



Forest corridors between the central Andes and the southern Atlantic Forest enabled dispersal and peripatric diversification without niche divergence in a passerine



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ABSTRACT

The central Andean rainforests and the Atlantic Forest are separated by the Chaco and the Cerrado domains. Despite this isolation, diverse evidence suggests that these rainforests have been connected in the past. However, little is known about the timing and geographic positions of these connections, as well as their effects on diversification of species. In this study, we used the Black-goggled Tanager (*Trichothraupis melanops*, Thraupidae) as a model to study whether the Andean and the Atlantic forests have acted as a refugia system, and to evaluate biogeographic hypotheses of diversification and connection between these rainforests. We compared alternative biogeographic scenarios by using Approximate Bayesian Computation (ABC), modeled range shifts across time, and assessed niche divergence between regions. The results indicated that the major phylogeographic gap within *T. melanops* is located between these rainforests. The ABC analysis supported peripatric diversification, with initial dispersal from the Atlantic Forest to the Andes during the Mid-Pleistocene. Also, the results supported an Andean-Atlantic forests connection through the current Cerrado-Chaco transition, linking the southern Atlantic Forest with the central Andes. Our findings, taken together with other studies, support that the connection between these biomes has been recurrent, and that has occurred mostly through the Cerrado and/or the Cerrado-Chaco transition. The data also support that the connection dynamic has played an important role in the biological diversification, by promoting peripatric divergence in some forest taxa restricted to both biomes.

1. Introduction

Shifts in the geographic distribution of forests have played an important role in the diversification of species by promoting vicariance and dispersal (Cadena et al., 2016; Prates et al., 2016). According to palynological evidence and paleoclimatic models, the glacial and interglacial cycles of the Quaternary not only favored divergence and secondary contact within the same biome (e.g., Carnaval and Moritz, 2008), but also enabled biotic exchange between disjunct regions (e.g., Costa, 2003; Prates et al., 2017).

Climate-mediated dispersal between biomes has been supported for a number of tropical rainforests in South America (e.g., Cadena et al., 2016; Sobral-Souza et al., 2015), including the central Andean

rainforests (also known as the Yungas) and the Atlantic Forest (e.g., Nores, 1992; Por, 1992; Silva, 1994), two of the World's most diverse ecosystems (Orme et al., 2005) (Fig. 1a). These two rainforest blocks are currently isolated by two domains that prevent dispersal of most rainforest-associated organisms: the Chaco and the Cerrado. The Chaco is covered by xerophytic vegetation (dry Chaco), as well as by grasslands, wetlands and gallery forests along its main rivers (humid Chaco). The Cerrado is mostly characterized by savannas, isolated patches of semideciduous forest and gallery forests along rivers.

Notwithstanding the isolation, there is evidence that central Andean and Atlantic forest biotas have been linked in the past. For instance, biotic exchange between these biomes is supported by disjunct distribution patterns of many species and lineages restricted to each biome

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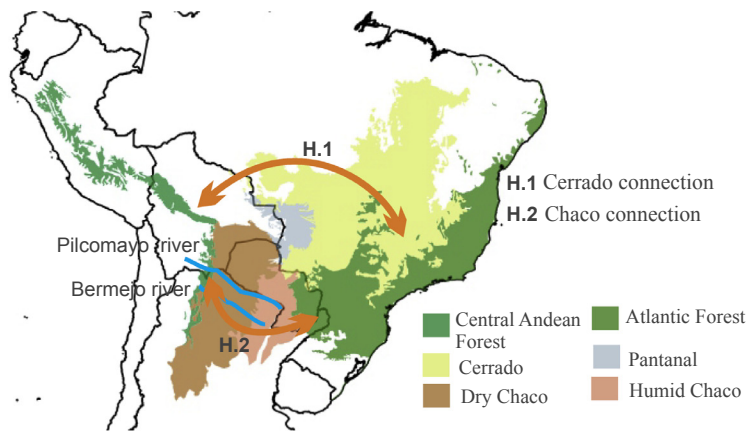
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a. Study region and working hypotheses



b. Geographic distribution and sampling localities of *T. melanops*

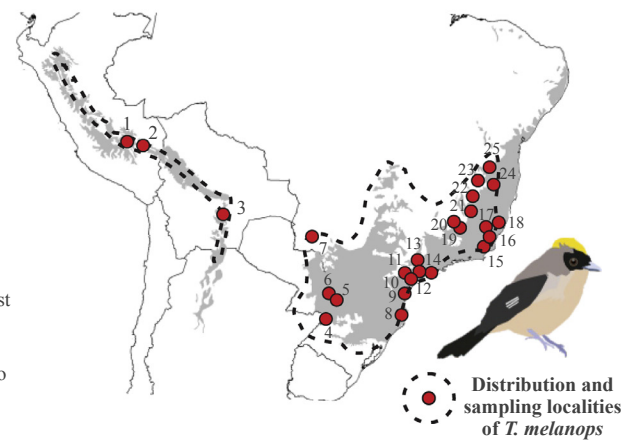


Fig. 1. Study region, working hypotheses and sampling localities for the phylogeographic study of *Trichothraupis melanops*. (a) Schematic representation of the working hypotheses of connection between the Andean and the Atlantic forest. H.1: Cerrado connection hypothesis (connection between the central or northern Andes with the central and/or southwestern Atlantic Forest), and H.2: Chaco connection hypothesis (connection between the southernmost Atlantic Forest with the central and/or the southern Andean tropical rainforests). (b) Geographic distribution of *T. melanops* and sampling localities for the phylogeographic study. See full identification of localities in Table A.1.

