JOURNAL OF
AVIAN BIOLOGY

Research

Ecological traits drive genetic structuring in two open-habitat birds from the morphologically cryptic genus *Elaenia* (Aves: Tyrannidae)

Eliane L. de Freitas, Leonardo Campagna, Bronwyn Butcher, Irby Lovette and Renato Caparroz

E. L. de Freitas (https://orcid.org/0000-0002-8072-3916) ✉ (elianeluizdefreitas@gmail.com) and R. Caparroz (https://orcid.org/0000-0003-4883-4023), Genetics and Biodiversity Laboratory, Dept of Genetics and Morphology, Univ. of Brasília – UnB, Brasília, Brazil. – L. Campagna, B. Butcher and I. Lovette, Fuller Evolutionary Biology Program, Cornell Lab of Ornithol., Ithaca, NY, USA and Dept of Ecology and Evolutionary Biology, Cornell Univ., Ithaca, NY, USA.

Journal of Avian Biology
2022: e02931
doi: 10.1111/jav.02931
Subject Editor: Lu Dong
Editor-in-Chief: Staffan Bensch
Accepted 17 December 2021

Understanding the relative contributions of the many factors that shape population genetic structuring is a central theme in evolutionary and conservation biology. Historically, abiotic or extrinsic factors (such as geographic barriers or climatic shifts) have received greater attention than biotic or intrinsic factors (such as dispersal or migration). This focus stems in part from the logistical difficulties in taking a comparative phylogeographic approach that contrasts species that have experienced similar abiotic conditions during their evolution yet differ in the intrinsic attributes that might shape their genetic structure. To explore the effects of intratropical migration on the genetic structuring of Neotropical birds, we chose two congeneric species, the lesser elaenia *Elaenia chiriquensis* and the plain-crested elaenia *E. cristata*, that are largely sympatric, and which have similar plumage, habitat preferences and breeding phenology. Despite these many commonalities, they differ in migratory behavior: *E. chiriquensis* is an intratropical migratory species while *E. cristata* is sedentary. We used a reduced representation genomic approach to test whether migratory behavior is associated with increased gene flow and therefore lower genetic population structure. As predicted, we found notably stronger genetic structuring in the sedentary species than in the migratory one. *E. cristata* comprises genetic clusters with geographic correspondence throughout its distribution, while there are no geographic groups within Brazil for *E. chiriquensis*. This comparison adds to the growing evidence about how intrinsic traits like migration can shape the genetic structuring of birds, and advances our understanding of the diversification patterns of the understudied, open habitat species from South America.

Keywords: ddRAD-Seq, ecological traits, flycatchers, genetic structure, migratory-sedentary behavior, Neotropics

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Introduction

How different factors contribute to shaping the genetic structure of populations is a central question in evolutionary and conservation biology (Foll and Gaggiotti 2006). The genetic structure of populations is determined by the combined effect of evolutionary forces acting within a population (e.g. mutation, genetic drift and selection) and by the mixing of genetic variation among populations through gene flow (Curnow and Wright 1978). The intensity of gene flow is influenced by a combination of abiotic or extrinsic factors (such as geographic barriers, geological or climatic shifts) (Mairal et al. 2017, Campillo et al. 2020) and/or biotic or intrinsic traits (such as reproductive behaviors, dispersal, migration and adaptation to local environmental conditions) (Burney and Brumfield 2009, Calderón et al. 2014, Smith et al. 2014, Nistelberger et al. 2015).

Phylogeographic studies have traditionally focused more on extrinsic factors, as addressing the effects of biotic factors is often challenging. One method of exploring the genetic effects of intrinsic traits involves comparing species that have experienced similar abiotic conditions during their evolution while diverging in the intrinsic attributes that are hypothesized to have influenced their genetic structuring (Papadopoulou and Knowles 2016).

In birds, migration is an intrinsic trait known to influence the spatial distribution of genetic variation (Arguedas and Parker 2000, Clegg et al. 2003, Irwin et al. 2011, Contina et al. 2019). Migration can be defined operationally as the short to large-scale, cyclic, seasonal movement of a population between breeding and non-breeding areas (Newton 2003). The level of genetic structure in migratory species (as well as in sedentary species) may be influenced by natal philopatry, which describes the tendency of individuals to return to their natal breeding grounds to reproduce (Weatherhead and Forbes 1994). High natal philopatry is expected to restrict gene flow among migrant groups, promoting stronger genetic structuring, whereas low natal philopatry facilitates gene flow, resulting in lower genetic structuring. Particularly in birds, studies have shown that natal philopatry is generally higher in sedentary populations compared to those that are migratory (Weatherhead and Forbes 1994). Furthermore, even when high natal philopatry was observed in migratory species, this occurred mostly in isolated populations on islands, and was hypothesized to be the product of a local adaptation (Wright and Mauck 1998, Förschler et al. 2010).

Most research on migratory birds has focused on species that breed in northern latitudes in North America (Nearctic migrants) and Eurasia (Palearctic migrants), and which migrate south during the Northern Hemisphere winter. Less work has been done on the 'Austral migrant' species of the Southern Hemisphere that migrate north during the Southern Hemisphere winter, and even fewer studies have addressed species that perform annual latitudinal movements on a smaller scale within the tropics, a phenomenon termed 'intratropical migration' (Hayes 1995, Jahn et al. 2020). Although there are more than 200 species of austral and intratropical migrants (Chesser 1994, Stotz et al. 1996, Jahn et al. 2006, 2020) in the Neotropical region, little is currently known about these migratory processes and their evolutionary consequences (Faaborg et al. 2010, Jahn et al. 2020).

Migratory behaviors often vary even among species of the same genus, as is the case in *Elaenia* flycatchers, a genus of 21 species occurring across Central and South America and the Caribbean. In this genus, some species are austral migrants (e.g. *E. chilensis* and *E. parvirostris*), others are intratropical migrants (e.g. *E. chiriquensis*) and others are sedentary (e.g. *E. cristata* and *E. obscura*) (Marini et al. 2009, Guaraldo et al. 2016, Somenzari et al. 2018). The lesser elaenia *E. chiriquensis* and the plain-crested elaenia *E. cristata* are sympatric across most of their geographic distributions (Fig. 1). They have similar preferences for breeding and foraging habitat (Cerrado sensu stricto, highly seasonal savanna), display similar and monomorphic plumage, and are mainly frugivorous (Hosner 2020, Hosner et al. 2020). Molecular phylogenetic studies show that these species represent independent lineages that are easily distinguished genetically (Rheindt et al. 2008). These species do not hybridize nor show high levels of incomplete lineage sorting, as is the case for other species pairs in the same genus (Rheindt et al. 2008, Tang et al. 2018).

