Received: 24 November 2022 | Revised: 21 September 2023 | Accepted: 25 September 2023

DOI: 10.1111/jbi.14738

Journal of Biogeography

WILEY

RESEARCH ARTICLE

The role of glaciations in the evolutionary history of a widely distributed Neotropical open habitat bird

Belén Bukowski[1] | Leonardo Campagna[2,3] | María José Rodríguez-Cajarville[1] | Gustavo S. Cabanne[1] | Pablo L. Tubaro[1] | Darío A. Lijtmaer[1]

[1]División Ornitología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-CONICET), Buenos Aires, Argentina

[2]Cornell Lab of Ornithology, Fuller Evolutionary Biology Program, Ithaca, New York, USA

[3]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York, USA

Correspondence

Belén Bukowski, División Ornitología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-CONICET), Buenos Aires, Argentina.

Email: belenbukowski@gmail.com

Funding information

Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación; Consejo Nacional de Investigaciones Científicas y Técnicas; Fundación Williams; Richard Lounsbery Fundation

Abstract

Aim: The Neotropics constitute the most biodiverse region of the world, yet its patterns of diversification and speciation differ among Neotropical areas and are not equally well understood. Particularly, avian evolutionary processes are understudied in the open habitats of temperate South America, where the role of glacial cycles is not clear. We analysed the evolutionary history of a Neotropical widespread bird species as a case study to evaluate its continental-scale patterns and processes of diversification, with a focus on Patagonia.

Location: Open habitats of the Neotropics.

Taxon: *Vanellus chilensis* (Aves, Charadriiformes).

Methods: We obtained reduced representation genomic and mitochondrial data from the four subspecies of *V. chilensis* to perform a phylogenetic/phylogeographical analysis and study the evolutionary history of the species. We complemented these analyses with the study of vocalizations, a reproductive signal in birds.

Results: The initial diversification event within *V. chilensis*, approximately 600,000 years ago, split a Patagonian lineage from one containing individuals from the rest of the Neotropics. We found considerable gene flow between these two lineages and a contact zone in northern Patagonia, and showed that genomic admixture extends to northwestern Argentina. Shallower divergence was detected between the two non-Patagonian subspecies, which are separated by the Amazon River. Vocalizations were significantly different between the two main lineages and were intermediate in their temporal and frequency characteristics in the contact zone.

Main Conclusions: Patagonian populations of *V. chilensis* are clearly differentiated from those of the rest of the Neotropics, possibly as a consequence of Pleistocene glaciations. A secondary contact zone in northern Patagonia with extensive gene flow among lineages appears to be the consequence of post-glacial, northward expansion of the Patagonian populations. Future analyses focused on the dynamics of the contact zone will allow us to establish whether the species continues to diverge or is homogenizing.

KEYWORDS

evolutionary history, gene flow, Patagonia, Pleistocene glaciations, refugia, secondary contact zone, vocalizations

1 | INTRODUCTION

The Neotropics constitute the most biodiverse region of the world (Rosenzweig, 1995), thus rendering the study of its patterns of diversification and speciation particularly relevant. Although the evolutionary history of Neotropial birds has been traditionally less studied than in other regions (Beheregaray, 2008; Reddy, 2014; Turchetto-Zolet et al., 2013), this started to change in the last couple of decades, mainly due to studies performed in the Andes (e.g. Brumfield & Edwards, 2007; Sedano & Burns, 2010; Weir, 2006), Amazonia (e.g. Aleixo & Rossetti, 2007; Naka & Brumfield, 2018; Ribas et al., 2012) and the Atlantic Forest (e.g. Cabanne et al., 2008; Lavinia et al., 2019; Trujillo-Arias et al., 2020). These studies started to shed light on the processes responsible for avian diversification in the region and made clear that they depend on a complex array of factors and their interaction (Rull, 2011; Rull & Carnaval, 2020; Turchetto-Zolet et al., 2013). Among these factors, the Andes Mountains stand out as they constitute a geographical barrier for lowland bird populations (Brumfield & Edwards, 2007; Fernandes et al., 2014; Weir & Price, 2011) and have also promoted diversification of highland taxa (Beckman & Wiit, 2015; Campagna et al., 2011; Weir, 2006). Other Neotropical drivers of diversification include wide rivers (Kopuchian et al., 2020; Naka & Brumfield, 2018; Ribas et al., 2012) and the open vegetation corridor that isolates Amazonia and the Andean forests from the Atlantic Forest (Cabanne et al., 2019; Lavinia et al., 2019; Trujillo-Arias et al., 2020).

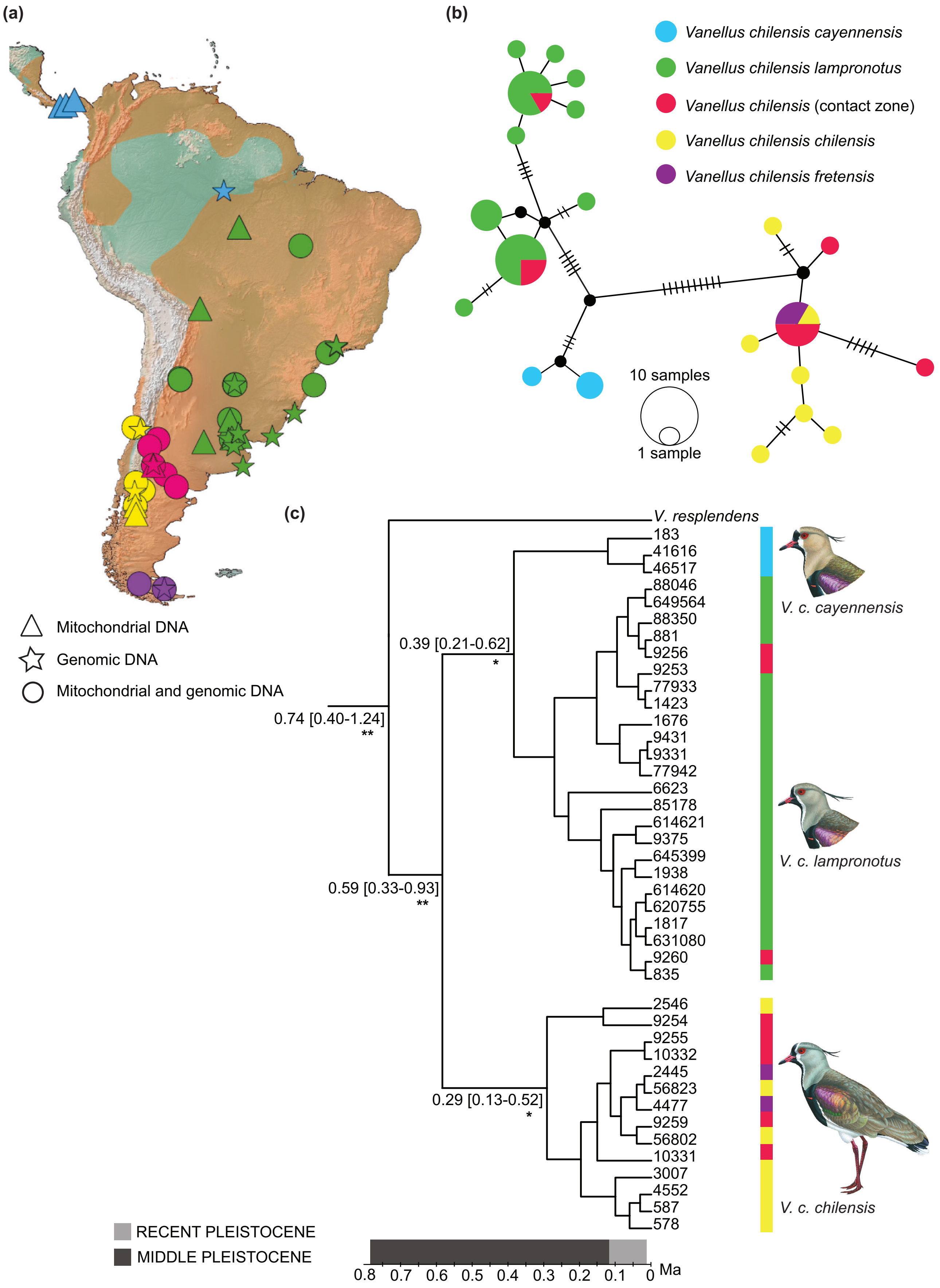
Pleistocene glacial cycles also fuelled avian diversification. In fact, they are considered a key speciation factor in the Nearctic and the Palearctic (particularly in boreal regions; Lovette, 2005), where the advance of the ice sheets isolated avian populations, promoting allopatric speciation (Lessa et al., 2003; Weir & Schluter, 2004) and resulting in a high proportion of recently diverged species with limited phylogeographical structure (Lijtmaer et al., 2011; Lovette, 2005). In the Neotropics, glacial cycles were also relevant for avian diversification, but not due to the advance of large ice sheets. Instead, Pleistocene climate oscillations triggered modifications in the vegetation and generated habitat fragmentation (mostly in tropical forests; e.g. Carnaval & Moritz, 2008; Pennington et al., 2000), promoting an increase both in phylogeographical structuring and speciation (e.g. Cabanne et al., 2008; Rull, 2011; Silva et al., 2019; Thom et al., 2020).

More similarly to the Northern Hemisphere, in Patagonia (the southern extreme of the continent) and in the highland habitats of the Andes, the direct advance of the ice and the expansion of glaciers during Pleistocene glaciations presumably isolated bird populations in refugia (Kopuchian et al., 2016; Weir, 2006) and generated an increase in their diversification rates (Jetz et al., 2012; Weir, 2006). However, southern South America has been comparatively much less studied than the rest of the Neotropics and the diversification processes in the region are still not as clear (Turchetto-Zolet et al., 2013). Studies performed in plants and vertebrates in Patagonia suggest that diversification patterns in this area were also more complex than in North America and were generated both by glacial cycles and pre-Pleistocene geological factors (Sánchez et al., 2021; Sérsic et al., 2011; Turchetto-Zolet et al., 2013). The effect of glaciations on Patagonian birds has been particularly understudied, but the few analyses performed so far suggest a marked effect of glaciations that affected several species (Acosta et al., 2020; Campagna et al., 2012; Kopuchian et al., 2016).

The main diversification drivers proposed for the Neotropics (such as the Andes Mountains and wide rivers) are expected to generate geographical isolation between populations and their consequent diversification in allopatry. However, genomic studies in birds and other animal groups clearly show that gene flow between diverging populations is much more common than traditionally considered (Lamichhaney et al., 2015; Poelstra et al., 2014; Pulido-Santacruz et al., 2019; Winker, 2021). Among other causes, this can be a consequence of speciation in sympatry or without clear geographical barriers (Barluenga et al., 2006; Lamichhaney et al., 2017; Martin et al., 2013; Rodríguez-Cajarville et al., 2022; Turbek et al., 2021) or the fact that barriers are more permeable than previously considered (Lavinia et al., 2019; Rodríguez-Gómez et al., 2013; Smith et al., 2014). In this context, the study of diversification and speciation in the Neotropics should not only assess the factors involved but also analyse how diversification occurred, if there is evidence of gene flow and, if this is the case, whether it occurred during diversification or as a consequence of secondary contact (Luzuriaga-Aveiga et al., 2021).

