

# Artículos

## HISTORICAL DEMOGRAPHY OF *Trichothraupis melanops* (AVES: THRAUPIDAE) AND THE PLEISTOCENE ATLANTIC FOREST BIOGEOGRAPHY

DEMOGRAFÍA HISTÓRICA DE *Trichothraupis melanops* (AVES: THRAUPIDAE) Y BIOGEOGRAFÍA DE LA SELVA ATLÁNTICA EN EL PLEISTOCENO

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**ABSTRACT:** The climatic cycles of the Pleistocene affected the distribution of the vegetation of different biomes, determining the distribution and evolution of the associated fauna. Many studies of Atlantic Forest (AF) organisms suggest that the Pleistocene glacial and interglacial cycles have produced demographically stable populations in the forest’s central region and unstable populations in its southern regions (known as the Carnival-Moritz model). We studied the phylogeographic structure of an AF passerine, the Black-goggled Tanager (*Trichothraupis melanops*, Thraupidae) and evaluated questions related to the history of the AF. We analyzed three independent genes, two nuclear and one mitochondrial, using population genetic methods based on summary statistics and traditional phylogeographic methods. Our results suggest that *T. melanops* shares phylogeographic features with other AF taxa. Even though an effective population size gradient was found between the central and southern populations, which is in agreement with the results of other phylogeographic studies and the forest refugia model (the Carnival-Moritz model), there is no evidence of a gradient of genetic diversity. In addition, we have found that *T. melanops* populations show the demographic dynamics expected according to the Carnival-Moritz model: the inland-southern population was found to be the one with the greatest signs of recent demographic expansion, compared to the central and coastal-southern populations. Although this species is ecologically generalist and not very sensitive to forest fragmentation and degradation, it has been impacted by the historical dynamism of the AF.

**KEYWORDS:** *Atlantic forest, Historical demography, Phylogeography, Pleistocene, Trichothraupis*

**RESUMEN:** Los ciclos climáticos del Pleistoceno afectaron la distribución de la vegetación de diferentes biomas, condicionando la distribución y evolución de la fauna asociada. Muchos estudios sobre organismos de la Selva Atlántica (SA) sugieren que los ciclos glaciales e interglaciales del Pleistoceno han producido poblaciones demográficamente estables en la región central de la selva y poblaciones inestables en sus regiones del sur (conocido como modelo Carnival-Moritz). Estudiamos la estructura filogeográfica de un paseriforme que se distribuye en la SA, el Frutero de Antojos Negros (*Trichothraupis melanops*, Thraupidae), y evaluamos preguntas relacionadas con la historia de la SA. Analizamos tres genes independientes, dos nucleares y uno mitocondri-

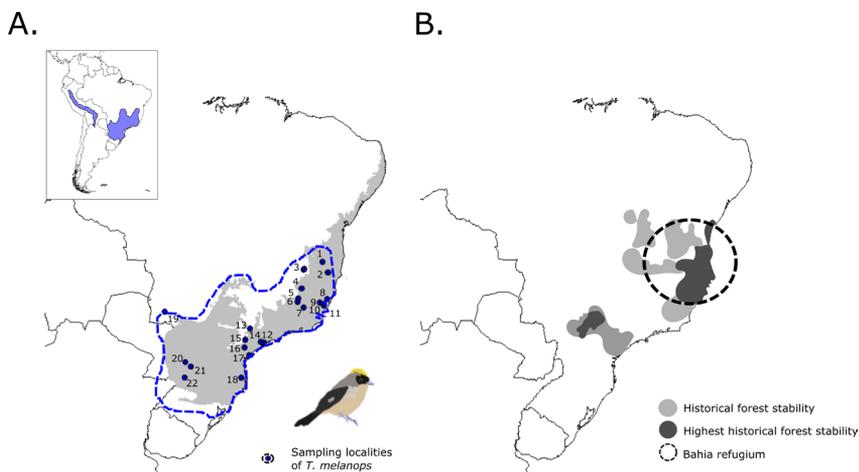
al, utilizando métodos de genética de poblaciones basados en estadísticas sumarias y métodos filogeográficos tradicionales. Nuestros resultados sugieren que *T. melanops* comparte características filogeográficas con otros taxones de la SA. Si bien se encontró un gradiente de tamaño poblacional efectivo entre las poblaciones del centro y del sur, lo que concuerda con los resultados de otros estudios filogeográficos y el modelo de refugios (el modelo Carnaval-Moritz), no hay evidencia de un gradiente de diversidad genética. Además, hemos encontrado que las poblaciones de *T. melanops* presentan la dinámica demográfica esperada por el modelo Carnaval-Moritz: la población del centro-sur resultó ser la que presenta mayores signos de expansión demográfica reciente, en comparación con las poblaciones central y de la costa sur. Aunque esta especie es ecológicamente generalista y poco sensible a la fragmentación y degradación forestal, ha sido impactada por el dinamismo histórico de la Selva Atlántica.