The most notable difference between these two *Elaenia* species is in their sedentary/migratory behavior. *E. cristata* lives in open savanna and can be found throughout the year across its entire distribution (Sick 1997, Hosner 2020). In contrast, *E. chiriquensis* can be found throughout the year in some smaller enclaves of savannas surrounded by forest in northern South America (hereafter only northern savannas) and increases its abundance in the center of the Cerrado (the tropical Brazilian savanna) during the breeding season between August and December (Medeiros and Marini 2007, Hosner et al. 2020). After breeding in the central Cerrado, *E. chiriquensis* moves north likely to the Amazonian region (Marini and Cavalcanti 1990). However, the exact migratory routes and the possible existence of resident populations in this species remain unclear.

Technological advances in massive parallel sequencing approaches have enabled fast and low-cost access to a high number of molecular markers, in a large number of individuals (Edwards et al. 2015, Goodwin et al. 2016), making it possible to analyze the genetic structure of populations more robustly (Lavretsky et al. 2019), including that of migratory birds (Kraus et al. 2011, 2013, Jonker et al. 2012, Ruegg et al. 2014, DeSaix et al. 2019, Delmore et al. 2020). Single nucleotide polymorphisms (SNP) are the main molecular marker used in studies that evaluate the genetic structure and levels of gene flow between populations of one or more species (Hohenlohe et al. 2010, Kopuchian et al. 2020), or that identify distinct groups within a migratory species (Kraus et al. 2013, Ruegg et al. 2014).

Here we used a reduced representation genomic approach (ddRAD-Seq) to study the effect of intratropical migration and sedentary behavior on the genetic structure of two Neotropical birds: the migrant (*E. chiriquensis*) and the sedentary (*E. cristata*). Our central hypothesis is that migratory behavior with

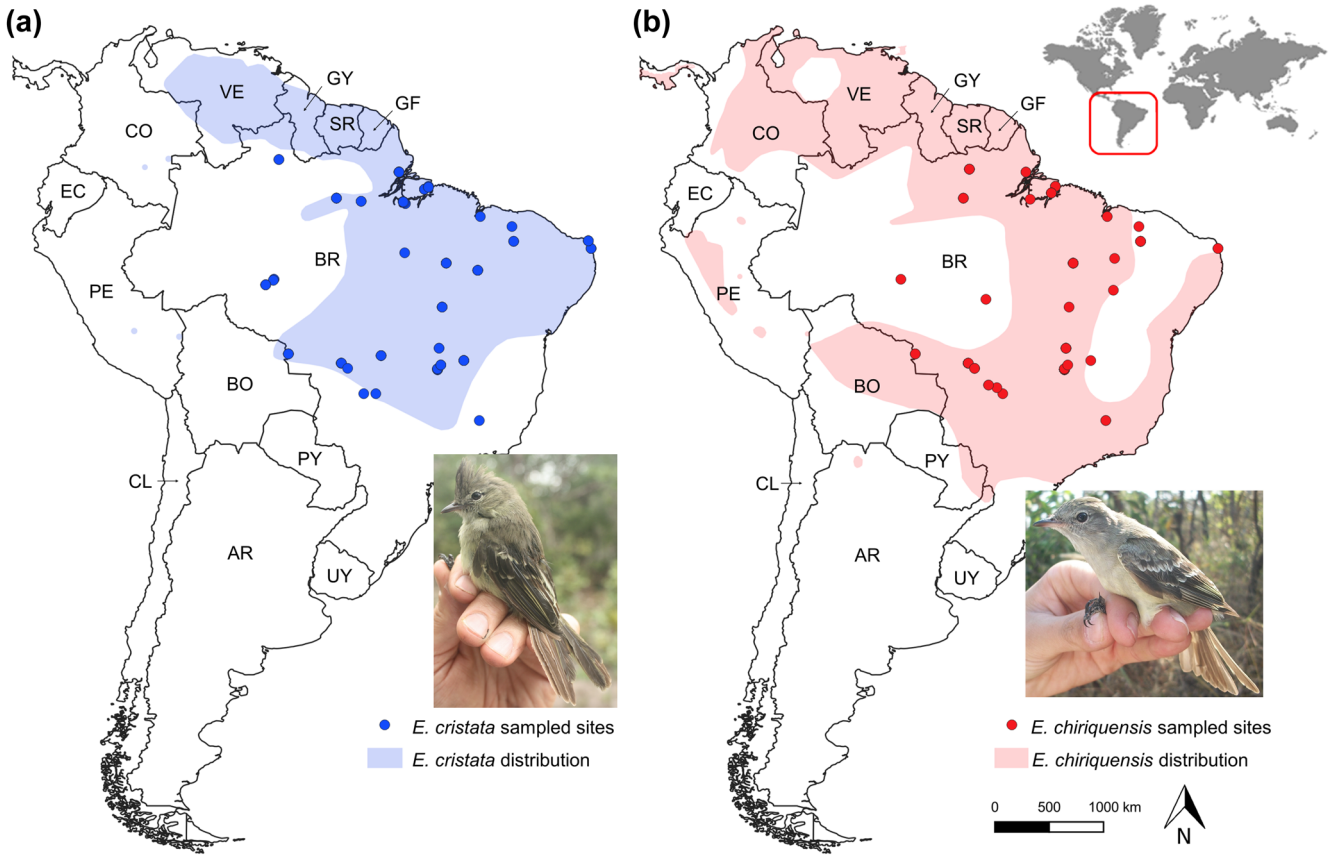

Figure 1. Geographical range distributions of *E. cristata* (a, blue) and *E. chiriquensis* (b, red) based on the International Union for Conservation of Nature (IUCN). Sampling sites are represented by color dots for each species (details in Table 1). Photos: (a) *E. cristata* from Mato Grosso – BR; Chapada dos Guimarães National Park (site 23; Fig. 3); (b) *E. chiriquensis* from Distrito Federal – BR; Protection Area Gama and Cabeça de Veado (site 29; Fig. 4).

lower natal philopatry will result in higher gene flow among populations, and therefore lower population structure in the migratory species compared to the sedentary one.

Material and methods

Species distribution and tissue sampling

We sampled 218 specimens (*E. cristata*, n = 98; *E. chiriquensis*, n = 120, but see the Results section for information about misidentifications) from 2003 to 2018 across 33 sites in South America (Supporting information, summarized in Table 1, Fig. 1). Specimens were captured in the field using mist-nest and banded to avoid duplicate sampling. Approximately 20 µl of blood was obtained from each individual using sterile needles and glass capillary tubes and stored in absolute ethanol at room temperature.