The Southern Lapwing (*Vanellus chilensis*; Charadriiformes) is a particularly pertinent species to study the influence of different diversification factors in the Neotropics. First, it is widespread and regionally common, distributed from southern Nicaragua (12° N) to Tierra del Fuego, Argentina (55° S; Piersman, 1996; Figure 1a), and found mainly in the lowlands but also in high

FIGURE 1 Distribution of *V. chilensis*, sampling information and analyses of mitochondrial DNA. (a) Distribution map (shaded in orange) showing the localities of sampled individuals, the subspecies to which they belong and the type of sequence obtained from each of them. Distribution was based on BirdLife International and NatureServe (2014). (b) Median-joining haplotype network of concatenated mDNA (COI + cyt *b*; 1238 bp). Circles represent haplotypes and their size is proportional to their frequency. The length of the branches connecting haplotypes is proportional to the number of nucleotide differences between them, which are indicated by the number of line marks on each branch. Colours represent the subspecies according to the scheme incorporated in the figure; note that the individuals from the contact zone between lineages were not assigned a subspecies and they are differentiated with a specific colour. Black circles represent unsampled hypothetical haplotypes. (c) Bayesian phylogenetic tree with divergence times obtained from the analysis of the concatenated mitochondrial dataset (1238 bp). The numbers near the nodes indicate the mean divergence time estimates and the numbers between square brackets correspond to the 95% highest posterior density intervals of those estimates. Divergence times and the numbers between square brackets are in millions of years ago. Posterior probability (PP) values are shown below the divergence times: **indicates maximum support (PP = 1.0); *denotes PP ≥ 0.95. The colours to the right of the tree identify the subspecies of each individual. Bird illustrations are from Santos (2020).


(a)
Mitochondrial DNA
Genomic DNA
Mitochondrial and genomic DNA
(b)
Vanellus chilensis cayennensis
Vanellus chilensis lampronotus
Vanellus chilensis (contact zone)
Vanellus chilensis chilensis
Vanellus chilensis fretensis
10 samples
1 sample
(c)
V. resplendens
183
41616
46517
88046
649564
88350
881
9256
9253
77933
1423
1676
9431
9331
77942
6623
85178
614621
9375
645399
1938
614620
620755
1817
631080
9260
835
2546
9254
9255
10332
2445
56823
4477
9259
56802
10331
3007
4552
587
578
0.74 [0.40-1.24]
**
0.59 [0.33-0.93]
**
0.39 [0.21-0.62]
*
0.29 [0.13-0.52]
*
V. c. cayennensis
V. c. lampronotus
V. c. chilensis
RECENT PLEISTOCENE
MIDDLE PLEISTOCENE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ma

grounds up to more than 3500 metres above sea level (Wiersma & Kirwan, 2018). Such a continent-wide distribution allows assessing the role of various diversification drivers at different spatial and temporal scales (Harvey & Brumfield, 2015; Lavinia et al., 2015; Lougheed et al., 2013). Second, it inhabits mainly open areas, including savannahs, steppes and pastures (Delfino & Caio, 2021; Jahn et al., 2017), which have been particularly poorly studied regarding their avian evolutionary patterns (Turchetto-Zolet et al., 2013; but see van Els et al., 2021).

Finally, by understanding the patterns of phenotypic and genetic variation within *V. chilensis*, it is possible to study how different diversification factors could have acted along its distribution. Specifically, based on morphological and vocal variation, four subspecies are currently recognized, which are separated into two groups: *V. c. chilensis* and *V. c. fretensis* are found in Patagonia (and also in central Chile), with the latter restricted to the southern extreme of South America, whereas *V. c. cayennensis* and *V. c. lampronotus* are found in the rest of the species' distribution (north and south of the Amazon River respectively; Clements et al., 2021; Fjeldså & Krabbe, 1990; Livezey, 2010; Wiersma & Kirwan, 2018). In fact, this phenotypic differentiation led some authors to propose that *V. chilensis* could actually include two different species (Fjeldså & Krabbe, 1990). Consistently with this grouping, analyses of mitochondrial DNA have shown that the Patagonian populations diverge by at least 1.5% genetic distance in the cytochrome c oxidase subunit I (COI) gene from the rest of the species (Elbourne, 2011; Kerr et al., 2009), even when there are currently no obvious geographical barriers between these groups. To the contrary, subspecies within each of the two main groups appear to be genetically similar (Elbourne, 2011; van Els et al., 2021).

In this context, we chose *V. chilensis* as a model to perform a continental-scale study of the patterns and processes of diversification in the Neotropics with a focus on Patagonia and the putative effects of glacial cycles. For this, we generated mitochondrial DNA information and nuclear genomic DNA data and analysed the tempo and mode of diversification of this species and evaluated the presence of gene flow between its different lineages. We complemented the genetic approach with a quantitative analysis of vocal data from the four subspecies and the contact zone between the two main groups.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling for genetic and genomic analyses

The dataset consisted of 58 specimens of *V. chilensis* from 47 collection sites covering the main distribution range of the species, with a focus on the southern cone of South America (Figure 1a; Table S1). *V. resplendens*, the sister species of *V. chilensis* (Livezey, 2010), was used as outgroup. Subspecies were determined according to the distribution range described by Piersman (1996) and Clements et al. (2021). Samples collected in northern Patagonia (north of Río Negro and Neuquén provinces in Argentina) and the southern region of central Argentina (south of Mendoza and Buenos Aires provinces) were not assigned a subspecies since this region represents the contact zone between *V. c. chilensis* and *V. c. lampronotus* and thus between the two main groups of the species (Clements et al., 2021; Elbourne, 2011; Fjeldså & Krabbe, 1990; Kerr et al., 2009; Livezey, 2010; Wiersma & Kirwan, 2018).

2.2 | Extraction, amplification and sequencing of mitochondrial DNA

DNA from fresh tissue (blood, liver or pectoral muscle) was extracted following the silica-based protocol described by Ivanova et al. (2006) using individual spin columns (Lijtmaer et al., 2012). We amplified two mitochondrial genes: COI (695 bp) and cytochrome *b* (cyt *b*, 1007 bp) with primer pairs BirdF1 (Hebert et al., 2004) and COIbirdR2 (Kerr et al., 2009) and L14841 (Kocher et al., 1989) and H16065 (Lougheed et al., 2000) respectively. The PCR cocktails and thermocycling profiles followed Lijtmaer et al. (2012) for COI and Arrieta et al. (2013) for cyt *b* and are detailed in Appendix S1 in Supporting Information. Sequencing of COI and cyt *b* was conducted at Macrogen (Seoul, Korea) and performed bidirectionally with the primers used for amplification. GenBank accession numbers are included in Table S1.

2.3 | Obtaining nuclear genomic markers through ddRADseq

We performed double-digest restriction site-associated DNA sequencing (ddRADseq; Peterson et al., 2012) following the protocol detailed in Thrasher et al. (2018). Sequencing was performed on an Illumina HiSeq 2500 lane (single-end, 150-bp) at the Cornell University Biotechnology Resource Center. Detailed information is provided in Appendix S1.

2.4 | Analyses of mitochondrial gene sequences

We edited and aligned COI and cyt *b* sequences using CODONCODE ALIGNER 4.0.4 (CodonCode Corporation). Analyses were performed using the concatenated sequences of COI and cyt *b*. We calculated the average p distances with MEGA 5.2 (Tamura et al., 2011) to analyse genetic variation and generated haplotype networks with the median joining algorithm implemented in POPART 1.0 (http://popart.otago.ac.nz). We conducted an analysis of molecular variance (AMOVA) in ARLEQUIN 3.5 (Excoffier & Lischer, 2010) to explore the distribution of genetic variation within *V. chilensis* and test whether there are differences in the frequency of mitochondrial haplotypes among subspecies. The Φ_{ST} values between pairs of subspecies were computed using uncorrected genetic distance matrices between haplotypes and significance was tested through 2000 random permutations.

We inferred phylogenetic trees through Bayesian methodology using MRBAYES 3.2.2 (Ronquist et al., 2012) and maximum parsimony in TNT 1.1 (Goloboff et al., 2003). The best-fit model of nucleotide substitution for each locus for the Bayesian analysis was selected using the Bayesian information criterion (BIC) implemented in JMODELTEST 2.1.1 (Darriba et al., 2012): HKY for COI and KHY+I for cyt *b* (Hasegawa et al., 1985). More details about these analyses are given in Appendix S1.

We generated a time-calibrated ultrametric tree with the Bayesian approach implemented in BEAUTI/BEAST 1.8 (Drummond et al., 2012) to estimate the age of the most recent common ancestor between *V. chilensis* and *V. resplendens* and the time of separation of the main mitochondrial lineages within *V. chilensis*. The mitochondrial markers were placed in separate partitions with unlinked substitution and clock models were selected with JMODELTEST (HKY for COI and HKY+I for cyt *b*). The tree models were linked since the mitochondria are a single unit of inheritance. Further details are provided in Appendix S1.

2.5 | Assembly and analyses of the nuclear ddRAD markers

We demultiplexed raw reads and applied standard quality filters in IPYRAD 0.7.28 (Eaton & Overcast, 2020; see Appendix S1). We only exported loci that were present in at least 80% of the samples to generate the final nuclear DNA dataset used for analyses.

We first performed a principal components analysis (PCA) to visualize genetic clustering using the packages 'SNPRelate' and 'gdsfmt' (Zheng et al., 2012) in R 3.5.1 (R Core Team, 2018). We used all the SNPs of the dataset (including multiple SNPs per locus), but because the PCA results are sensitive to missing data, SNPs missing from at least one individual were removed (missing rate = 0). To study the population structure of the species, we used STRUCTURE 2.3.4 (Pritchard et al., 2000) to assign individuals to genetic clusters (K), by using unlinked SNP as exported by IPYRAD. Further details about Structure analysis are given in Appendix S1.

We estimated divergence time, effective population size and gene flow with G-PHOCS 1.2.3 (Gronau et al., 2011). Because of the computationally intensive nature of this analysis, we restricted our dataset to a maximum of 10 individuals per subspecies, selecting the samples based on their location to maintain the widest possible geographical coverage within each of the subspecies (see further details in Appendix S1).

Spatial variation in gene flow, the consequently inferred location of barriers to gene flow and genetic diversity were calculated and visualized using the program Estimated Effective Migration Surfaces (EEMS; Petkova et al., 2016). EEMS methodological details are provided in Appendix S1.

2.6 | Geographical cline analyses of the mitochondrial and nuclear markers

We fit a series of equilibrium geographical cline models using the R package 'hzar' (Derryberry et al., 2014) to assess whether clinal patterns differed between mitochondrial and nuclear DNA. We determined the position of each population along a 1D linear transect estimated as the latitudinal distance from the southernmost sample (Table S2). For each molecular dataset, we chose the model that best fit the data using the Akaike information criterion corrected for small sample size (AICc). See details about these analyses in Appendix S1.

2.7 | Vocal analyses

We analysed 104 independent vocalizations sampled from the four subspecies and the contact zone between lineages in the northern region of Patagonia (Table S3).