**PALABRAS CLAVE:** *Demografía histórica, Filogeografía, Pleistoceno, Selva Atlántica, Trichothraupis*

The Atlantic Forest (AF) is distributed along the eastern coast of Brazil, northeastern Argentina, and eastern Paraguay (Figure 1A). This biome is one of the most diverse and threatened ecosystems in the world (Galindo Leal and Câmara 2003). The climatic oscillations that occurred during the late Pleistocene affected the demography, population diversification and distribution of the organisms that inhabit the AF, and thus have driven the evolution of the high biological diversity of the biome. Up to now, most studies on the impacts of climatic fluctuations on AF organisms focused on species that are specialists in well-preserved forests (Cabanne et al. 2008, Thom et al. 2021), while there are few studies on organisms that are generalists or less sensitive to environmental disturbance (Batalha-Filho et al. 2012, Lima-Rezende et al. 2022, Bukowski et al. 2023).

The climatic cycles of the Pleistocene promoted speciation due to population isolation and divergence

in refugia during periods such as glacial maxima (Haffer 1969, Moritz et al. 2000, Haffer and Prance 2001). Some studies in the AF reveal the importance of geographic isolation and climate change in genetic diversification when evaluating phylogeographic divergence (Cabanne et al. 2008, Batalha-Filho et al. 2012, Cabanne 2019, Trujillo et al. 2020). In the AF, climatic fluctuations have had a significant impact on the distribution and continuity of the forest. The Carnaval and Moritz (2008) model (from now on Carnaval-Moritz model) states that expansions and contractions of the AF, associated with Pleistocene climatic fluctuations, fragmented and reduced the area occupied by forest organisms, affecting the demographic patterns of population structure and genetic variation. By applying ecological niche modeling to historical climate data, Carnaval and Moritz (2008) predicted how species distributions may have shifted in response to changing climate conditions. They fo-



**Figure 1.** Distribution of the Atlantic Forest, study area of the genetic study of *Trichothraupis melanops* and working hypothesis. A. Distribution of the Atlantic Forest (grey area) and of *Trichothraupis melanops*. We also present collection sites for the genetic samples. See detail in the Appendix. The inset map indicated the study area. B. Working hypothesis on the location of historically stable areas for the AF during the last 21 000 years. The darker the area the higher the forest stability. The circle indicates the area of highest forest stability (Bahia refugium). Modified from Carnaval and Moritz (2008).

cused on variations in biodiversity patterns and species ranges, highlighting how different species would have responded to glacial and interglacial periods. To validate their predictions, Carnaval and Moritz (2008) also compared their modeled results with genetic data from modern populations of various species, that provided insights into historical population dynamics and genetic diversity. The model predicts that during glacial maxima, the center of the AF was a refugium (Bahía refugium, Figure 1B), while the southern region of the biome was the most unstable in terms of continuity and forest coverage (south of Rio de Janeiro and Espírito Santo). Refugia are areas where species could persist during unfavorable glacial periods. Specifically, the model predicts the Bahía refugium 21 000 years ago, during the last glacial maximum. This refugium would have provided relatively stable and suitable climates for certain species to survive when large parts of the AF became inhospitable due to colder and drier conditions. According to Carnaval-Moritz model, most AF organisms should have been equally affected by the biome fragmentation during the Pleistocene, so they are expected to share a single phylogeographic history. Specific predictions are: a) populations of those organisms associated with the AF will share geographical patterns of genetic structure, b) the central populations (associated with the Bahía refugium) will be demographically stable and have the greatest genetic diversity and effective size, while c) those in the south will be less genetically diverse, will have a smaller effective size, and will show signs of demographic expansion. Consequently, d) it is also expected to find a genetic diversity gradient between the Bahia region and the AF south, the latter populations being the ones with the least diversity.