The specimens were identified in the field using slight morphological differences (Hosner 2020, Hosner et al. 2020) or through vocalization whenever possible. Briefly, the diagnostic characters for *E. cristata* are mainly an elongated crown of feathers building a conspicuous crest, without a white coronal patch, and two broad, well-marked wing bars (Fig. 1a). While *E. chiriquensis* exhibits slightly elongated crown feathers forming a less pronounced crest with a variably sized white coronal patch occasionally hidden, and it also has two broad wing bars and whitish edges on the remiges (Fig. 1b). Vocalizations were easy to distinguish by ear between species and when the captured individual vocalized, we also compared this vocalization with recorded vocalizations for the species available in databases such as eBird (<https://ebird.org>) or Xeno-canto (<www.xeno-canto.org>).

To increase the geographic coverage of our sampling, we obtained 108 *Elaenia* tissue samples from ornithological collections for a total of 326 samples (Supporting information).

In both species, most of the individuals (89% for *E. chiriquensis*, and 79% for *E. cristata*) were sampled during the breeding season (August–February), usually during the same expedition in each site (Supporting information).

DNA extraction and quality control

Total genomic DNA was extracted following a phenol, chloroform, isoamyl alcohol protocol (as in Friesen et al. 1997), or using the PureLink Genomic DNA Mini Kit

Table 1. Description of the samples of *E. cristata* and *E. chiriquensis* used in the genetic analyses after removing misidentified or samples with > 80% of missing data. Locality number (No.) corresponding to those shown in Fig. 3 and 4. Sample size for each locality is also shown.

No.	State; collection site	Latitude	Longitude	*E. cristata*	*E. chiriquensis*
1	Roraima; Viruá National Park	1.42	−60.98	2	0
2	Amapá; Cerrado Experiment Field of the Embrapa Amapá	0.39	−51.05	16	2
3	Pará; Óbidos	0.63	−55.72	0	7
4	Pará; Oriximiná	−1.76	−56.22	10	4
5	Pará; Monte Alegre	−2.02	−54.18	3	0
6	Pará; Portel	−1.85	−50.70	10	1
7	Pará; Marajó Island	−0.78	−48.61	10	12
8	Pará; Parauapebas	−6.28	−50.58	1	0
9	Amazonas; Manicoré	−8.47	−61.39	4	1
10	Rondônia; Machadinho	−8.92	−62.07	5	0
11	Maranhão; Urbano Santos	−3.28	−44.33	1	0
12	Maranhão; Chapada das Mesas National Park	−7.13	−47.15	8	11
13	Piauí; Pirarucura	−4.11	−41.71	0	1
14	Piauí; Castelo do Piauí	−5.33	−41.57	2	3
15	Piauí; Guadalupe	−6.74	−43.72	0	1
16	Piauí; Serra das Confusões	−9.37	−43.82	0	1
17	Piauí; Uruçuí	−7.73	−44.54	7	0
18	Rio Grande do Norte; Rio Fogo	−5.29	−35.40	10	0
19	Rio Grande do Norte; Parnamirim	−5.92	−35.17	6	2
20	Tocantins; Ponte Alta do Tocantins	−10.76	−47.48	4	1
21	Mato Grosso; Guarantã do Norte	−10.12	−54.36	0	1
22	Mato Grosso; Vila Bela da Santíssima Trindade	−14.63	−60.20	10	3
23	Mato Grosso; Chapada dos Guimarães National Park	−15.40	−55.83	10	10
24	Mato Grosso; Itiquira	−17.21	−54.14	0	2
25	Mato Grosso; Araguaia	−17.42	−53.45	0	1
26	Mato Grosso; Nova Xavantina	−14.78	−52.53	5	0
27	Goiás; Emas National Park	−17.92	−52.97	2	1
28	Goiás; Chapada dos Veadeiros National Park	−14.16	−47.74	2	8
29	Distrito Federal; Protection Area Gama and Cabeça de Veado	−15.92	−47.87	15	73
30	Distrito Federal; Águas Emendadas Ecological Station	−15.55	−47.59	3	1
31	Minas Gerais; Grande Sertão Veredas National Park	−15.18	−45.69	9	1
32	Minas Gerais; Mateus Leme	−20.14	−44.45	0	2
33	Minas Gerais; Serra Azul	−20.14	−44.41	1	0
Total				156	150

(Invitrogen, Carlsbad, CA, USA) (for museum tissue samples), following manufacturer instructions. Genomic DNA quality and concentrations were verified on a 1% agarose gel stained with ethidium bromide and using the Qubit™ dsDNA BR Assay Kit (ThermoFisher, Waltham, MA, USA), respectively.

Species identification through mitochondrial DNA

As the two *Elaenia* species analyzed are challenging to distinguish morphologically, we confirmed the field identifications using diagnostic variation in the mitochondrial gene NADH dehydrogenase subunit-2 (ND2). Polymerase chain reaction (PCR) amplifications were performed with the primer pair LMET and H6313 (Sorenson et al. 1999) in a final volume of 20 µl using the Phusion High-Fidelity PCR Kit (New England BioLabs, Ipswich, Massachusetts, USA), 10 mM each dNTP, 10 µM of each primer (forward and reverse), 0.4 U of Taq Polymerase and 30–80 ng of genomic DNA. The temperature cycling involved the first step at 98°C for 30 s, followed by 30 cycles of 10 s at 98°C, 30 s at 54°C and 30 s at 72°C, followed by a final extension step of 72°C for 10 min.

PCR products were then treated with exonuclease (EXO) and shrimp alkaline phosphatase (SAP) (ThermoFisher) in a final volume of 1 µl with 10 U μl^{-1} of EXO and 1.0 U μl^{-1} of SAP per 10 µl of PCR product, heated in a thermocycler at 37°C for 30 min, then held at 90°C for 10 min. PCR products were then sequenced at the Cornell University Biotechnology Resource Center Genomics Facility and MACROGEN Inc. Sequencing results were verified using Geneious Prime 2019.0.4 (<www.geneious.com>). The species were identified by comparing the ND2 sequence obtained from each sample with the database nucleotide collection (GenBank) using a Standard Nucleotide BLAST-blastn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) optimized for highly similar sequences (Megablast) (Morgulis et al. 2008).

ddRADseq dataset

We generated ddRADseq loci following the approach outlined by Peterson et al. (2012) with modifications as described by Thrasher et al. (2018). Briefly, we digested each sample with *SbfI* and *MspI* and ligated adapters that allowed multiplexing. The libraries, each containing approximately

20 samples, were size-selected and PCR-enriched, incorporating the Illumina HiSeq adapters (Illumina, San Diego, California, USA). Finally, all groups of samples were combined in equimolar proportions and sequenced, single end 100 bp, on two lanes of an Illumina HiSeq 2500.

