds. Ecography 33, 333–342.

Smithe, F.B., 1975. Naturalist's color guide. American Museum of Natural History, New York.
Stiles, F.G., Skutch, A.F., Gardner, D., 1989. A Guide to the Birds of Costa Rica. Comstock Publishing Associates Cornell University Press, Ithaca, New York.
Swofford, R., 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
Todd, W.E.C., 1929. A review of the Vireonine genus Pachysylvia. Proc. Biol. Soc. Wash. 42, 181–206.
Tuomisto, H., Van doninck, J., Ruokolainen, K., Moulatlet, G.M., Figueiredo, F.O.G., Sirén, A., Cárdenas, G., Lehtonen, S., Zuquim, G., 2019. Discovering floristic and geoecological gradients across Amazonia. J. Biogeogr. 46, 1734–1748.
Wallace, A.R., 1852. On the monkeys of the Amazon. Alfred Russel Wallace Writings 3.
Wang, X., Edwards, R.L., Auler, A.S., Cheng, H., Kong, X., Wang, Y., Cruz, F.W., Dorale, J. A., Chiang, H.-W., 2017. Hydroclimate changes across the Amazon lowlands over the past 45,000 years. Nature 541, 204–207.
Weir, J.T., Faccio, M.S., Pulido-Santacruz, P., Barrera-Guzman, A.O., Aleixo, A., 2015. Hybridization in headwater regions, and the role of rivers as drivers of speciation in Amazonian birds. Evolution 69, 1823–1834.
Weir, J.T., Price, M., 2011. Andean uplift promotes lowland speciation through vicariance and dispersal in *Dendrocincla* woodcreepers. Mol. Ecol. 20, 4550–4563.
Weir, J.T., Schluter, D., 2008. Calibrating the avian molecular clock. Mol. Ecol. 17, 2321–2328.
Wilman, H., Belmaker, J., Simpson, J., de la Rosa, C., Rivadeneira, M.M., Jetz, W., 2014. EltonTraits 1.0: Species-level foraging attributes of the world's birds and mammals. Ecology 95, 2027.
Yoder, A.D., Olson, L.E., Hanley, C., Heckman, K.L., Rasoloarison, R., Russell, A.L., Ranivo, J., Soarimalala, V., Karanth, K.P., Raselimanana, A.P., Goodman, S.M., 2005. A multidimensional approach for detecting species patterns in Malagasy vertebrates. Proc. Natl. Acad. Sci. 102, 6587–6594.
Zamudio, K.R., Bell, R.C., Mason, N.A., 2016. Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. PNAS 113, 8041–8048.
Zink, R.M., Remsen Jr, J.V., 1986. Evolutionary processes and patterns of geographic variation in birds. Curr. Ornithol. 4.
Zucker, M.R., Harvey, M.G., Oswald, J.A., Cuervo, A.M., Derryberry, E., Brumfield, R.T., 2016. The Mouse-colored Tyrannulet (*Phaeomyias murina*) is a species complex that includes Cocos Flycatcher (*Nesotriccus ridgwayi*), an island form that underwent a population bottleneck. Mol. Phylogenet. Evol. 101, 294–302.