We studied the historical demography of the Black-goggled Tanager (*Trichothraupis melanops*, Aves: Thraupidae), an ecologically generalist bird, to evaluate predictions of the hypothesis about the impact of Pleistocene climatic fluctuations on the AF organisms as described by the Carnaval-Moritz model. *T. melanops* is a monotypic species distributed in the understory of humid and montane forests in eastern and southern Brazil, eastern Paraguay, and northeastern Argentina (in the AF) (Figure 1A), as well as in a narrow strip of Andean rainforest between southern Peru and northwestern Argentina (Ridgely and Tudor 2009). In a study on the evolution between the Andes and the AF, Trujillo et al. (2018) showed that the Andean population of the species is genetically isolated from the AF populations and from the gallery forests in the Cerrado and/or Chaco. This species is a good model to

study biogeographical events in the AF because it is abundant, not threatened (IUCN 2021) and forest dependent (Trujillo et al. 2018). Also, it is a species with moderate to low sensitivity to habitat disturbance (Ridgely and Tudor 2009), so its response to habitat natural modifications linked to climate global cycles could be different from that of species more sensitive to habitat changes (e.g., Cabanne et al. 2008) due to its diet flexibility, tolerance for modified habitats, dispersal abilities or competitive interactions (Ribon 2003, dos Anjos 2006) during the Pleistocene glacial cycles.

## METHODS

### Genetic dataset

We studied DNA sequences (51 specimens from 22 localities) collected in a previous study (Trujillo et al. 2018) to evaluate population genetic structure and historical demography of the AF population of *T. melanops* (see details of the samples and sampling localities in Appendix A and B). The studied genetic dataset corresponded to three independent markers: Cytochrome b (Cyt b, 930 bp), intron 9 of the Low-density lipoprotein receptor linked to the Z chromosome (VLDL, 448 bp), and intron 11 of Glyceraldehyde-3-phosphate dehydrogenase (G3-pdh, 330 bp).

### Genetic analyses

We used CLUSTAL in MEGA v7.0.26 (Sudhir et al. 2016) to align sequences. To detect population structure, we used Geneland (Guillot et al. 2005) in R Studio (RStudio Team 2015). In Geneland, the most likely number of populations was estimated by 5 runs of 20 million iterations each, independent for each marker. With this information, we performed new runs for each marker, setting the number of most likely populations obtained and performing 5 runs of 20 million iterations each. Then, we selected the results of the runs with the highest posterior probability for each marker. To build haplotype networks we used the Median Joining algorithm in PopArt (Bandelt et al. 1999).

To evaluate population assignment and isolation by geographic distance, we performed a partial Mantel test in Vegan (Oksanen et al. 2020) between the pairwise genetic distance between individuals, and predictors such as geographical distances between individuals and an indicator matrix of population identity. The genetic distance matrices were generated using the Pairwise Distances function (parameters: p-distance, delete substitutions and complete dele-

tion of gaps) in MEGA v7.0.26. The identity matrix was assembled considering the presence or absence of the samples from a certain locality within the populations defined by Geneland. Also, an analysis of molecular variance (AMOVA) was performed to evaluate genetic differentiation among populations detected by Geneland in Arlequin v3.5.2.2 (Excoffier and Lischer 2010).