After assessing read quality with FastQC (<www.bioinformatics.babraham.ac.uk/projects/fastqc>), we used FASTX-Toolkit (<http://hannonlab.cshl.edu/fastx_toolkit>) to trim sequences to 98 bp to discard lower-quality base calls at the 3′ end of the sequence. Subsequently, we used FASTX-Toolkit to retain reads without a single base below a Phred quality score of 10 and with at least 95% of bases with quality above 20. We demultiplexed reads using the 'process_radtags' program from the STACKS ver. 2.41 bioinformatics pipeline (Catchen et al. 2011, 2013), discarding reads that did not pass the Illumina filter, had barcode contamination, lacked an *SbfI* cut site or one of the unique barcodes used for multiplexing at the 5′ end. We obtained an average of 550 757 (± 263 678 reads) quality-filtered reads per individual (Supporting information).

We assembled the reads from both species into RADseq loci using the de novo pipeline from STACKS. We conducted a sensitivity analysis by testing different values for coverage (m = 5, 10, 20 and 30) as suggested by Rochette and Catchen (2017) yet did not find substantial differences in the number of loci recovered. Parameters were therefore set to a minimum coverage of 5 (m), up to seven differences between alleles of the same locus (M) and seven differences among aligned loci of different individuals (n). This combination of parameters produced an average coverage per locus ranging from 11.24 to 68.55x, with an overall average of 29.61x (± 9.2). We exported SNPs using the program 'populations' in STACKS for all the samples combined (n = 326) and again for each species separately, *E cristata* (n = 156) and *E. chiriquensis* (n = 150). We retained loci that were present in at least 80% of the individuals and exported both one SNP per RADSeq locus (to avoid including linked loci) and all SNP per RADSeq loci.

Principal component analysis

We conducted a principal component analysis of the SNP dataset using the package SNPRelate (Zheng et al. 2012) in R (<www.r-project.org>) to assess possible groupings among individuals. For this analysis, we exported SNPs using 'populations' from STACKS in four different ways: 1) for all the specimens (n = 326 and 5938 SNPs); 2) removing both all the specimens misidentified in the field (Results) and samples with more than 80% of missing data for all loci (n = 306 and 6458 SNPs); 3) only with *E. cristata* individuals (n = 156 and 6447 SNPs); and 4) only *E. chiriquensis* samples (n = 150 and 9671 SNPs).

Phylogenetic analyses

We built a phylogenetic tree using RAxML ver. 8.2.9 (Stamatakis 2014) for both species using the SNP dataset in Phylip format (variant sites only), and the following parameters: 500 replicates of rapid bootstrap analysis, and the *ASC_GTRGAMMA* model with the Lewis correction for ascertainment bias.

Population genetic structure

We performed Structure analyses using the SNP datasets obtained for each species separately and one SNP per locus to avoid the effect of linkage (2814 SNPs for *E. cristata*, and 2765 for *E. chiriquensis*). We conducted analyses at K values ranging from 1 to 10 for *E. cristata* and 1 to 5 for *E. chiriquensis*, with 10 replicate runs at each value. We also performed a Structure analysis for a subsample of *E. cristata* from central Brazil (localities, 8, 11, 12, 14, 17, 20, 23, 26, 27, 28, 29, 30, 31 and 33; Fig. 3c), with K values ranging from 1 to 6. Each run included 500 000 iterations of burn-in followed by 1 000 000 sampling iterations. We estimated the best value of K using the method of Evanno et al. (2005). Using the groups from the Structure results, we calculated F_{ST} among populations within *E. cristata* using the 'populations' module of STACKS (parameter 'fststats'). Due to the weak genetic structure observed in *E. chiriquensis*, we did not estimate the F_{ST} values for this species as we did not have clearly defined populations.

The level of intraspecific genetic structure was also assessed using haplotype information in fineRAD Structure ver. 0.3 and RADpainter (Lawson et al. 2012, Malinsky et al. 2018). Briefly, this algorithm works in four steps: 1) it calculates the co-ancestry matrix using the SNP dataset, 2) clusters individuals based on the co-ancestry matrix, 3) builds a dendrogram tree; and 4) plots results using the fineRADstructurePlot.R script in R (<www.r-project.org>). Haplotype datasets were produced with 'populations' in STACKS, for each species separately, and without a filter for minor allele frequency.

Results

Elaenia species identification

Of the 326 specimens analyzed in our study (including samples from both museum collections and wild birds), 273 (83.74%) had a portion of the mitochondrial ND2 gene amplified successfully. From the 186 specimens morphologically identified by our team in the field and with successfully amplified ND2 sequences, 10 (5.4%) were misidentified to species. From the 87 tissue samples requested from ornithological collections with ND2 data, 18 (20.7%) were misidentified to species. Among these 28 misidentified specimens, 12 genetically identified *E. chiriquensis* individuals were previously identified as *E. cristata*, seven specimens previously identified as *E. cristata* were genetically *E. chiriquensis* and nine have been genetically identified as other species of *Elaenia* and even another morphologically similar tyrant flycatcher *Sublegatus arenarum*. While high, this field identification error rate is not surprising given the high phenotypic similarity of these species and the fact that samples were