Recordings were in 'wav' format and spectrograms were generated and analysed using RAVENPRO 1.5 (http://www.birds.cornell.edu/raven). Songs in this species consist of a repetition of a single note with multiple harmonics. We measured five variables on each spectrogram related to this repetitive note: the fundamental frequency, the bandwidth of the note, the mean duration of the note, the mean duration of the interval between the notes and the number of frequency inflexions of the note. We performed a PCA and used the nonparametric Kruskal–Wallis test to assess differences among lineages in their PC scores. We also performed a complementary one-way analysis of variance (ANOVA), followed by Bonferroni's contrasts using the individual five song variables. Details about these analyses are given in Appendix S1.

Finally, we choose the song variable that differed the most between subspecies and we fit cline models to this vocal variable along the sampling transect using the R package 'hzar' mentioned above (Table S4; Derryberry et al., 2014). We selected the best fit model based on the comparison of AICc values. Further details are provided in Appendix S1.

3 | RESULTS

3.1 | Mitochondrial genetic analyses

3.1.1 | Genetic diversity and population structure

We analysed the concatenated mitochondrial sequences (COI + cyt *b* = 1238 bp) of 41 individuals representing the four subspecies and the contact zone between the two main lineages in central Argentina (Figure 1a; Table S1). Our results showed a clear differentiation between the subspecies from Patagonia (*V. c. chilensis* and *V. c. fretensis*) and individuals from the rest of the Neotropics (*V. c. lampronotus* and *V. c. cayennensis*) with a mean genetic differentiation between these two main lineages of 1.5% (Table 1). Lower levels of differentiation were observed between *V. c. lampronotus* and *V. c. cayennensis* (1% mean divergence). On the other hand, the mean genetic distance between the two subspecies from Patagonia was 0.2%, which was the same distance found within *V. c. chilensis* and indicates almost no differentiation between these subspecies.

TABLE 1 Pairwise comparisons among the four subspecies of *Vanellus chilensis* based on mitochondrial DNA (COI + cyt *b*). Above the diagonal: pairwise Φ_{ST} values with significant values ($p < 0.05$) in bold. Diagonal: mean uncorrected genetic distances within each subspecies. Below the diagonal: mean uncorrected genetic distances between pairs of subspecies. Genetic distances are expressed in percentage values. Individuals from the contact zone between lineages were not included in this comparison.

Subspecies	*V. c. cayennensis*	*V. c. lampronotus*	*V. c. chilensis*	*V. c. fretensis*
V. c. cayennensis	0.10	**0.70**	**0.84**	0.94
V. c. lampronotus	1.00	0.30	**0.82**	**0.81**
V. c. chilensis	1.30	1.70	0.20	0.03
V. c. fretensis	1.20	1.60	0.20	0.00

Both the haplotype network (Figure 1b; Figure S1) and the AMOVA (Table 1) uncovered a marked phylogeographical structure. The network showed a clear divergence between the Patagonian lineage and the rest of the species. Even though there is no structure within Patagonia, as *V. c. chilensis* and *V. c. fretensis* lack differentiation and share haplotypes, there is some degree of differentiation between *V. c. lampronotus* and *V. c. cayennensis* and there are two differentiated groups within *V. c. lampronotus*. Consistently, Φ_{ST} values from the AMOVA were high between all the pairs of subspecies (Φ_{ST} 0.7–0.94) with the only exception of the extremely low value (0.03) between the two Patagonian subspecies (see Table 1). Aside from this latter case, Φ_{ST} values were significant in all but one of the comparisons: *V. c. fretensis* and *V. c. cayennensis*, from the extremes of the species distribution, had the highest Φ_{ST} value (0.94) but likely lacked significance because of their small sample sizes. The haplotype network also shows that the contact zone included individuals from the two main lineages, which in fact shared haplotypes with representatives of *V. c. lampronotus*, *V. c. chilensis* and *V. c. fretensis*. Moreover, individuals from both lineages were sampled in the same localities, very close to each other.

3.1.2 | Phylogenetic analyses and diversification dating

Bayesian and maximum parsimony (MP) reconstructions recovered *V. chilensis* as monophyletic in relation to its sister species *V. resplendens* (Figure S2). The time-calibrated ultrametric phylogeny (Figure 1c) shows that the split between them occurred recently, well within the Pleistocene (0.74 million years ago (Ma); 95% HDP: 0.40–1.24). These reconstructions show two reciprocally monophyletic clades (Figure S2; Figure 1c): one including the subspecies from Patagonia (*V. c. chilensis* and *V. c. fretensis*) and the other with the subspecies from the rest of the Neotropics (*V. c. lampronotus* and *V. c. cayennensis*). The split between them, which was the first split within the species, occurred in the Middle Pleistocene, approximately 0.59 Ma (95% HPD: 0.33–0.93 Ma). Subsequently, the subspecies from central South America (*V. c. lampronotus*) and the northern Neotropics (*V. c. cayennensis*) separated approximately 0.39 Ma (95% HPD: 0.21–0.62 Ma). The contact zone includes individuals from both lineages and no separation can be observed between the two Patagonian subspecies.

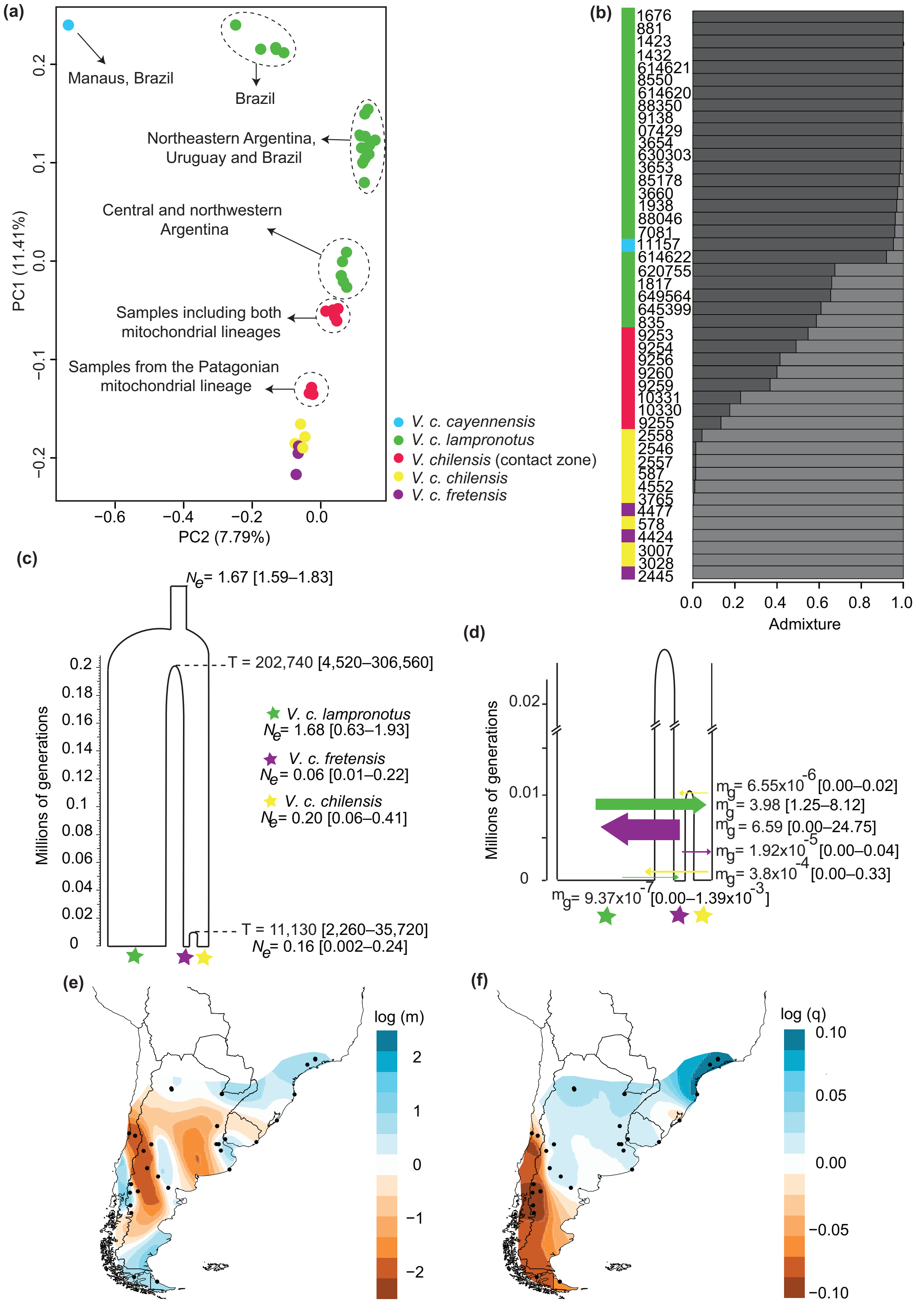
3.2 | Nuclear genomic analyses

We studied reduced-representation genomic loci from 45 specimens belonging to the four subspecies of *V. chilensis* that were collected in 31 localities from Brazil, Uruguay, Chile and Argentina, and in the contact zone between lineages (Figure 1a; Table S1). The de novo assembly produced a total of 8239 RADseq loci (21,111 SNPs) present in at least 80% of the individuals. This dataset was used to perform the analyses described below (with specific modifications when needed for particular analyses; see Appendix S1).

3.2.1 | Population structure

Nuclear genomic variation was congruent with the results described for mitochondrial DNA, showing a clear differentiation between the Patagonian lineage and the rest of the species. First, a PCA (Figure 2a) clearly separated these two lineages. This analysis also shows the contrasting little differentiation between the two Patagonian subspecies and the marked differentiation between *V. c. cayennensis* and *V. c. lampronotus*. The latter also showed considerable internal variation, which could be due to its

FIGURE 2 Genomic analyses of *V. chilensis*. (a) Principal component analysis (PCA) based on 2338 SNPs. The geographical origin of the samples of *V. c. lampronotus* and the mitochondrial lineages of samples from the contact zone are indicated in the graph. (b) Structure plot for $K = 2$ based on 7028 SNPs (one random SNP per RAD locus). (c) G-PhoCS estimates of divergence times (T; in millions of generations) and current and ancestral effective population sizes (Ne; in millions of individuals), with their 95% confidence intervals, based on 8239 RADseq loci (21,111 SNPs). (d) Detail with the migration rates (m_g; in migrants per generation) with their 95% confidence intervals. (e) Estimated effective migration rates (m) inferred by EEMS. Orange represents areas of low migration relative to the average, and blue indicates areas of higher migration. (f) Effective diversity rates (q) as inferred by EEMS, with orange representing areas of lower diversity and blue representing higher diversity.