To estimate summary statistics of neutrality and demography (neutrality-demographic tests), we used DNAsp v6 (Rozas et al. 2017). To study the genetic diversity of the populations delimited by Geneland, the haplotypic ( $H_d$ ) and nucleotide ( $\pi$ ) diversities were calculated, as well as the number of haplotypes and segregating sites. To evaluate historical demography and possible variations in population size, we performed Tajima's  $D$  (Tajima 1989), Fu's  $F_s$  (Fu 1997) and Ramos-Onsins  $R_2$  (Ramos-Onsins and Rozas 2002) tests. A statistically significant result of these tests implies rejection of the null hypothesis of demographic neutrality and/or stability. To differentiate demographic effects from natural selection, we performed the DH and DHEW compound tests (Zeng 2006, 2007a). Both tests merge different statistics and evaluate the null hypothesis of neutrality, without being sensitive to demographic effects. Tajima's  $D$  and  $H$  are tests that detect positive selection with high probability, but each one is sensitive to different demographic processes and, therefore, DH is statistically specific to study positive natural selection (Zeng 2006). The DHEW test combines the DH test with the Ewens-Watterson EW test (Watterson 1978). The EW test is powerful detecting positive selection, population expansion, and bottlenecks. Results obtained by the DHEW tests are highly powerful since they combine the DH specificity to detect selection with the statistical power of EW. Significant values are expected for both tests, DH and DHEW, only if there has been a positive selection (Zeng 2007b). These tests were performed with Zeng's DH program (<https://github.com/drkaizeng/publications-and-software>), using *Eucometis penicillata*, a sister species to *T. melanops*, as outgroup (Burns and Racicot 2009), and a significance limit of 0.01 (Yednock and Neigel 2014).

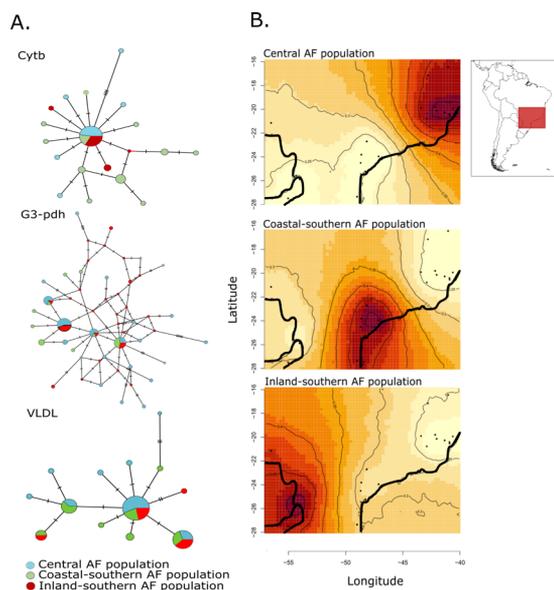
Finally, to evaluate the demographic variation between regions we reconstructed the effective population size of each population using the Extended Bayesian Skyline Plot (EBSP) in BEAST v2.6.3 (Bouckaert 2019). Samples from the different populations were analyzed separately, with 10 million iterations of the MCMC algorithm per run and different nucleotide substitution models for each marker. For Cyt b and G3-pdh, we used the molecular evolution model GTR,

while for VLDL the estimates were obtained using HKY, following Trujillo et al. (2018). All parameters were estimated from data, except Cyt b substitution rate per site, which was set at 0.0105 substitutions per site per million generations (Papadopoulou et al. 2010). We evaluated population growth by obtaining the ratio between population effective sizes of different periods in comparison to the present. We chose the specific periods of time based on previous information related to forest fragmentation due to climate oscillations (Rull and Carnaval 2020).

## RESULTS

### Phylogeographical patterns

We have studied Cyt b, G3-pdh and VLDL DNA sequences of a sample of 51 *T. melanops* from the AF. We obtained haplotype networks of these sequences that indicated a moderate population structure across the study area (Figure 2A). Geneland analysis divided the *T. melanops* samples of Cyt b into three genetic clusters, with high posterior probability of assignment (Figure 2B). These populations were named "Central", "Coastal-southern" and "Inland-southern". Samples from Minas Gerais and Espírito Santo (Brazil) belong to the Central population. Samples from São Paulo, Santa Catarina and Paraná (Brazil) belong to the



**Figure 2.** Population genetic structure of *T. melanops* based on three independent genetic markers. **A)** Median joining network of mtDNA Cyt b sequences, nuclear DNA G3-pdh and VLDL. **B)** Geneland analysis and population genetic structure based on Cyt b sequences. Dark red-brown colors indicate highest probability of association of samples to each population.