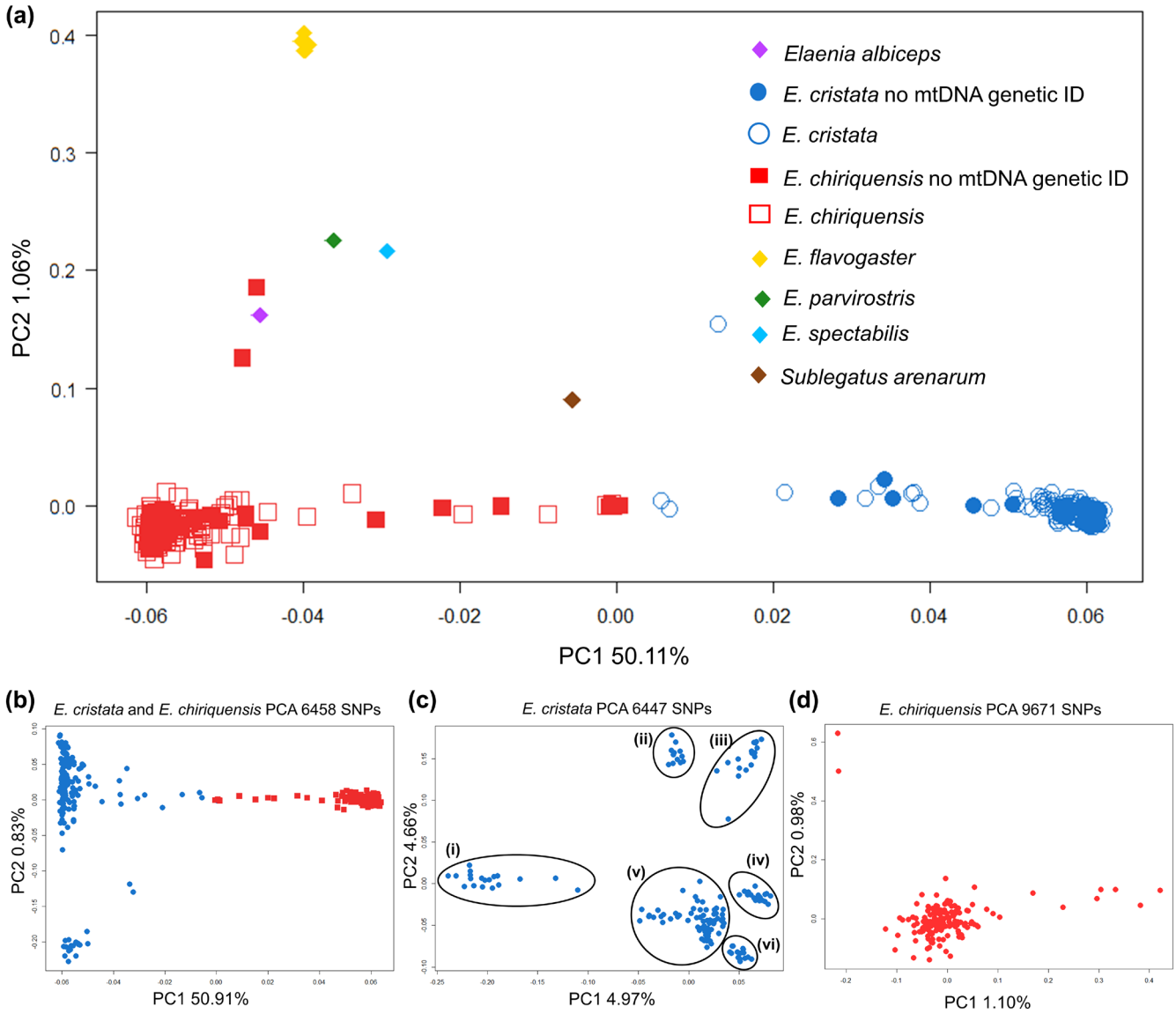

Figure 2. Principal component analyses (PCAs) derived from different sets of SNPs and *Elaenia*/*Sublegatus* samples (symbols). (a) 5983 SNPs from 326 samples. Diamond symbols refer to other species detected after genetic identification. Closed circles refer *E. cristata* species with no mtDNA genetic identification while open circles refer to samples with mitochondrial genetic identification. Closed squares refer to *E. chiriquensis* without genetic identification, and open squares to those with the genetic ID (see Methods, for details). (b) 6458 SNPs from 156 *E. cristata* and 150 *E. chiriquensis* samples after removing nine misidentified samples and samples with missing data > 80%. (c) 6447 SNPs from 156 *E. cristata*. The ellipses indicate groups with geographic correspondence, localities numbers are described in Table 1: (i) sites 9, 10 and 22; (ii) 1 and 4; (iii) 2 and 5; (iv) 6 and 7; (v) Brazilian central region; (vi) sites 18 and 19. (d) 9671 SNPs from 150 *E. chiriquensis*.

collected by many different people with varied levels of experience with these taxa. The PCA results based on the SNP data from all our samples and colored based on the mtDNA genetic identification show how errors can occur when using only morphological characters to identify species in the genus *Elaenia* (Fig. 2a, Supporting information). We determined that this larger dataset included representatives of six *Elaenia* species (*E. cristata*, *E. chiriquensis*, *E. albiceps*, *E. flavogaster*, *E. parvirostris* and *E. spectabilis*), and two samples from another flycatcher species *Sublegatus arenarum*. Because of this rate of misidentification, we only included in our final genomic analyses the *Elaenia* samples (n = 33) for which we did not confirm the field identification with mitochondrial DNA (failed to be amplified) if they grouped diagnostically in the PCA with those with confirmed identifications (Fig. 2a).

There is no overlap between *E. cristata* and *E. chiriquensis* in the PCA based on the SNP dataset derived from these two species alone (Fig. 2b). In this analysis, the first two principal components explain 51.17% of the total variation. To explore patterns of intraspecific variation we ran subsequent analyses on samples from each of these species alone. The PCA from *E. cristata* (the sedentary species; Fig. 2c) explained