(a)
PC1 (11.41%)
PC2 (7.79%)
0.2
0.1
0.0
−0.1
−0.2
−0.6
−0.4
−0.2
0.0
Manaus, Brazil
Brazil
Northeastern Argentina, Uruguay and Brazil
Central and northwestern Argentina
Samples including both mitochondrial lineages
Samples from the Patagonian mitochondrial lineage
V. c. cayennensis
V. c. lampronotus
V. chilensis (contact zone)
V. c. chilensis
V. c. fretensis
(b)
1676
881
1423
1432
614621
8550
614620
88350
9138
07429
3654
630303
3653
85178
3660
1938
88046
7081
11157
614622
620755
1817
649564
645399
835
9253
9254
9256
9260
9259
10331
10330
9255
2558
2546
2557
587
4552
3765
4477
578
4424
3007
3028
2445
0.0
0.2
0.4
0.6
0.8
1.0
Admixture
(c)
N_e= 1.67 [1.59–1.83]
T = 202,740 [4,520–306,560]
Millions of generations
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
V. c. lampronotus
N_e= 1.68 [0.63–1.93]
V. c. fretensis
N_e= 0.06 [0.01–0.22]
V. c. chilensis
N_e= 0.20 [0.06–0.41]
T = 11,130 [2,260–35,720]
N_e= 0.16 [0.002–0.24]
(d)
Millions of generations
0.02
0.01
0
m_g= 6.55x10^{-6} [0.00–0.02]
m_g= 3.98 [1.25–8.12]
m_g= 6.59 [0.00–24.75]
m_g= 1.92x10^{-5} [0.00–0.04]
m_g= 3.8x10^{-4} [0.00–0.33]
m_g= 9.37x10^{-7} [0.00–1.39x10^{-3}]
(e)
log (m)
2
1
0
−1
−2
(f)
log (q)
0.10
0.05
0.00
−0.05
−0.10

wide distribution, as shown by the fact that its internal clustering corresponds to the geographical location of each individual (see details in Figure 2a). The Structure analysis also supported the two main groups, as $K=2$ was the model with the highest likelihood (Figure S3) and the two genomic clusters correspond to the mitochondrial lineages (Figure 2b). Consistently, the G-PhoCS analysis (Figure 2c) indicated that the first split that originated these two main lineages took place roughly 200,000 generations ago (i.e. around 600,000 years ago assuming that the generation time for non-passerines can be estimated to be 3 years on average based on the results of Saether et al., 2005). The two Patagonian subspecies are inferred to have diverged from each other much more recently, around 11,000 generations ago (we could not infer the divergence between *V. c. lampronotus* and *V. c. cayennensis* because we had genomic data from only one individual of the latter).

3.2.2 | Admixture and gene flow among lineages

Various analyses evidenced the presence of intense gene flow between the two main lineages. First, in the PCA, the individuals from the contact zone were intermediate between those of the two lineages. These individuals from the contact zone were divided into two groups, but these did not correspond to their geographical origin as clearly as in the case of *V. c. lampronotus* (Figure 2a). The specimens that were grouped closer to *V. c. chilensis* and *V. c. fretensis* belong to the Patagonian mitochondrial lineage, whereas the cluster placed closer to *V. c. lampronotus* included specimens of both mitochondrial lineages (including the aforementioned cases of individuals from different lineages that were collected in the same localities). In turn, the Structure analysis confirmed the presence of genomic admixture in the individuals of the contact zone (Figure 2b). Moreover, individuals with mixed genomic content were not limited to the contact area, as there were also specimens of *V. c. lampronotus* from northwestern Argentina with a considerable proportion of its genome belonging to the Patagonian cluster (see Figure 2b; Table S1). Finally, we also found evidence of high levels of gene flow between lineages using G-PhoCS (Figure 2d). Gene flow from the Patagonian lineage to the more widespread lineage was ~1.7× higher than in the opposite direction.

The EEMS analysis suggested a barrier to migration in northern Patagonia with a south-east to north-west orientation that separates the Patagonian populations from those of *V. c. lampronotus* (Figure 2e). This indicates that individuals on either side of this barrier are less connected through migration than expected under pure isolation by distance. This is consistent with the results based on mitochondrial DNA, which indicated the same geographical area of contact. In addition, this analysis showed that the populations from Patagonia and central Chile had much lower genetic diversity than those from the rest of the species distribution (Figure 2f).

3.2.3 | Effective population size

The G-PhoCS analysis estimated that the current effective population size of *V. c. lampronotus* (1.68 million individuals) is similar to the ancestral effective population size of the species (1.67 million individuals) and much larger than that of the Patagonian subspecies (200,000 individuals of *V. c. chilensis* and only 60,000 individuals of *V. c. fretensis*; see Figure 2c).

3.3 | Mitochondrial and nuclear DNA geographical clines

The first model (fixed scaling and no tails) was selected as the best fit model for both the mitochondrial and nuclear clines (Table S5). When we compared modelled clines along the south–north transition of the subspecies, variation in mitochondrial and nuclear markers showed geographical discordance. In fact, the nuclear cline was less steep and its centre was located approximately 300 km northwards compared to the centre of the mitochondrial cline (Figure 3a; Table S5), highlighting that gene flow between lineages extends beyond northern Patagonia for nuclear DNA (this same result was obtained when we modelled the clines using only the specimens for which we obtained both nuclear and mitochondrial DNA; data not shown).

3.4 | Vocalization analyses

Both a PCA and the analysis of individual song variables showed significant differences in vocalizations among subspecies. The PCA generated three PCs that explained almost 85% of song variation. PC1 correlated with the mean duration of the note and its number of inflexions, PC2 correlated with the fundamental frequency and bandwidth and PC3 correlated with the interval between notes (Table S6). Subspecies statistically differed in PC1 and PC2 (Kruskal–Wallis test; $p < 0.01$), whereas no differences were found for PC3 ($p = 0.32$), indicating that subspecies differed in note duration, the number of inflexions in the notes and their frequencies, but not in the separation between notes (see Table S7). Pairwise comparisons showed that *V. c. cayennensis* and *V. c. lampronotus* significantly differed from each other for PC1 and PC2 ($p < 0.01$ in both cases), and that they also differed from the Patagonian subspecies (*V. c. chilensis* and *V. c. fretensis*) for either PC1, PC2 or both, depending on the comparison (Figure 4; Table S7). The two Patagonian subspecies did not differ in any of the PCs (Figure 4; Table S7), consistent with the lack of DNA differentiation between them. The results using the individual song variables were consistent with the PCA, showing significant differentiation between the two main lineages and between *V. c. lampronotus* and *V. c. cayennensis*, but no differences between the Patagonian subspecies (Tables S8 and S9).

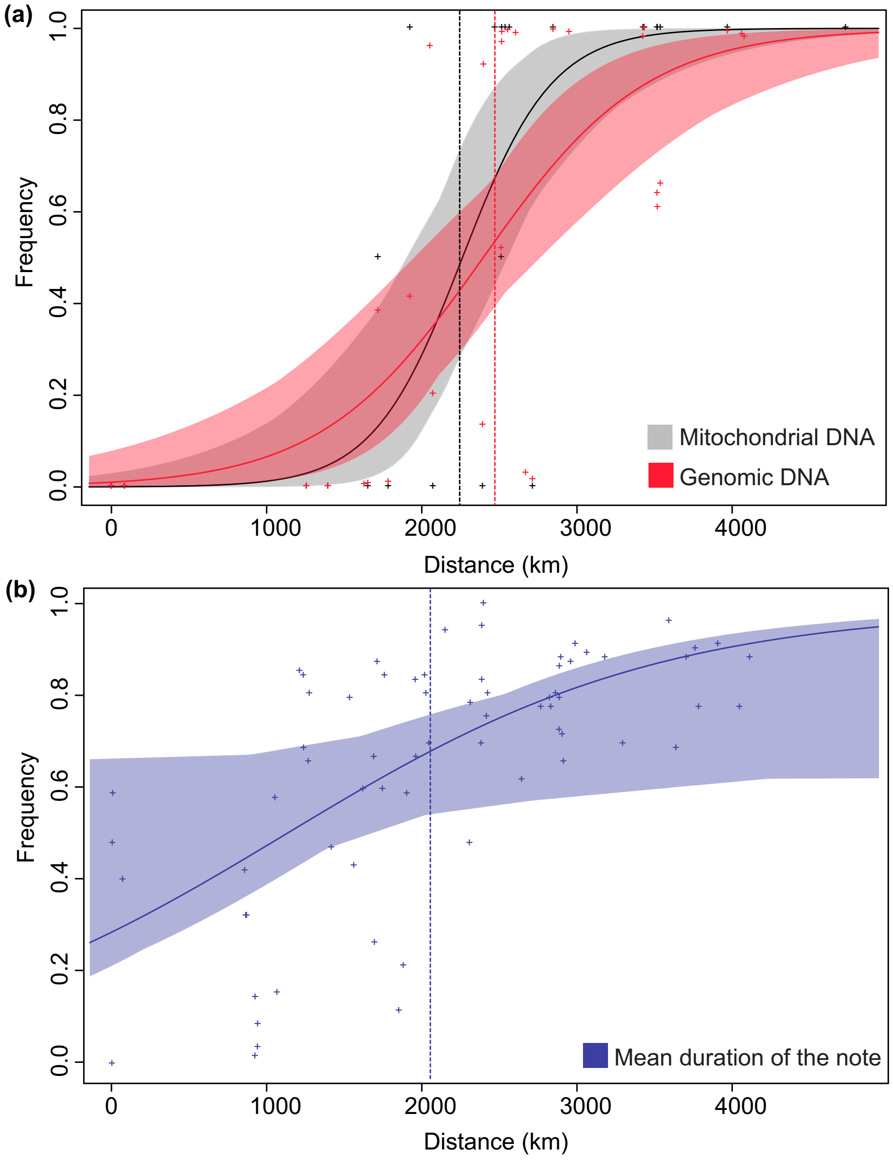


FIGURE 3 Geographical clines estimated using HZAR for mitochondrial and nuclear DNA and the song variable mean duration of the note of *V. chilensis*. Distance on the *x*-axis is latitudinal distance in kilometres from the southernmost population. (a) Best-fit geographical clines shown for mitochondrial (grey) and nuclear (red) DNA. Vertical dashed lines indicate the location of the centre for each cline (black for mitochondrial cline and red for nuclear cline). (b) Best-fit geographical cline shown for the song variable mean duration of the note (blue). The vertical dashed line indicates the location of the centre of the cline.

We analysed the characteristics of the songs used in the area of contact between the two main lineages to compare the results with the mitochondrial and genomic DNA patterns. In this area, four of the five song variables (fundamental frequency, duration of the note, duration of the interval between notes and number of inflexions) showed intermediate values between those of *V. c. chilensis* and *V. c. lampronotus*, the two subspecies that come into contact (Figure 4; Table S8). Moreover, the songs in this area significantly differed in most variables from the songs of *V. c. lampronotus* and *V. c. chilensis* (Table S9). Consistently, the vocalizations of the contact zone were intermediate between those of the two main lineages in the PCA (Figure 4a), significantly differing from the songs of *V. c. lampronotus* in PC1 and from those of *V. c. chilensis* in PC2 (Figure 4b; Table S7).

Finally, we performed a vocal geographical cline analysis using the song variable mean duration of the note, which we chose because it showed significant differences between all pairs of subspecies (with the sole exception of the two Patagonian subspecies) and between the songs of the contact zone and those of *V. c. lampronotus* and *V. c. chilensis* (Table S9). The model with none fitting exponential tails was selected as the best fit model based on the lowest AICc score (Table S5). Compared to the DNA clines, the vocal cline showed more variation along the transect and a more gradual, less steep increase from the Patagonian lineage in Santa Cruz province (Argentina) to *V. c. lampronotus* in Matto Grosso (Brazil), with an estimated centre located 158 km southwards to the centre of the mitochondrial cline (Figure 3b).