Coastal-southern population. Samples from Misiones (Argentina) and Mato Grosso do Sul (Brazil) belong to the Inland-southern population. Geneland analysis with both nuclear markers did not support any specific cluster.

AMOVA results with Cyt b indicated a significant  $\Phi_{ST}$  ( $\Phi_{ST}=0.147$ ,  $p=0.017$ ), describing that 15% of the molecular variation was attributed to differences among populations. The AMOVA results with VLDL or G3-pdh were not significant ( $p>0.05$ ).

The partial Mantel tests did not support isolation by geographic distance for any of the studied genes ( $p>0.05$ , controlling for population). In addition, it indicated that the metric population explained genetic variation only in the case of Cyt b ( $r=0.2276$ ;  $p=0.001$ , controlled for geographic distances). Therefore, these analyses indicated that the population structure observed in Cyt b sequences was significant and not an artifact of an uneven geographic sampling. Thus, we considered three genetic clusters for downstream analyses (i.e., Central, Coastal-southern, and Inland-southern).

### Diversity and demography

Diversity indices were not clearly different among the three populations (Table 1). Therefore, no gradient between populations was distinguished for the indices of haplotype diversity and nucleotide diversity. Summary statistics (Table 1) yielded significant negative values of Fu's  $F_s$  for the Central and Coastal-southern AF populations for the three analyzed markers. Tajima's  $D$  was significantly negative for the Central population for all three markers, and the

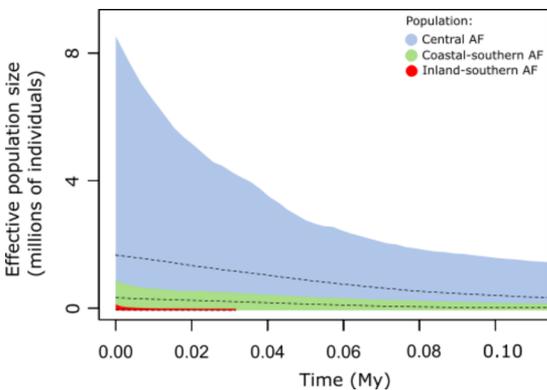
Coastal-southern population for VLDL. Finally, Ramos-Onsins'  $R_2$  was significant for the Central and Coastal-southern populations according to the three markers. In addition, the value of  $R_2$  was significant for the Inland-southern population according to Cyt b. The values of the DH and DHEW statistics were all non-significant, indicating that the significant results of the previous tests were due to demographic effects and not to bias from neutrality.

The Extended Bayesian Skyline Plot analyses estimated a current effective population size of 1 660 000 individuals in the Central population, 330 000 individuals in the Coastal-southern and 18 000 individuals in the Inland-southern population (Figure 3, Table 2). We quantified the population growth by comparing the present effective size in relation to three periods during the last 30 000 years, which indicated a tendency of the Inland-southern AF to be the population with the largest growth, and of the Central AF to be the most stable population (Figure 3, Table 2). Table 2 presents the confidence intervals associated with  $N_e$  and growth rates. While there may be overlap in the confidence intervals, exact values are derived from effective sizes estimated through Bayesian methods and represent the highest posterior probability, indicating that overlapping intervals do not necessarily imply the absence of differences.

### DISCUSSION

We evaluated the hypothesis of the impact of Pleistocene climatic fluctuations on the Atlantic Forest organisms during the late Pleistocene by conducting a population genetic study of *Trichothraupis melanops*, an ecologically generalist species. We found three genetically distinct populations, with a different population effective size, being the Central AF population the largest, followed by the Coastal-southern and Inland-southern populations. The historical demography of these populations was compatible with an expansion scenario in the AF inland-south, as well as with a smaller population growth in the Central and Coastal-southern region. The results indicated that *T. melanops* shared a population genetic structure, along with demographic dynamics, with other organisms in the region, partially agreeing with the model proposed by Carnaval and Moritz.

The phylogeographic study of the AF *T. melanops* populations supported the Carnaval-Moritz model as a) the bird's populations shared geographical patterns of genetic structure with other species in the region, b) the Central population (associated with the Bahía refugium) was the largest in population size and the



**Figure 3.** Historical demography of *T. melanops* in the Atlantic Forest based on Extended Bayesian Skyline Plot and on three independent markers. The dashed line represents the median effective population. Confidence intervals are based on the 95% highest posterior density. Time is in million years (My).