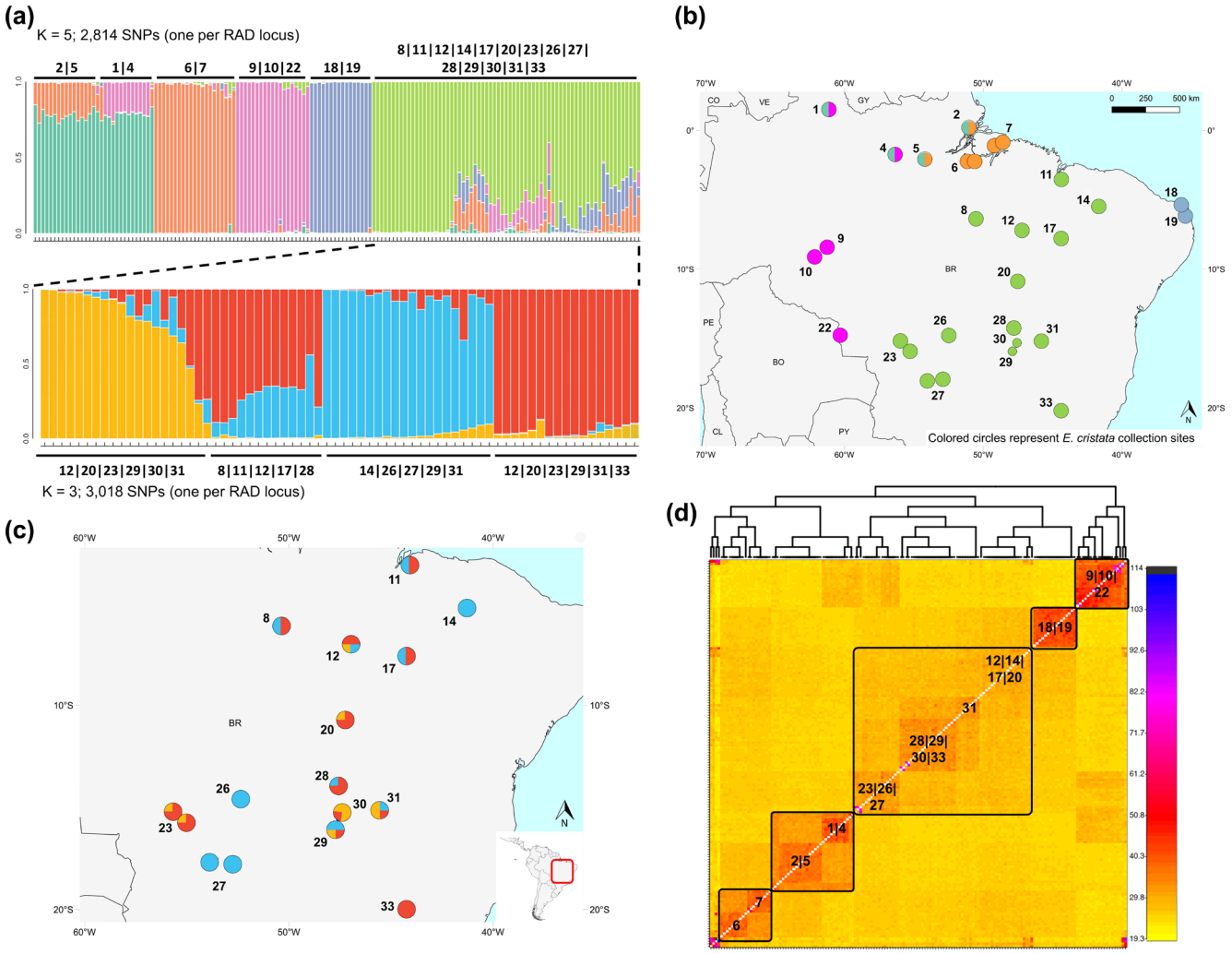

Figure 3. Pattern of genetic structure in the resident species (*E. cristata*). (a) Structure analysis based on 2814 SNPs (one per RAD locus) and 156 specimens showing five populations (K = 5) across all sampled sites (top). When we looked into the Brazilian central region ('green group') in further detail, the 70 sampled specimens were grouped into three populations (K = 3) in an analysis based on 3018 SNPs (bottom). (b) Geographical distribution of genetic clusters detected in the Structure analysis across all sampled sites; colored circles represent groups according to the Structure results; numbers correspond to sampled sites described in Table 1. (c) Geographical distribution of genetic clusters detected in the Structure analysis across sites in central Brazil. (d) The fineRADstructure plot derived from haplotype data (n = 156) indicating the clusters formed according to geographic location (numbers correspond to the localities shown on the maps).

9.63% of the variation and included several distinct clusters in the space of the two first principal components, whereas the equivalent plot for *E. chiriquensis* (the migratory species; Fig. 2d) explained only 2.08% of the total genetic variation and had no apparent clustering.

Genetic clusters and moderate structure in the sedentary species

The results from the PCA were consistent with those from the remaining analyses, where *E. cristata* and *E. chiriquensis* consistently showed distinct patterns of population structure. Structure results showed that in the sedentary species (*E. cristata*), the K values with the highest likelihood were 3 and 5, with the K = 5 pattern showing genetic clusters that can be explained geographically (Fig. 3a, Supporting information). The five clusters correspond to two groups in the northern savannas, one in the southwestern Amazon Forest, one in the extreme northeast of Brazil, and a larger group including all other collection locations from the Cerrado. The Structure results obtained when analyzing individuals from this last large group alone also indicated the possibility for finer population structure, with evidence for three additional clusters (Fig. 3a, Supporting information). The central portion (site 29, Fig. 3c) contains admixed individuals with the genetic composition of all three groups, while some localities such as Nova Xavantina and Emas National Park show less evidence of admixture (sites 26 and 27; Fig. 3c). We obtained similar results in our fineRAD Structure analysis (Fig. 3d), observing five co-ancestry groups with additional

genetic sub-structuring within two of them. Finally, phylogenetic analysis with RAxML showed the unrooted *E. cristata* tree with four well-supported branches (bootstrap > 85) (Supporting information). These branches corresponded to specific geographic regions shown in the map (Fig. 3b).

The mean pairwise F_{ST} among the five genetic populations resulting from Structure for *E. cristata* ranged from 0.028 to 0.109 (Supporting information). The lowest values are derived from the comparisons between the population in the central region of the distribution and other populations (Supporting information).

Few genetic clusters and low structure in the migratory species

In contrast to the results from the sedentary species, for the migratory lesser elaenia *E. chiriquensis*, PCA analysis grouped samples into one cluster (Fig. 2d). Similarly, Structure results supported K = 2 (Fig. 4a, Supporting information) with most of our sampling localities belonging to a single genetic population, but with a discrete separation in a group around the Amazon rainforest, formed by individuals from Óbidos, Oxiriminá and Manicoré (sites 3, 4 and 9; Fig. 4b). The fineRAD Structure results also recovered this group, and an additional one which included individuals from Ilha do Marajó (site 7; Fig. 4b). The RAxML analysis did not show any well-supported clades (Supporting information).

Discussion

Assuming that both *Elaenia* species studied here, which have highly overlapping ranges, have experienced a similar historical geographic and climatic context (extrinsic factors which could shape their population structure), our results suggest that their intrinsic sedentary/migratory behavior has influenced their genetic structure. As we predicted, the sedentary species showed greater genetic structuring compared to the migratory one, across a wide area of South America where both species are sympatric (Fig. 1).

Patterns of genetic structure for *E. chiriquensis*

The low genetic structure found for migratory *E. chiriquensis* had already been preliminarily observed using other marker types (Bates et al. 2003, Rheindt et al. 2015) and suggests substantial recent gene flow throughout the species' range, which is primarily in South America. As described in previous studies (Marini and Cavalcanti 1990, Medeiros and Marini 2007, De Paiva and Marini 2013), the abundance of this species increases significantly between August and December in the south-central region of the distribution in areas with Cerrado sensu stricto vegetation, a habitat described as preferential for nest building by the species. After breeding, studies suggest that birds fly to areas in the north of the distribution, becoming absent in some regions in the southern Cerrado during the non-breeding period (Marini and Cavalcanti 1990).

Some authors have considered *E. chiriquensis* as partially migratory (Somenzari et al. 2018) based on the absence of records in the central Cerrado between June and August (Marini and Cavalcanti 1990), while individuals have been observed in northern Brazil throughout the year. Intraspecific differences in migratory behavior among individuals within the species can lead to temporal and spatial reproductive asynchrony promoting divergence of neutral genetic variation between sedentary and migrant populations (Burney and Brumfield 2009). For instance, migratory behavior was recently identified as a driver for the diversification of subspecies of an austral migratory bird species (*Tyrannus savanna*, Gómez-Bahamón et al. 2020).

Although a high degree of gene flow was observed in the sampled migratory populations of Lesser Elaenia, we still observed a modest level of genetic structuring among the populations from the northern (site 7, Fig. 4c) and western portion of the sampled area (sites 3, 4 and 9, Fig. 4c). The cluster analyses showed two or three populations (Structure, and FineRAD Structure, respectively) in this periphery of the Amazonian region: one population was formed by sites 3, 4 and 9, and another population by site 7 (Fig. 4). However, some admixed individuals of these populations were also collected in the central Cerrado (see site 29 in Fig. 3a) during the breeding season. Even though our data suggests a weak evidence of structuring for *E. chiriquensis* in our sampling of migratory populations (Guaraldo et al. 2021), we can't rule out the possibility of potential structuring among the resident populations in the non-sampled regions of its distribution. Future studies should include individuals from populations of *E. chiriquensis* in regions of Central America to confirm if this pattern of low genetic structure applies to the entire species. The absence of migration in some populations can lead to a decrease in gene flow and, consequently, higher population structure, as seen in the congeneric *E. cristata*.