4 | DISCUSSION

We used mitochondrial and nuclear genomic data and vocalizations to study the evolutionary history of *V. chilensis* and the processes involved in its diversification. Our findings indicate that despite its currently continuous distribution, there is a clear split between the Patagonian populations and the rest of the species, which occurred

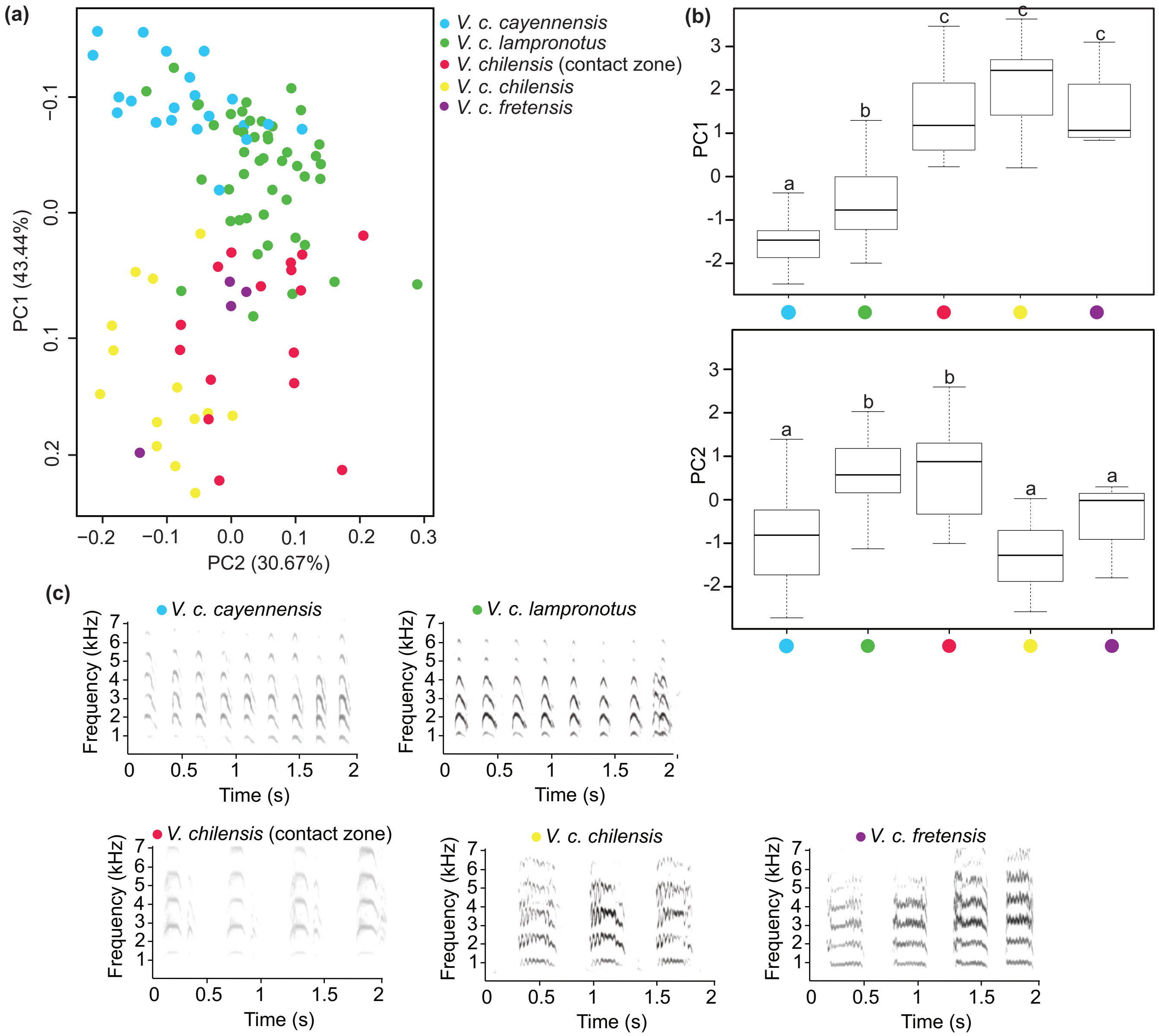


FIGURE 4 Analyses of the vocalizations of *V. chilensis*. (a) Song differentiation among subspecies based on a PCA performed with the song variables (fundamental frequency, bandwidth, mean duration of the note, mean duration of the interval between notes and number of inflexions of the note). (b) Boxplots showing the distribution of PC1 and PC2 values for the four subspecies and the individuals from the contact zone. Significant comparisons between pairs of subspecies and between them and individuals from the contact zone are identified with different letters. (c) Representative spectrograms of the four subspecies and the contact zone.

fairly recently, in the Middle Pleistocene. The high level of gene flow between these two main lineages is evident both in terms of their genomic and vocal characteristics. These findings and their implications are discussed in turn below.

First, our mitochondrial DNA results indicate a recent origin for *V. chilensis*, placing the split from *V. resplendens*, its sister species (Livezey, 2010), only 0.74 million years ago. This result is consistent with the tempo that we found for the internal diversification of the species based both on mitochondrial and nuclear genomic DNA (see below), and also with the internal divergences found by van Els et al. (2021). Even though this result contrasts with the phylogeny by Barth et al. (2013), in which the split between these two species was placed around 8 Ma, this difference could be due to the fact that their phylogeny used the general calibration by Jetz et al. (2012). A study incorporating more *V. resplendens* individuals and more markers could shed some light on this discrepancy, ideally in the context of a molecular phylogeny of the genus *Vanellus*, which has never been performed.

The mitochondrial DNA also indicates that about 600,000 years ago, shortly after the initial diversification event, *V. chilensis* divided into its two main current lineages, the Patagonian lineage in Chile and southern Argentina and the lineage distributed in the rest of the Neotropics. Consistently, the various analyses based on the nuclear genome also identified these same two main genomic clusters.

Moreover, the estimation of the divergence time between these lineages using genomic data was concordant with that suggested by the mitochondrial DNA: even though the G-PhoCS analysis had a broad confidence interval, it suggested that this split could have taken place approximately 200,000 generations ago and the generation time for non-passerines could be estimated to be around 3 years on average based on the data published by Saether et al. (2005).

Patagonia was strongly affected by glaciations during the Pleistocene and the advance of ice sheets in the steppe and downhill of the Andes (Rabassa, 2008; Rabassa et al., 2000, 2011), in many cases, isolated different organisms in refugia of suitable habitat (Lessa et al., 2010; Nuñez et al., 2011; Sánchez et al., 2021; Sérsic et al., 2011; Weir, 2006). This could have been the case for the Patagonian populations of *V. chilensis*, triggering this initial diversification in allopatry and the split into the two main current lineages. A confirmation of this scenario would require further analyses with a denser sampling in Patagonia and the evaluation of specific predictions of alternative hypotheses. One such alternative diversification explanation could be related to the fact that *V. chilensis* includes migrant populations in Patagonia and sedentary populations in the rest of its distribution. Changes in migratory behaviour can lead to diversification and speciation, a mechanism that has recently gained momentum (Gómez-Bahamón et al., 2020; Rolland et al., 2014; Winger et al., 2014) and could be particularly addressed in future analyses of this species.

The two Patagonian subspecies lack mitochondrial or nuclear genetic differentiation and showed very low genetic diversity. To the contrary, the lineage occupying lower latitudes of the Neotropics did show higher internal variation and, in fact, its two subspecies differ by around 1% mitochondrial genetic distance. Because these subspecies are separated by the Amazon River (Fjeldså & Krabbe, 1990; Livezey, 2010; Wiersma & Kirwan, 2018), one of the most relevant barriers to gene flow in the Neotropics (Aleixo & Rossetti, 2007; Naka & Brumfield, 2018; Ribas et al., 2012), we could postulate that this was likely the factor causing this differentiation. However, the northernmost subspecies (*V. c. cayennensis*) was represented in our dataset by the mitochondrial DNA of just a few individuals from Central America and genomic data from only one individual from Manaus, Amazonas state in Brazil. Therefore, it is difficult to draw finer conclusions about how the Amazon River could have acted as a diversification factor.

The Andes constitute another key diversification factor in the Neotropics (Brumfield & Edwards, 2007; Weir, 2006; Weir & Price, 2011) but do not appear to be a main driver for *V. chilensis* diversification, given that the species is mostly distributed to the east of the Andes, except for the northern and southern extremes of its distribution (see Figure 1a). However, the mountains appear to be a barrier to gene flow in the southern cone of South America. In the first place, the Patagonian subspecies *V. c. chilensis* is present in the northern extreme of the species distribution in Chile, to the west of the Andes, but at the same latitude *V. c. lampronotus* is present to the east of the Andes in Argentina. Moreover, our analyses confirm this differentiation: the mitochondrial DNA of specimens from Santiago de Chile and Valparaíso correspond to the Patagonian lineage, whereas birds from Mendoza province, at the same latitude in Argentina, have mitochondrial sequences characteristic of *V. c. lampronotus*. Furthermore, there are no signs of admixture in the genomic DNA obtained in these localities (see Figure 2b). On the other hand, our limited sampling in the northern portion of the species' distribution prevents us from drawing conclusions about the role of the Andes Mountains in northern South America.

Our data confirmed the presence of a contact zone between the two main lineages in northern Patagonia. Both mitochondrial lineages were found in some localities of this area and the genomic data showed the presence of individuals with mixed genomic content, indicating gene flow between lineages (see Figure 2). If the initial diversification of these lineages occurred in allopatry (a possibility mentioned above), gene flow is a consequence of secondary contact after the post-glacial expansion of the Patagonian population. This means that the divergence in allopatry was not sufficient to result in complete reproductive isolation. This lack of complete isolation is reasonable considering the recent divergence between the two lineages (Lijtmaer et al., 2003; Price & Bouvier, 2002) and is a pattern commonly found in the Neotropics when lineages that diverged in the Pleistocene come into secondary contact (Luzuriaga-Aveiga et al., 2021; Rocha et al., 2020).

Our results are consistent with a northward Patagonian lineage expansion as a possible cause for the secondary contact, as the G-PhoCS analysis indicated that gene flow is more intense from the Patagonian lineage to *V. c. lampronotus* than in the opposite direction (see Figure 2d). This pattern, however, can also be caused by other factors, such as differences in population sizes, and it should therefore be analysed in more detail. Irrespective of the direction of the expansion that caused the secondary contact, there is a decoupling in the patterns found for the nuclear and mitochondrial DNA. This can be observed in the DNA clines, which show that the centre of the nuclear DNA cline is shifted 300 km north of the centre of the mitochondrial DNA cline (Figure 3a). In addition, this difference between the nucleus and the mitochondria is evidenced by the fact that the individuals that were sampled in the same localities of the contact zone and belonged to the different mitochondrial lineages had very similar genomic DNA. This difference in mitochondrial and nuclear DNA patterns could potentially be driven by differences in selection for the two types of DNA or by male-biased dispersal (Toews & Brelsford, 2012). Given the relevance of co-adapted systems between the mitochondria and the nucleus and the role of mito-nuclear incompatibilities in the speciation process (Hill, 2015, 2016; Toews & Brelsford, 2012; Trier et al., 2014), this decoupling deserves a more thorough analysis in the future. For instance, it would be interesting to analyse whether in the birds of the contact zone and northwestern Argentina nuclear genes with mitochondrial function show the variants that would be expected according to their mitochondrial lineage (Morales et al., 2018).