**Table 1.** Neutrality-demography tests of populations of *T. melanops* based on three independent genetic markers. Sample size in number of DNA sequences (N), Tajima's D, Fu's Fs, Ramos-Onsins R<sub>2</sub>, DH and DHEW neutrality tests of Zeng (p-values indicated), number of polymorphic sites (S), number of haplotypes (H), haplotype (Hd) and nucleotide ( $\pi$ ) diversities are presented. P-values are within parentheses. Significant values are indicated in bold (p-value<=0.05 for Tajima's D and Ramos-Onsins' R<sub>2</sub>; p-value<=0.02 for Fu's Fs; p-value<=0.01 for DH and DHEW).

Marker	Statistic	Population		
		Central	Coastal-southern	Inland-southern
Cyt b	N	15	18	11
	D	-1.898 <b>(0.01)</b>	-0.826 (0.22)	-1.114 (0.21)
	Fs	-2.718 <b>(0.02)</b>	-5.082 <b>(0.001)</b>	-1.525 (0.03)
	R <sub>2</sub>	0.110 <b>(0.04)</b>	0.091 <b>(0.02)</b>	0.140 <b>(0.02)</b>
	DH	(0.0363)	(0.1185)	(0.0547)
	DHEW	(0.0104)	(0.1746)	(0.0354)
	S	10	9	3
	H	7	10	4
	$\pi$	0.00173 ± 0.00160	0.00234 ± 0.00142	0.00081 ± 0.00079
	Hd	0.657 ± 0.138	0.908 ± 0.044	0.600 ± 0.154
VLDL	N	24	32	18
	D	-1.396 <b>(0.05)</b>	-1.472 <b>(0.05)</b>	0.193 (0.61)
	Fs	-6.601 <b>(0.0001)</b>	-5.101 <b>(0.002)</b>	-0.161 (0.46)
	R <sub>2</sub>	0.071 <b>(0.002)</b>	0.067 <b>(0.03)</b>	0.161 (0.63)
	DH	(0.0718)	(0.0981)	(0.3753)
	DHEW	(0.0456)	(0.0682)	(0.6101)
	S	8	10	5
	H	10	10	5
	$\pi$	0.00264 ± 0.00217	0.00286 ± 0.00230	0.00338 ± 0.00173
	Hd	0.746 ± 0.091	0.764 ± 0.072	0.771 ± 0.051
G3-pdh	N	36	30	24
	D	-1.576 <b>(0.03)</b>	-1.367 (0.07)	-1.033 (0.16)
	Fs	-13.523 <b>(0.0002)</b>	-8.449 <b>(0.0003)</b>	-3.753 (0.03)
	R <sub>2</sub>	0.069 <b>(0.05)</b>	0.072 <b>(0.04)</b>	0.108 (0.12)
	DH	(0.4028)	(0.2339)	(0.1135)
	DHEW	(0.2835)	(0.3383)	(0.0726)
	S	33	19	12
	H	24	16	10
	$\pi$	0.01596 ± 0.00838	0.00893 ± 0.00554	0.01000 ± 0.00515
	Hd	0.946 ± 0.025	0.929 ± 0.028	0.850 ± 0.080

most stable demographically, and c) the Inland-southern population showed the strongest signs of recent population expansion. However, regarding genetic diversity pattern across populations, no clear results

were obtained since no diversity gradient was found between the Bahia region and the southern AF region.

We found that the population genetic structure of *T. melanops* was comparable to that of other organisms

**Table 2.** Effective population size and population growth to the present of populations of *T. melanops* at different time periods, as estimated by Bayesian Skyline Plot. 95% confidence intervals are indicated.