The generally low genetic structure observed in the migratory species is consistent with the expectation of low natal philopatry as described by Weatherhead and Forbes (1994). This type of pattern occurs when individuals in a given area migrate to multiple areas in successive reproductive seasons. Occurrence data of this species throughout the year suggest that migrants come from the north of the distribution, where records of the species presence are constant throughout the year (Marini and Cavalcanti 1990), and reproduce in different regions in the south-central portion of the distribution. Furthermore, isotopic data indicate that the migrant *E. chiriquensis* exhibits a niche-following behavior (it seeks similar resources throughout the annual cycle), suggesting that despite flying north, it spends the winter in savanna areas (Guaraldo et al. 2016). Similarly, our data suggest a connection between savanna patches in northern South America and the central Cerrado region (Fig. 4). In fact, the genetic differentiation of the migrant populations that occur in these savanna patches which are interlocked in a forest environment may be associated with greater natal philopatry due to their isolation. Isolated migrant populations of passerines often show

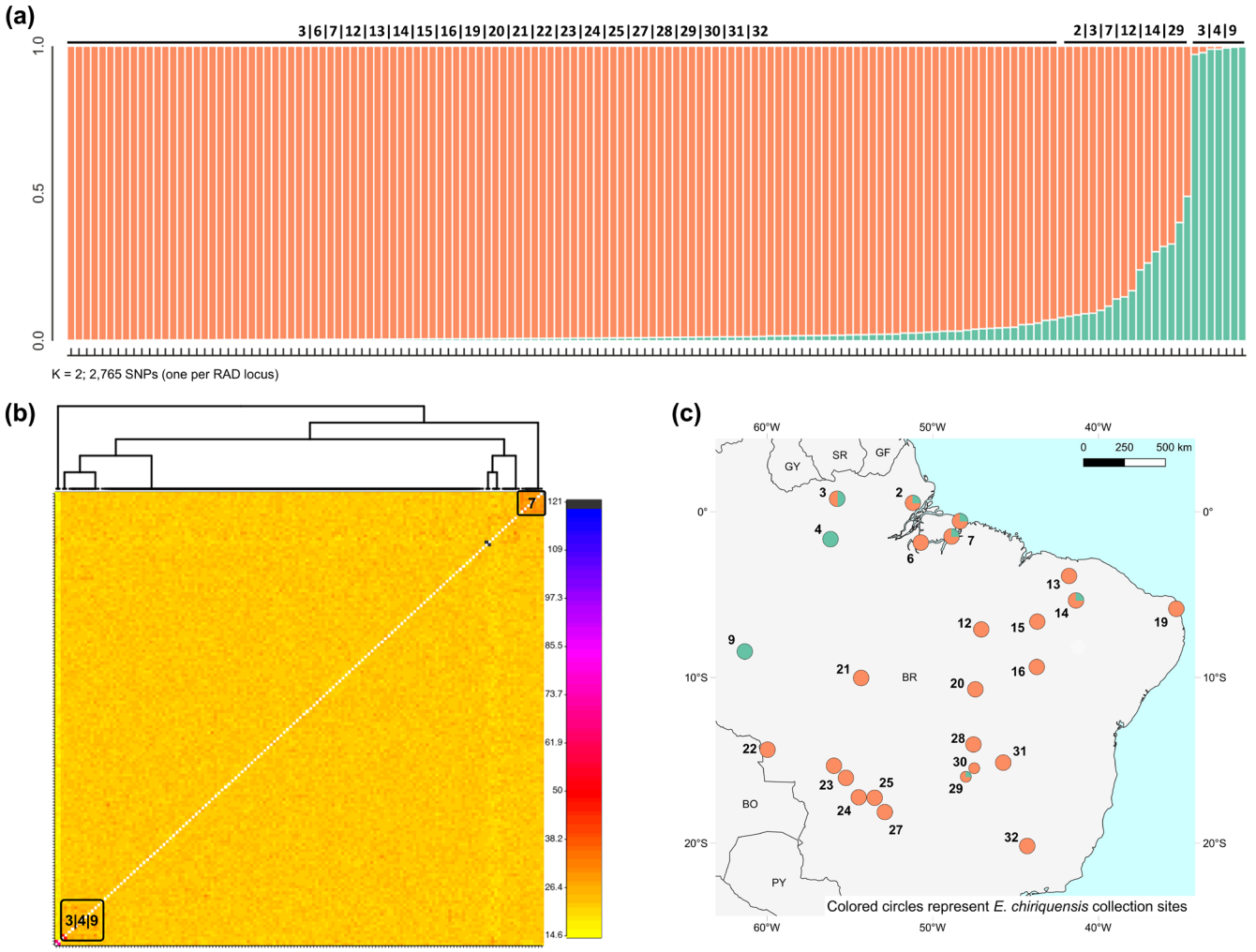

Figure 4. Pattern of genetic structure in the migratory species (*E. chiriquensis*). (a) The Structure analysis based on 2765 SNPs (one per RAD locus) and 150 specimens showed 2 populations (K=2) across all sampled sites. (b) The fineRADstructure plot derived from haplotype data (n=150) indicating the clusters formed according to geographic location (the numbers correspond to sampled sites shown on the maps). (c) Geographic distribution of genetic clusters detected in the Structure analysis across all sampled sites; colored circles represent groups according to the Structure results (not proportional to the number of sampled individuals); numbers correspond to sampled sites described in Table 1.

considerably higher philopatry than non-isolated populations (Weatherhead and Forbes 1994, Wright and Mauck 1998).

Alternatively, the Amazon rainforest could be a partially effective geographic barrier for this species, limiting migration and consequently decreasing gene flow among the migrant populations. The congruence of the genetic clusters formed in the northern savannas and peri-Amazonian area in both *Elaenia* species studied suggests the presence of an extrinsic barrier locally driving the genetic structure in these two species. In fact, these two factors (philopatry and a geographic barrier) are not mutually exclusive and may be acting together to shape the genetic structure of these species, and the relative contribution of each should be better investigated.

Genetic structuring in *E. cristata*

As expected, the sedentary species showed greater genetic structure, and we identified five genetic populations (Fig. 3; K=5; pairwise F_{ST} ranges from 0.028 to 0.109): two of them occurred in patches of savannas nested in the Amazonian region and the other three in southwestern, central and northeastern South America. The different populations of the north have a strong geographic association with the different savanna areas, indicating that the processes that led to the disjunct formation of these areas may have also influenced these avian population dynamics.