The G-PhoCS analysis indicated that gene flow considerably exceeds one migrant per generation (see Figure 2d), the traditionally considered threshold above which lineages cannot differentiate through genetic drift (Wright, 1931). However, the advent of genomics has shown that differentiation and subsequent speciation in the presence of gene flow is far more common than traditionally believed, particularly when selection plays a relevant role (Feder et al., 2012; Lamichhaney et al., 2015; Turbek et al., 2021; Winker, 2021). In this context, the high levels of gene flow between *V. chilensis* lineages do not necessarily mean that they are undergoing a homogenization process.

The pattern of vocal variation was consistent with the genetic results, with significant differences between the two lineages and the presence of intermediate vocalizations in the contact zone. Notably, this latter result was not due to an intermediate average of vocal features in the contact zone because of the presence of representatives from both lineages, but instead reflects the fact that the songs of some of the individuals presented intermediate characteristics in this area (see Figures 3b and 4). In fact, the vocal cline showed a clear latitudinal gradient in the mean duration of the note, which was the song feature that most consistently differed between subspecies. Moreover, this clinal pattern corresponded to the transition between the two genetic lineages and its centre was located close (158 km southwards) to the mitochondrial DNA cline centre (Figure 3b). The considerable variation in this vocal feature along the sampled localities and its less steep cline could be related to the multiple aspects that can affect innate songs and their consequent polygenic control (e.g. Acero-Murcia et al., 2021; Podos & Warren, 2007). Although this concordance between the genetic and vocal results could be expected, particularly in a species that does not learn its songs, this result is relevant for various reasons: (1) it provides additional evidence of the presence of gene flow in the contact zone between lineages, constituting a tangible, phenotypic outcome of the presence of admixed genomic content, (2) it explicitly shows the similarity between song and gene geographical structuring, a pattern that has not been frequently demonstrated (Acero-Murcia et al., 2021; Brumfield, 2005) and (3) it adds to the relatively small body of cases in which the presence of intermediate songs has been clearly established in contact/hybrid zones (e.g. Gee, 2005; Shipilina et al., 2017). Regarding the latter aspect, vocal differences between recently diverged lineages that are in contact are crucial for maintaining their genetic integrity and preventing the homogenization of their genomic content (Cowles & Uy, 2019; Turbek et al., 2021; Uy et al., 2018) but the presence of intermediate songs in the contact zone may undermine their role for species recognition. Future studies that analyse the effect of intermediate songs for species recognition, establish the fitness of the individuals with admixed genomic content and assess the role played by selection could help to elucidate whether these two lineages of recent origin will continue to diversify or are on a path towards homogenization.

ACKNOWLEDGEMENTS

We thank the collectors and staff of the institutions that loaned tissue samples used in this study: Zoological Museum Copenhagen; Royal Ontario Museum; Instituto Nacional de Pesquisas da Amazônia; Museu de Zoologia da Universidade de São Paulo; Museu Nacional de Rio de Janeiro; American Museum of Natural History; Burke Museum; Smithsonian Institution, National Museum of Natural History and Louisiana State University Museum of Natural Science. We also thank the Macaulay Library of Natural Sounds and the Florida Museum of Natural History for loaning sound recordings, as well as the recordists who deposited their *Vanellus* recordings in Xeno-Canto. We specially thank Natalia Trujillo-Arias and Laura Barone from the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' for their help in the field. We also thank Bronwyn Butcher from the Fuller Evolutionary Biology Lab (Cornell Lab of Ornithology) for her guidance and help in the generation of the genomic data. For granting relevant permits we thank the Offices of Fauna of Buenos Aires, Mendoza and Río Negro provinces in Argentina (permit numbers: 74/2017, 1243/2017 and 1280/2017). This study was funded by the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) from Argentina, the Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación from Argentina, the Richard Lounsbery Fundation and Fundación Williams. Finally, we thank Michael N. Dawson (Chief Editor) and Camila Ribas (Editor) for their valuable comments. The suggestions of two anonymous reviewers greatly improved this article.

CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

The ddRAD data will be publicly available in Dryad once the manuscript is accepted (doi: https://doi.org/10.5061/dryad.b2rbnzsm1). The sequences of mitochondrial DNA will be publicly available in GenBank on 1 October 2023. The GenBank accession numbers are detailed in Supplementary information, Table S1.

ORCID

Belén Bukowski https://orcid.org/0000-0002-5227-4797

REFERENCES

Acero-Murcia, A. C., Raposo do Amaral, F., de Barros, F. C., da Silva Ribeiro, T., Miyaki, C. Y., & Maldonado-Coelho, M. (2021). Ecological and evolutionary drivers of geographic variation in songs of a Neotropical suboscine bird: The Drab-Breasted Bamboo Tyrant (*Hemitriccus diops*, Rhynchocyclidae). *American Ornithological Society*, *138*, 1–15. https://doi.org/10.1093/ornithology/ukab003

Acosta, I., Cabanne, G. S., Noll, D., González-Acuña, D., Pliscoff, P., & Vianna, J. A. (2020). Patagonian glacial effects on the endemic Green-backed Firecrown, *Sephanoides sephaniodes* (Aves: Trochilidae): Evidence from species distribution models and molecular data. *Journal of Ornithology*, *162*, 289–301. https://doi.org/10.1007/s10336-020-01822-4

Aleixo, A., & Rossetti, D. d. F. (2007). Avian gene trees, landscape evolution, and geology: Towards a modern synthesis of Amazonian historical biogeography? *Journal of Ornithology*, *148*, 443–453. https://doi.org/10.1007/s10336-007-0168-7

Arrieta, R. S., Lijtmaer, D. A., & Tubaro, P. L. (2013). Evolution of postzygotic reproductive isolation in galliform birds: Analysis of first and second hybrid generations and backcrosses. *Biological Journal of the Linnean Society*, *110*(3), 528–542. https://doi.org/10.1111/bij.12153

Barluenga, M., Stoking, K. N., Salzburger, W., Muschick, M., & Meyer, A. (2006). Sympatric speciation in Nicaraguan Crater Lake cichlid fish. *Nature*, *439*(7077), 719–723. https://doi.org/10.1038/nature04325

Barth, J. M. I., Matschiner, M., & Robertson, B. C. (2013). Phylogenetic position and subspecies divergence of the Endangered New Zealand Dotterel (*Charadrius obscurus*). *PLoS One*, *8*(10), e78068. https://doi.org/10.1371/journal.pone.0078068

Beckman, E. J., & Wiit, C. C. (2015). Phylogeny and biogeography of the New World siskins and goldfinches: Rapid, recent diversification in the Central Andes. *Molecular Phylogenetics and Evolution*, *87*, 28–45. https://doi.org/10.1016/j.ympev.2015.03.005

Beheregaray, L. B. (2008). Twenty years of phylogeography: The state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology*, *17*(17), 3754–3774. https://doi.org/10.1111/j.1365-294X.2008.03857.x

Brumfield, R. T. (2005). Mitochondrial variation in Bolivian populations of the Variable Antshrike (*Thamnophilus caerulescens*). *The Auk*, *122*(2), 414–432. https://www.jstor.org/stable/4090436

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*(2), 346–367. https://doi.org/10.1111/j.1558-5646.2007.00039.x

Cabanne, G. S., d'Horta, F. M., Sari, E. H. R., Santos, F. R., & Miyaki, C. Y. (2008). Nuclear and mitochondrial phylogeography of the Atlantic forest endemic *Xiphorhynchus fuscus* (Aves: Dendrocolaptidae): Biogeography and systematics implications. *Molecular Phylogenetics and Evolution*, *49*(3), 760–773. https://doi.org/10.1016/j.ympev.2008.09.013

Cabanne, G. S., Campagna, L., Trujillo-Arias, N., Naoki, K., Gomez, I., Miyaki, C. Y., Santos, F. R., Dantas, G. P. M., Aleixo, A., Claramunt, S., Rocha, A., Caparroz, R., Lovette, I. J., & Tubaro, P. L. (2019). Phylogeographic variation within the Buff-browed Foliage-gleaner (Aves: Furnariidae: *Syndactyla rufosuperciliata*) supports an Andean-Atlantic forests connection via the Cerrado. *Molecular Phylogenetics and Evolution*, *133*, 198–213. https://doi.org/10.1016/j.ympev.2019.01.011

Campagna, L., Geale, K., Handford, P., Lijtmaer, D. A., & Tubaro, P. L. (2011). A molecular phylony of the Sierrafinches (*Phrygilus*, Passeriformes): Extreme polyphyly in a group of Andean specialist. *Molecular Phylogenetics and Evolution*, *61*, 521–533. https://doi.org/10.1016/j.ympev.2011.07.011

Campagna, L., St Clair, J. J. H., Lougheed, S. C., Woods, R. W., Imberti, S., & Tubaro, P. L. (2012). Divergence between passerine populations from the Malvinas – Falkland Islands and their continental counterparts: A comparative phylogeographical study. *Biological Journal of the Linnean Society*, *106*(4), 865–879. https://doi.org/10.1111/J.1095-8312.2012.01898.X

Carnaval, A. C., & Moritz, C. (2008). Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. *Journal of Biogeography*, *35*(7), 1187–1201. https://doi.org/10.1111/j.1365-2699.2007.01870.x

Clements, J. F., Schulenberg, T. S., Iliff, M. J., Billerman, S. M., Fredericks, T. A., Gerbracht, J. A., Lepage, D., Sullivan, B. L., & Wood, C. L. (2021). The eBird/Clements checklist of Birds of the World: v2021. https://www.birds.cornell.edu/clementschecklist/download/

Cowles, S. A., & Uy, J. A. C. (2019). Rapid, complete reproductive isolation in two closely related Zosterops white-eye bird species despite broadly overlapping ranges. *Evolution*, *73*(8), 1647–1662. https://doi.org/10.1111/evo.13797

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772. https://doi.org/10.1038/nmeth.2109

Delfino, H. C., & Caio, J. C. (2021). Macro and micro-habitat selection by *Vanellus chilensis* (aves: Charadriiformes) in southern Brazil. *El Hornero*, *36*(1), 17–26.

Derryberry, E. P., Derryberry, G. E., Maley, J. M., & Brumfield, R. T. (2014). HZAR: Hybrid zone analysis using an R software package. *Molecular Ecology Resources*, *14*(3), 652–663. https://doi.org/10.1111/1755-0998.12209

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*(8), 1969–1973. https://doi.org/10.1093/molbev/mss075

Eaton, D. A. R., & Overcast, I. (2020). ipyrad: Interactive assembly and analysis of RADseq data sets. *Bioinformatics*, *36*(8), 2592–2594. https://doi.org/10.1093/bioinformatics/btz966

Elbourne, R. (2011). COI barcoding of the Shorebirds: Rates of evolution and the identification of species. *Ecology and Evolutionary Biology University of Toronto*, 1–154.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Feder, J. L., Egan, S. P., & Nosil, P. (2012). The genomics of speciation with-gene-flow. *Trends in Genetics*, *28*(7), 342–350. https://doi.org/10.1016/j.tig.2012.03.009

Fernandes, A. M., Wink, M., Sardelli, C. H., & Aleixo, A. (2014). Multiple speciation across the Andes and throughout Amazonia: The case of the spot-backed antbird species complex (*Hylophylax naevius*/*Hylophylax naevioides*). *Journal of Biogeography*, *41*(6), 1094–1104. https://doi.org/10.1111/jbi.12277

Fjeldså, J., & Krabbe, N. (1990). *Birds of the high Andes: A Manual to the Birds of the Temperate Zone of the Andes and Patagonia*, *South America*. In Zoological Museum, University of Copenhagen and Apollo Books (Ed.), Copenhagen and Svendborg, Denmark.