Time period	Population					
	Central AF		Coastal-southern AF		Inland-southern AF	
	Ne	Population growth	Ne	Population growth	Ne	Population growth
Present	1.66 (0.09– 8.53)		0.33 (0.01–0.99)		0.018 (2.05x10 <sup>9</sup> – 0.21)	
0.01 MYA	1.49 (0.27–6.11)	1.11 (0.03–1.40)	0.29 (0.05–0.68)	1.13 (0.02–1.46)	0.015 (8.45x10 <sup>8</sup> –0.15)	1.2 (0.024–1.4)
0.02 MYA	1.36 (0.23–5.11)	1.22 (0.39 –1.67)	0.25 (0.03–0.63)	1.32 (0.33–1.57)	0.012 (8.45x10 <sup>8</sup> – 0.12)	1.5 (0.024– 1.75)
0.03 MYA	1.20 (0.10–4.25)	1.38 (0.90–2.00)	0.204 (0.0023–0.58)	1.6 (0.006–1.7)	0.008 (8.45x10 <sup>8</sup> – 0.05)	2.25 (0.024–4.2)

from the AF (Costa 2003, Batalha-Filho et al. 2013, Costa and Leite 2013), which suggests a shared biogeographic history among them. The Geneland analysis for Cyt b supported three populations of *T. melanops* (Figure 2B), indicating that there is genetic differentiation across AF regions, also supported by AMOVA. Moreover, despite the uneven geographic sampling, we found that genetic differences between these populations were not explained by isolation by distance.

The different populations of *T. melanops* are apparently limited or associated to important geographic landmarks of the AF. Several authors define the central AF, as limited in the south by the Doce River, as the turning point between populations and lineages in the biome (Cracraft and Prum 1988, Costa 2003, Cabanne et al. 2008, Carnaval et al. 2009). The northernmost population of *T. melanops* was located to the north of that river. The Coastal-southern population occupied the southern Serra do Mar mountain chain, an important region in the biome because of its high levels of endemism (Cracraft and Prum 1988, Rull and Carnaval 2020), while the Inland-southern population was associated with the interior of the continent, to regions with semideciduous forests. It is worth noting that other species from the region also have their phylogeographic patterns associated with these geographic landmarks (e.g., Cabanne et al. 2008, Carnaval et al. 2009, Batalha-Filho et al. 2013), thus

*T. melanops* and these other species might have been affected by the same evolutionary forces.

The variation of population effective size of *T. melanops* across the study region supported the Carnaval-Moritz model, which proposes that the populations located in the north and central zone of the biome will be the largest. However, the genetic diversity values for each region did not clearly differ between populations (Table 1). Results of the Extended Bayesian Skyline Plot suggested moderate differences in effective size among populations (Figure 3, Table 2). Despite differences are relatively small, as predicted, the Central and Coastal-southern populations presented the largest population effective size, while the smallest population was the Inland-southern. The population dynamic was also in accordance with the Carnaval-Moritz model, with the Inland-southern population showing the highest signs of recent population expansion, compared to the Central and Coastal-southern populations (Table 2). The Inland-southern population showed a population growth of 2.25 times in the last 30 000 years, while the highest growth in the Central and Coastal-southern populations was 1.6 times. In addition, when comparing population growth during the last 0.1 million years, the northernmost population was the one with the shallower growth in relation to present, which is also in agreement with predictions and with what is

observed in other birds of the region (e.g., Cabanne et al. 2008, Batalha-Filho et al. 2013, Raposo do Amaral et al. 2021). These shallow population growth indicators may be related to the fact that the studied species has moderate to low sensitivity to habitat disturbance (Ridgely and Tudor 2009), so its response to habitat natural modifications linked to climate global cycles could be different from that of species more sensitive to habitat changes (e.g., Cabanne et al. 2008).

In conclusion, we found in *T. melanops* a population genetic structure with evidence of shallow demographic change during the end of the Pleistocene, which is expected to be associated to the forest dynamisms imposed by global climate cycles. Most studies on the impacts of climatic fluctuations on AF organisms focus on endemic species that are specialist of well-preserved forests (Cabanne et al. 2008, Batalha-Filho et al. 2012), while there are few studies of organisms like *T. melanops*, which are ecologically flexible and less sensitive to environmental disturbances (Ribon 2003, dos Anjos 2006). The results indicated *T. melanops* population changes in agreement with the Carnaval-Moritz model and are comparable to what was observed in other species of the region that are more sensitive to habitat change. Thus, even though the species is ecologically less sensitive to habitat modifications, habitat change in the AF during the end of the Pleistocene seems to have been strong enough to have impacted its population structure and demography.