Despite observing greater genetic structuring in central and northeastern South America for this sedentary species, the magnitude of the genetic difference among these populations is small, consistent with recent isolation and/or some ongoing gene flow. One of these populations occurs in the northeast, in the Caatinga, the largest patch of the Seasonally Dry Tropical Forest of South America with a predominance of xeric vegetation (for details, Werneck 2011), while the other populations inhabit the Cerrado. Genetically differentiated populations occurring in the different open vegetation

biomes of South America have been identified in several organisms (Wuster et al. 2005, Ramos et al. 2007), including in other birds (Rocha et al. 2020).

Most previous studies of differentiation in Neotropical birds from open areas have found evidence for Pleistocene climatic oscillations as a driver of intraspecific divergence (Lima-Rezende et al. 2019b, Rocha et al. 2020, Ritter et al. 2021). For instance, the genetic differentiation of narrow-billed woodcreeper *Lepidocolaptes angustirostris* populations seems to have occurred in allopatry in stable areas that formed during Pleistocene climatic fluctuations (Rocha et al. 2020). Similarly, studies of other bird species that occur in the Cerrado have identified a weak intraspecific genetic structure, possibly due to the increase in gene flow between populations promoted by the expansion of climatically suitable areas for these species during the Pleistocene (Lima-Rezende et al. 2019a, Rocha et al. 2020).

In this context, it is important to highlight that our study assumes that the two species studied underwent similar historical demographic processes, but we can't completely rule out that these species, despite being ecologically similar, did not experience different geographic or climatic contexts at different times in the past. For instance, these two bird species might have occurred in distinct stable areas during the Pleistocene climatic oscillations, but the current intense gene flow promoted by migratory behavior in *E. chiriquensis* may have erased the genetic signature of historical isolation among previously isolated populations.

Elaenia sp. misidentification

About 8.5% (n=28) of the specimens collected for this study were misidentified in the field at the time of sample collection, which is not surprising given their morphological similarity (Fig. 1). Most of the cases involved the two focal species, with some *E. cristata* identified in the field being genetically identified as *E. chiriquensis* and vice versa. In a few cases (n=9), other species of *Elaenia* and even another morphologically similar tyrant flycatcher *Sublegatus arenarum* were misidentified as the two focal species of this study. This non-trivial field identification error rate underscores the difficulty with working in this challenging group of morphologically cryptic species. The genus *Elaenia* comprises 21 species that are all quite similar morphologically, and their misidentification has been widely reported (Traylor 1982, Hosner Hosner 2004, Winkler et al. 2020). For instance, Rheindt et al. (2015), in a similar study using the ND2 mtDNA gene, also found one misidentified *Elaenia* sample (*E. c. albivertex* labeled as *E. flavogaster*) out of 13 samples obtained from ornithological collections.

In general, field identification of *Elaenia* species is based either on the birds' vocalizations or on the species' geographical distribution (Sick 1997). Criteria based on geographic distribution can be challenging when species are sympatric or when there are migratory species involved. *Elaenia cristata* is thought to be partially migratory in some regions (Hosner 2020), such as in the Mato Grosso state in Brazil. We found 12 cases of misidentified *E. cristata* labeled as *E. chiriquensis*, three of them in Mato Grosso state and five in Pará state, localities that may be in the migratory routes of other species of *Elaenia*. Therefore, misidentification between sedentary and migratory *Elaenia* species can lead to the eventual misinterpretation of migratory behavior. Owing to the difficulty of using morphology to identify *Elaenia* species, we encourage using complementary species identification methods, such as DNA barcode approaches that have been successfully applied in the identification of many Tyrannidae species (Kerr et al. 2007, Chaves et al. 2008).

Conclusions

Our study adds evidence on how migratory behavior, as an intrinsic factor, can shape the genetic structure of Neotropical bird species and improves our understanding of the diversification patterns of open habitat South American species. As expected, migratory behavior can lead to a weak genetic structure, likely the product of substantial ongoing gene flow among populations. Similar patterns may exist among the other 200 species of migratory Neotropical birds, in contrast to the high levels of geographic structuring known to exist within many sedentary Neotropical species.

Acknowledgements – We are grateful to the directors and employees of all of our sampling locations for assistance during fieldwork and the curators of the Ornithological Collections in Brazil for providing tissues samples for this research. We also thank the Laboratório de Genética e Biodiversidade/UnB team, especially Gislaine Fernandes, for assistance during fieldwork and the Cornell Lab of Ornithology team. We thank Getúlio Oliveira Jr. for helpful comments on earlier drafts of the manuscript.

Funding – ELF was supported by scholarship CAPES (no. 1583777) and CNPq (no. 140440/2017-8), also CAPES PDSE no. 47/2017 awards 88881.190324/2018-01. This study is part of the GENPAC (564036/2010-2) and PPBio (457444/2012-6) projects supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)/Ministério da Ciência, Tecnologia e Inovação (MCTI)/Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Permits - Trapping and tissue sampling were allowed by the Research Ethics Committees of University of Brasília (UnBDOC no. 75111/2013) by the ICMBio/Sistema de Autorização e Informação em Biodiversidade, and Centro Nacional de Pesquisa e Conservação de Aves Silvestres (CEMAVE)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)/Sistema Nacional de Anilhamento (SNA).

Conflict of interest – The authors declare that they have no conflicts of interest.

Author contributions

Eliane L. Freitas: Conceptualization (lead); Data curation (lead); Formal analysis (equal); Funding acquisition (equal); Investigation (lead); Methodology (lead); Project administration (supporting); Software (equal); Visualization (equal); Writing – original draft (lead); Writing – review and editing

(supporting). **Leonardo Campagna**: Conceptualization (supporting); Data curation (equal); Formal analysis (lead); Funding acquisition (supporting); Investigation (equal); Methodology (equal); Software (lead); Supervision (equal); Writing – original draft (supporting); Writing – review and editing (lead). **Bronwyn Butcher**: Conceptualization (supporting); Data curation (equal); Formal analysis (equal); Funding acquisition (supporting); Investigation (supporting); Methodology (equal); Resources (supporting); Software (equal); Supervision (supporting); Writing – review and editing (equal). **Irby Lovette**: Conceptualization (supporting); Data curation (supporting); Formal analysis (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (lead); Resources (lead); Software (equal); Supervision (lead); Writing – original draft (supporting); Writing – review and editing (lead). **Renato Caparroz**: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (lead); Resources (lead); Software (equal); Supervision (lead); Writing – original draft (supporting); Writing – review and editing (lead).

Transparent Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/jav.02931>.

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.jdfn2z3cf> (Freitas et al. 2022).

Supporting information

The supporting information associated with this article is available from the online version.

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