Gee, J. M. (2005). No species barrier by call in an avian hybrid zone between California and Gambel's quail (*Callipepla californica* and *C. Gambelii*). *Biological Journal of the Linnean Society*, *86*(2), 253–264. https://doi.org/10.1111/j.1095-8312.2005.00538.x

Goloboff, P., Farris, J., & Nixon, K. (2003). T.N.T.: Tree analysis using new technology. Program and documentation. http://www.zmuc.dk/public/phylogeny

Gómez-Bahamón, V., Márquez, R., Jahn, A. E., Miyaki, C. Y., Tuero, D. T., Laverde-R, O., Restrepo, S., & Cadena, C. D. (2020). Speciation associated with shifts in migratory behavior in an avian radiation. *Current Biology*, *30*, 1312–1321. https://doi.org/10.1016/j.cub.2020.01.064

Gronau, I., Hubisz, M. J., Gulko, B., Danko, C. G., & Siepel, A. (2011). Bayesian inference of ancient human demography from individual genome sequences. *Nature Genetics*, *43*(10), 1031–1034. https://doi.org/10.1038/ng.937

Harvey, M. G., & Brumfield, R. T. (2015). Genomic variation in a widespread Neotropical bird (*Xenops minutus*) reveals divergence, population expansion, and gene flow. *Molecular Phylogenetics and Evolution*, *83*, 305–316. https://doi.org/10.1016/j.ympev.2014.10.023

Hasegawa, M., Kishino, K., & Yano, T. (1985). Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, *22*(2), 160–174. https://doi.org/10.1007/BF02101694

Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS Biology*, *2*(10), e312. https://doi.org/10.1371/journal.pbio.0020312

Hill, G. E. (2015). Mitonuclear ecology. *Molecular Biology and Evolution*, *32*(8), 1917–1927. https://doi.org/10.1093/molbev/msv104

Hill, G. E. (2016). Mitonuclear coevolution as the genesis of speciation and the mitochondrial DNA barcode gap. *Ecology and Evolution*, *6*(16), 5831–5842. https://doi.org/10.1002/ece3.2338

Ivanova, N. V., de Waard, J. R., & Hebert, P. D. N. (2006). An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, *6*(4), 998–1002. https://doi.org/10.1111/J.1471-8286.2006.01428.X

Jahn, O., Ganchev, T. D., Marques, M. I., & Schuchmann, K.L. (2017). Automated sound recognition provides insights into the behavioral ecology of a tropical bird. *PLoS One*, *12*(1), e0169041. https://doi.org/10.1371/journal.pone.0169041

Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature*, *491*(7424), 444–448. https://doi.org/10.1038/nature11631

Kerr, K. C. R., Lijtmaer, D. A., Barreira, A. S., Hebert, P. D. N., & Tubaro, P. L. (2009). Probing evolutionary patterns in neotropical birds through DNA barcodes. *PLoS One*, *4*(2), e4379. https://doi.org/10.1371/journal.pone.0004379

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(16), 6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Kopuchian, C., Campagna, C., Lijtmaer, D. A., Cabanne, G. S., García, N. C., Lavinia, P. D., Tubaro, P. L., Lovette, I., & Di Giacomo, A. S. (2020). A test of the riverine barrier hypothesis in the largest subtropical river basin in the neotropics. *Molecular Ecology*, *29*(12), 2137–2149. https://doi.org/10.1111/mec.15384

Kopuchian, C., Campagna, L., Di Giacomo, A., Wilson, R. E., Bulgarella, M., Petracci, P., Mazar Barnett, J., Matus, R., Blank, O., & McCracken, K. G. (2016). Demographic history inferred from genome-wide data reveals two lineages of sheldgeese endemic to a glacial refugium in the southern Atlantic. *Journal of Biogeography*, *43*(10), 1979–1989. https://doi.org/10.1111/jbi.12767

Lamichhaney, S., Berglund, J., Almén, M. S., Maqbool, K., Grabherr, M., Martinez-Barrio, A., Promerová, M., Rubin, C.-J., Wang, C., Zamani, N., Grant, B. R., Grant, P. R., Webster, M. T., & Andersson, L. (2015). Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature*, *518*(7539), 371–375. https://doi.org/10.1038/nature14181

Lamichhaney, S., Han, F., Webster, M., Andersson, L., Grant, B., & Grant, P. (2017). Rapid hybrid speciation in Darwin's finches. *Science*, *359*(6372), 224–228. https://doi.org/10.1126/science.aao4593

Lavinia, P. D., Barreira, A. S., Campagna, L., Tubaro, P. L., & Lijtmaer, D. A. (2019). Contrasting evolutionary histories in neotropical birds: Divergence across an environmental barrier in South America. *Molecular Ecology*, *28*(7), 1730–1747. https://doi.org/10.1111/mec.15018

Lavinia, P. D., Escalante, P., García, N. C., Barreira, A. S., Trujillo-Arias, N., Tubaro, P. L., Naoki, K., Miyaki, C. Y., Santos, F. R., & Lijtmaer, D. A. (2015). Continental-scale analysis reveals deep diversification within the polytypic red-crowned ant tanager (*Habia rubica*, Cardinalidae). *Molecular Phylogenetics and Evolution*, *89*, 182–193. https://doi.org/10.1016/j.ympev.2015.04.018

Lessa, E. P., Cook, J. A., & Patton, J. L. (2003). Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(18), 10331–10334. https://doi.org/10.1073/pnas.1730921100

Lessa, E. P., D'Elia, G., & Pardiñas, U. F. J. (2010). Genetic footprints of Late Quaternary climate change in the diversity of Patagonian-Fueguian rodents. *Molecular Ecology*, *19*(15), 3031–3037. https://doi.org/10.1111/j.1365-294X.2010.04734.x

Lijtmaer, D. A., Kerr, K. C. R., Barreira, A. S., Hebert, P. D. N., & Tubaro, P. L. (2011). DNA barcode libraries provide insight into continental patterns of avian diversification. *PLoS One*, *6*, e20744.

Lijtmaer, D. A., Kerr, K. C. R., Stoeckle, M. Y., & Tubaro, P. L. (2012). DNA barcoding birds: From field collection to data analysis. In W. J. Kress & D. L. Erickson (Eds.), *DNA barcodes: methods and protocols. Methods in molecular biology* (Vol. *858*, pp. 127–152). Springer Science Business Media LLC.

Lijtmaer, D. A., Mahler, B., & Tubaro, P. L. (2003). Hybridization and postzygotic isolation patterns in pigeons and doves. *Evolution*, *57*(6), 1411–1418. https://doi.org/10.1111/j.0014-3820.2003.tb00348.x

Livezey, B. (2010). Phylogenetics of modern shorebirds (Charadriiformes) based on phenotypic evidence: Analysis and discussion. *Zoological Journal of the Linnean Society*, *160*(3), 567–618. https://doi.org/10.1111/j.1096-3642.2010.00635.x

Lougheed, S. C., Campagna, L., Dávila, J. A., Tubaro, P. L., Lijtmaer, D. A., & Handford, P. (2013). Continental phylogeography of an ecologically and morphologically diverse Neotropical songbird, Zonotrichia capensis. *BMC Evolutionary Biology*, *13*, 58. https://doi.org/10.1186/1471-2148-13-58

Lougheed, S. C., Freeland, J. R., Handford, P., & Boag, P. T. (2000). A molecular phylogeny of warbling-finches (Poospiza): Paraphyly in a Neotropical Emberizid genus. *Molecular Phylogenetics and Evolution*, *17*(3), 367–378. https://doi.org/10.1006/mpev.2000.0843

Lovette, I. J. (2005). Glacial cycles and the tempo of avian speciation. *Trends in Ecology and Evolution*, *20*(2), 57–59. https://doi.org/10.1016/j.tree.2004.11.011

Luzuriaga-Aveiga, V. E., Ugarte, M., & Weir, J. T. (2021). Distinguishing genomic homogenization from parapatric speciation in an elevationally replacing pair of Ramphocelus tanagers. *Molecular Ecology*, *30*(21), 5517–5529. https://doi.org/10.1111/mec.16128

Martin, S. H., Dasmahapatra, K. K., Nadeau, N. J., Salazar, C., Walters, J. R., Simpson, F., Blaxter, M., Manica, A., Mallet, J., & Jiggins, C. D. (2013). Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Research*, *23*(11), 1817–1828. https://doi.org/10.1101/gr.159426.113

Morales, H. E., Pavlova, A., Amos, N., Major, R., Kilian, A., Greening, C., & Sunnucks, P. (2018). Concordant divergence of mitogenomes and a mitonuclear gene cluster in bird lineages inhabiting different climates. *Nature Ecology and Evolution*, *2*, 1258–1267. https://doi.org/10.1038/s41559-018-0606-3

Naka, L. N., & Brumfield, R. T. (2018). The dual role of Amazonian rivers in the generation and maintenance of avian diversity. *Science Advances*, *4*(8), 1–13. https://doi.org/10.1126/sciadv.aar8575

Nuñez, J. J., Wood, N. K., Rabanal, F. E., Fontanella, F. M., & Sites, J. W., Jr. (2011). Amphibian phylogeography in the antipodes: Refugia and postglacial colonization explain mitochondrial haplotype distribution in the Patagonian frog *Eupsophus calcaratus* (Cycloramphidae). *Molecular Phylogenetics and Evolution*, *58*(2), 343–352. https://doi.org/10.1016/j.ympev.2010.11.026

Pennington, R. T., Prado, D. E., & Pendry, C. A. (2000). Neotropical seasonally dry forests and Quaternary vegetation change. *Journal of Biogeography*, *27*(2), 261–263. https://doi.org/10.1046/j.1365-2699.2000.00397.x

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Petkova, D., Novembre, J., & Stephens, M. (2016). Visualizing spatial population structure with estimated effective migration surfaces. *Nature Genetics*, *48*(1), 94–100. https://doi.org/10.1038/ng.3464

Piersman, T. (1996). Family Charadriidae. In J. del Hoyo, A. Elliott, & J. Sargatal (Eds.), *Handbook of the birds of the world* (Vol. *3*, pp. 384–443). Lynx Edicions.

Podos, J., & Warren, P. S. (2007). The evolution of geographic variation in birdsong. *Advances in the Study of Behavior*, *37*, 403–458. https://doi.org/10.1016/S0065-3454(07)37009-5

Poelstra, J. W., Vijay, N., Bossu, C. M., Lantz, H., Ryll, B., Müller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M. G., & Wolf, J. B. (2014). The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. *Science*, *344*(6190), 1410–1414. https://doi.org/10.1126/science.1253226

Price, T. D., & Bouvier, M. M. (2002). The evolution of F1 postzygotic incompatibilities in birds. *Evolution*, *56*(10), 2083–2089. https://doi.org/10.1111/j.0014-3820.2002.tb00133.x

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Pulido-Santacruz, P., Aleixo, A., & Weir, J. T. (2019). Genomic data reveal a protracted window of introgression during the diversification of a neotropical woodcreeper radiation. *Evolution*, *74*(5), 842–858. https://doi.org/10.1111/evo.13902

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rabassa, J. (2008). The late Cenozoic of Patagonia and Tierra del Fuego. In J. Rabassa (Ed.), *Developments in quaternary science* (Vol. *11*, p. 524). Elsevier Science.