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**Appendix A.** Samples used for genetic and demographic analyses of *T. melanops*. Abbreviations for tissue catalogue ID are: MSB:Bird, Museum of Southwestern Biology, University of New Mexico, USA; MACN-Or-ct, Bird tissue collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina; MG, Museu Paraense Emilio Goeldi, Belém, Brazil; LGEMA, Laboratório de Genética e Evolução Molecular de Aves, Universidade de São Paulo, Brazil, and B, Laboratorio de Biodiversidade e Evolução Molecular, Universidade Federal Minas Gerais, Brazil. Population assignment abbreviations mean: C: central AF; CoS: coastal-southern AF; IS: inland-southern AF.

Locality number	Locality	Tissue ID	Population assignment
1	Leme do Prado, Minas Gerais, Brazil	B3224	C
2	Jequitinhonha, Minas Gerais, Brazil	B1077	C
3	Turmalina, Minas Gerais, Brazil	B1078	C
4	Caratinga, Minas Gerais, Brazil	B3222	C
5	Simonésia, Minas Gerais, Brazil	B1072	C
6	Viçosa, Minas Gerais, Brazil	B1254	C
7	Santa Bárbara, Minas Gerais, Brazil	B0476	C
8	Santa Teresa, Espírito Santo, Brazil	B1265	C
9	Domingos Martins, Espírito Santo, Brazil	B1818	C
10	Conceição do Castelo, Espírito Santo, Brazil	B1402	C
11	Alfredo Chaves, Espírito Santo, Brazil	B1366	C
12	PETAR, Núcleo Caboclos, São Paulo, Brazil	B1367	C
13	P.E. Serra do Mar, Núcleo Curucutú, São Paulo, Brazil	B3300	C
		B3297	C
		B3298	C
		B3301	C
		B3264	C
		LGEMA 1603	CoS
		LGEMA 15091	CoS
		LGEMA 15199	CoS
		LGEMA 15200	CoS
		LGEMA 1576	CoS
		LGEMA 15193	CoS
		LGEMA 15205	CoS

Locality number	Locality	Tissue ID	Population assignment
14	Juquitiba, São Paulo, Brazil	LGEMA 1141	CoS
		LGEMA 1156	CoS
		LGEMA 2073	CoS
15	Fazenda Barreiro Rico, Anhembi, São Paulo, Brazil	LGEMA 2074	CoS
		LGEMA 2089	CoS
		LGEMA 2972	CoS
16	Buri, São Paulo, Brazil	LGEMA 2975	CoS
		LGEMA 14652	CoS
17	Guaraqueçaba, Utinga, Paraná, Brazil	LGEMA 14707	CoS
		LGEMA 14586	CoS
		LGEMA 14588	CoS
18	Nova Trento, Santa Catarina, Brazil	LGEMA 14642	CoS
		LGEMA 145643	CoS
		MG51927	IS
19	Bonito, Mato Grosso do Sul, Brazil	MG51928	IS
		MG51929	IS
		MG51930	IS
20	Paraje María Soledad, General Belgrano, Misiones, Argentina	MACN-Or-ct 1969	IS
		MACN-Or-ct 2851	IS
		MACN-Or-ct 2865	IS
21	P.N Iguazú, Iguazú, Misiones, Argentina	MACN-Or-ct 3581	IS
		MACN-Or-ct 6827	IS
		MACN-Or-ct 7001	IS
22	CIAR, Oberá, Misiones, Argentina	MACN-Or-ct 7004	IS
		MACN-Or-ct 6971	IS
		MACN-Or-ct 1984	IS
		MACN-Or-ct 1987	IS
	Outgroup: <i>Eucometis penicillata</i>	MACN-Or-ct 5950	

**Appendix B.** Samples used for genetic and demographic analyses of *T. melanops*. Population assignment abbreviations mean: C: central AF; CoS: coastal-southern AF; IS: inland-southern AF.

	C	CoS	IS	Total
Number of individuals	17	20	14	51
Number of Cyt b sequences	15	18	11	44
Number of G3-pdh sequences	36	30	24	90
Number of VLDL sequences	24	32	18	74