Rabassa, J., Coronato, A., Bujalesky, G., Salemme, M., Roig, C., Meglioli, A., Heusser, C., Gordillo, S., Roig, F., Borromei, A., & Quattrocchio, M. (2000). Quaternary of Tierra del Fuego, southernmost South America: An updated review. *Quaternary International*, *68*, 217–240. https://doi.org/10.1016/S1040-6182(00)00046-X

Rabassa, J., Coronato, A., & Martínez, O. (2011). Late Cenozoic glaciations in Patagonia and Tierra del Fuego: An updated review. *Biological Journal of the Linnean Society*, *103*(2), 316–335. https://doi.org/10.1111/j.1095-8312.2011.01681.x

Reddy, S. (2014). What's missing from avian global diversification analyses? *Molecular Phylogenetics and Evolution*, *77*, 159–165. https://doi.org/10.1016/j.ympev.2014.04.023

Ribas, C. C., Aleixo, A., Nogueira, A. C. R., Miyaki, C. Y., & Cracraft, J. (2012). A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society B*, *279*(1729), 681–689. https://doi.org/10.1098/rspb.2011.1120

Rocha, A. V., Cabanne, G. S., Aleixo, A., Silveira, L. F., Tubaro, P., & Caparroz, R. (2020). Pleistocene climatic oscillations associated with landscape heterogeneity of the South American dry diagonal explains the phylogeographic structure of the narrow-billed woodcreeper (*Lepidocolaptes angustirostris*, Dendrocolaptidae). *Journal of Avian Biology*, *51*(9), e02537. https://doi.org/10.1111/jav.02537

Rodríguez-Cajarville, M. J., Campagna, L., Alves Lima-Rezende, C., Carboni, M., Tubaro, P. L., & Cabanne, G. S. (2022). Genomic and phenotypic divergence-with-gene-flow across an ecological and elevational gradient in a neotropical bird. *Journal of Biogeography*, *49*(8), 1535–1548. https://doi.org/10.1111/jbi.14449

Rodríguez-Gómez, F., Gutiérrez-Rodríguez, C., & Ornelas, J. F. (2013). Genetic, phenotypic and ecological divergence with gene flow at the Isthmus of Tehuantepec: The case of the azure-crowned hummingbird (*Amazilia cyanocephala*). *Journal of Biogeography*, *40*(7), 1360–1373. https://doi.org/10.1111/jbi.12093

Rolland, J., Jiguet, F., Jønsson, K. A., Condamine, F. L., & Morlon, H. (2014). Settling down of seasonal migrants promotes bird diversification. *Proceedings of the Royal Society B*, *281*, 20140473. https://doi.org/10.1098/rspb.2014.0473

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Rosenzweig, M. L. (1995). *Species diversity in space and time*. Cambridge University Press.

Rull, V. (2011). Neotropical biodiversity: Timing and potential drivers. *Trends in Ecology and Evolution*, *26*(10), 508–513. https://doi.org/10.1016/j.tree.2011.05.011

Rull, V., & Carnaval, C. (2020). *Neotropical diversification: Patterns and processes*. Springer.

Saether, B. E., Lande, R., Engen, S., Weimerskirch, H., Lillegård, M., Altwegg, R., Becker, P. H., Bregnballe, T., Brommer, J. E., McCleery, R. H., Merilä, J., Nyholm, E., Rendell, W., Robertson, R. R., Tryjanowski, P., & Visser, M. E. (2005). Generation time and temporal scaling of bird population dynamics. *Nature*, *436*(7047), 99–102. https://doi.org/10.1038/nature03666

Sánchez, K. I., Avila, L. J., Sites, J. W., Jr., & Morando, M. (2021). An integrative approach to address species limits in the southernmost members of the *Liolaemus kingii* group (Squamata: Liolaemini). *Molecular Phylogenetics and Evolution*, *157*, 107046. https://doi.org/10.1016/j.ympev.2020.107046

Santos, E. S. A. (2020). Southern Lapwing (*Vanellus chilensis*). In T. S. Schulenberg (Ed.), *Birds of the World*. Cornell Lab of Ornithology. https://doi.org/10.2173/bow.soulap1.01

Sedano, R. E., & Burns, K. J. (2010). Are the northern Andes a species pump for Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves: Thraupini). *Journal of Biogeography*, *37*(2), 325–343. https://doi.org/10.1111/j.1365-2699.2009.02200.x

Sérsic, A. N., Cosacov, A., Cocucci, A. A., Johnson, L. A., Pozner, R., Avila, L. J., Sites, J. W., Jr., & Morando, M. (2011). Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society*, *103*(2), 475–494. https://doi.org/10.1111/j.1095-8312.2011.01656.x

Shipilina, D., Serbyn, M., Ivanitskii, V., Marova, I., & Backström, N. (2017). Patterns of genetic, phenotypic, and acoustic variation across a chiffchaff (*Phylloscopus collybita abietinus/tristis*) hybrid zone. *Ecology and Evolution*, *7*(7), 2169–2180. https://doi.org/10.1002/ece3.2782

Silva, S. M., Peterson, A. T., Carneiro, L., 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., … Aleixo, A. (2019). A dynamic continental moisture gradient drove Amazonian bird diversification. Science. *Advances*, *5*, eaat5752. https://doi.org/10.1126/sciadv.aat5752

Smith, B. T., McCormack, J. E., Cuervo, A. M., Hickerson, M. J., Aleixo, A., Cadena, C. D., Pérez-Emán, J., Burney, C. W., Xie, X., Harvey, M. G., Faircloth, B. C., Glenn, T. C., Derryberry, E. P., Prejean, J., Fields, S., & Brumfield, R. T. (2014). The drivers of tropical speciation. *Nature*, *515*, 406–409. https://doi.org/10.1038/nature13687

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution*, *28*(10), 2731–2739. https://doi.org/10.1093/molbev/msr121

Thom, G., Xue, A. T., Sawakuchi, A. O., Ribas, C. C., Hickerson, M. J., Aleixo, A., & Miyaki, C. (2020). Quaternary climate changes as speciation drivers in the Amazon floodplains. *Science Advances*, *6*, eaax4718. https://doi.org/10.1126/sciadv.aax4718

Thrasher, D. J., Butcher, B. G., Campagna, L., Webster, M. S., & Lovette, I. J. (2018). Double-digest RAD sequencing outperforms microsatellite loci at assigning paternity and estimating relatedness: A proof of concept in a highly promiscuous bird. *Molecular Ecology Resources*, *18*(5), 953–965. https://doi.org/10.1111/1755-0998.12771

Toews, D. P. L., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, *21*(16), 3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x

Trier, C. N., Hermansen, J. S., Sætre, G.-P., & Bailey, R. I. (2014). Evidence for mito-nuclear and sex-linked reproductive barriers between the hybrid Italian Sparrow and its parent species. *PLoS Genetics*, *10*, e1004075. https://doi.org/10.1371/journal.pgen.1004075

Trujillo-Arias, N., Rodríguez-Cajarville, M. J., Sari, E., Miyaki, C. Y., Santos, F. R., Witt, C. C., Barreira, A. S., Gómez, I., Naoki, K., Tubaro, P. L., & Cabanne, G. S. (2020). Evolution between forest macrorefugia is linked to discordance between genetic and morphological variation in Neotropical passerines. *Molecular Phylogenetics and Evolution*, *149*, 106849. https://doi.org/10.1016/j.ympev.2020.106849

Turbek, S. P., Browne, M., Di Giacomo, A. S., Kopuchian, C., Hochachka, W. M., Estalles, C., Lijtmaer, D. A., Tubaro, P. L., Silveira, L. F., Lovette, I. J., Safran, R. J.,Taylor, S. A., & Campagna, L. (2021). Rapid speciation via the evolution of pre-mating isolation in the Iberá Seedeater. *Science*, *371*(6536), eabc0256. https://doi.org/10.1126/science.abc0256

Turchetto-Zolet, A. C., Pinheiro, F., Salgueiro, F., & Palma-Silva, C. (2013). Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology*, *22*(5), 1193–1213. https://doi.org/10.1111/mec.12164

Uy, J. A. C., Irwin, D. E., & Webster, M. S. (2018). Behavioral isolation and incipient speciation in birds. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 1–24. https://doi.org/10.1146/annurev-ecolsys-110617-062646

van Els, P., Zarza, E., Rocha Moreira, L., Gómez-Bahamón, V., Santana, A., Aleixo, A., Ribas, C. C., Sena do Rêgo, P., Pérsio Dantas Santos, M., Zyskowski, K., Prum, R. O. & Berv, J. (2021). Recent divergence and lack of shared phylogeographic history characterize the diversification of neotropical savanna birds. *Journal of Biogeography*, *48*(2), 1–14. https://doi.org/10.1111/jbi.14065

Weir, J. T. (2006). Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. *Evolution*, *60*(4), 842–855. https://doi.org/10.1111/j.0014-3820.2006.tb01161.x

Weir, J. T., & Price, M. (2011). Andean uplift promotes lowland speciation through vicariance and dispersal in *Dendrocincla* woodcreepers. *Molecular Ecology*, *20*(21), 4550–4563. https://doi.org/10.1111/j.1365-294X.2011.05294.x

Weir, J. T., & Schluter, D. (2004). Ice sheets promote speciation in boreal birds. *Proceedings of the Royal Society B*, *271*(1551), 1881–1887. https://doi.org/10.1098/rspb.2004.2803

Wiersma, P., & Kirwan, G. M. (2018). Southern Lapwing (*Vanellus chilensis*). In J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, & E. de Juana (Eds.), *Handbook of the birds of the world alive*. Lynx Edicions.

Winger, B. M., Barker, F. K., & Ree, R. H. (2014). Temperate origins of long-distance seasonal migration in New World songbirds. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(33), 12115–12120. https://doi.org/10.1073/pnas.1405000111

Winker, K. (2021). An overview of speciation and species limits in birds. *Ornithology*, *138*, ukab006. https://doi.org/10.1093/ornithology/ukab006

Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, *16*(2), 97–259. https://doi.org/10.1093/genetics/16.2.97

Zheng, X., Levine, D., Shen, J., Gogarten, S., Laurie, C., & Weir, B. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

BIOSKETCH

Belén Bukowski is interested in the study of the evolutionary history of the Neotropical avifauna. This work represents a component of her PhD research at the Ornithology Division of the MACN on the study of the patterns and processes responsible for the diversification, and possible early stages of speciation, of Neotropical birds.

Author contributions: Belén Bukowski, Darío A. Lijtmaer and Pablo L. Tubaro conceived the ideas; Belén Bukowski and María José Rodríguez-Cajarville conducted the fieldwork and collected the data with additional material from collaborators; Belén Bukowski analysed the data with assistance from Leonardo Campagna and Gustavo S. Cabanne and Belén Bukowski and Darío A. Lijtmaer led the writing with assistance from all co-authors.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bukowski, B., Campagna, L., Rodríguez-Cajarville, M. J., Cabanne, G. S., Tubaro, P. L., & Lijtmaer, D. A. (2023). The role of glaciations in the evolutionary history of a widely distributed Neotropical open habitat bird. *Journal of Biogeography*, *00*, 1–16. https://doi.org/10.1111/jbi.14738