(e.g., the passerines *Phylloscartes ventralis* and *Poecilotriccus plumbeiceps*; Ridgely and Tudor, 2009), and by paleopalynological and paleoclimatic data suggesting that climatic variation through time has favored pulses of humid forest expansion through the Chaco and/or the Cerrado (e.g., Ledru, 1991; Oliveira-Filho and Ratter, 1995; Zurita et al., 2014). However, additional studies are needed to have a better comprehension of the timing, geographic positions and about during which climatic periods (glacial or interglacial period) occurred these connections, as well as about their effects on diversification of species (but see Trujillo-Arias et al., 2017).

The connection dynamics between these rainforests could have driven biotic diversification by promoting some processes such as: (a) vicariance of ancestral ranges due to the formation of climate-associated barriers (e.g., establishment of xerophytic forests); (b) peripatric diversification (*sensu* Coyne and Orr, 2004), with dispersal and colonization of regions with similar environmental conditions (which could drive divergence by drift), or with colonization of regions with different ecological conditions (novel habitats, which could trigger divergent selection and ecological diversification); and (c) secondary contacts, which could have triggered reinforcement and divergence. In addition, it is unknown whether there is a general pattern on the dispersal direction between regions (i.e., from the Andes to the Atlantic Forest or vice versa). Palynological and paleoclimatic studies (e.g., Cabanne et al., 2016; Oliveira-Filho and Ratter, 1995; Sobral-Souza et al., 2015) suggest that the Atlantic Forest and some of its organisms expanded their ranges into the Cerrado and towards the Andes during the Pleistocene, unlike the Andean organisms that seem to have more stable ranges (but see Colinvaux et al., 1996). Along the Andes, climatic changes might have had a smaller impact on rainforest ranges than in the lowland regions, because in the mountains rainforest blocks followed the altitudinal shifts of their climatic belts (Fjelds a et al., 2012). Therefore, because cycles of range expansions could have been broader and stronger in the Atlantic Forest, driving greater opportunities of dispersal from this biome, we hypothesize that most organisms that recently (i.e., during the Pleistocene) dispersed between regions show a shared pattern of dispersal from the Atlantic Forest to the Andes.

If the forest dynamics (cycles of connection and isolation) between the Andean and the Atlantic forests has been an important driver of speciation in the Neotropics, the species that are present in both biomes should show their major phylogeographic gap between regions, indistinctly if it occurred by vicariance or peripatry. Alternatively, if the

dynamics of connection resulted in high and constant rates of historical gene flow, enough to preclude divergence, the organisms shared by the two biomes should not exhibit population differentiation between regions. Besides, if differences in climatic conditions between these rainforests triggered diversification, the populations in both regions might show divergent climatic niches.

To date, two connection routes between the Andean and Atlantic forests have been proposed (Fig. 1a). One route could have occurred during glacial periods, through gallery forests in the Cerrado or through the southern edge of the Amazon forest, linking the Andes with the central Atlantic Forest (henceforth, Cerrado connection hypothesis). This hypothesis is based on Silva (1994, 1996), which document the occurrence of Atlantic Forest birds in the gallery forests of the Cerrado region. A second route could have occurred during interglacial periods and through gallery forests of the main rivers of the Chaco region (i.e., Bermejo and Pilcomayo Rivers) (Nores, 1992; Por, 1992), linking the southernmost Atlantic Forest with the southern Andean tropical rainforest (henceforth, Chaco connection hypothesis). It is worth noting that Nores (1992) and Silva (1994) considered these hypotheses as mutually exclusive. Evidence supporting each of the mentioned connections is mixed; for instance, some studies support the presence of humid forest in the Cerrado in the past (e.g. Cabanne et al., 2016; Ledru, 1993, 1991; Oliveira-Filho and Ratter, 1995; Peres et al., 2015), whereas the evidence for a Chaco connection is scarce, with a few studies supporting expansions of humid forests through this biome (Turchetto-Zolet et al., 2016; Zurita et al., 2014). Recently, Trujillo-Arias et al. (2017) performed a paleodistribution and phylogeographic study of a forest bird (*Arremon flavirostris*, Emberizidae) and proposed that the connection between these biomes was double, through a major corridor across the Cerrado during glacial periods, and through a minor corridor across the Chaco, during interglacial periods. However, because of the small number of taxa studied, additional research is needed to understand the history of connection between these biomes.

Here, we studied the Black-goggled Tanager (*Trichothraupis melanops*, Thraupidae) to evaluate hypotheses of connection between the Andean and the Atlantic Forest. *T. melanops* is monotypic and a good model to evaluate these hypotheses because it inhabits the understory of humid and montane forests, from the east slope of the central Andes (elevation range from 500 m to 2400 m) and from the Atlantic Forest (elevation range until 1550 m). It is also a good model because it is found in some of the gallery forests and forest patches of the wet Chaco,

and of the Cerrado, that could have been directly involved in the corridors that linked these rainforests (Ridgely and Tudor, 2009) (Fig. 1b).

The main objective of this study is to address whether the Andean and the Atlantic forests act as a refugia system, as well as to evaluate biogeographic hypotheses of diversification and connection between them. To achieve our goal we performed phylogeographic analyses and tested demographic scenarios by using Approximate Bayesian Computation (ABC, Beaumont et al., 2002). We also used Ecological Niche Model approaches to evaluate range shifts through time and possible climatic niche divergence between the populations of *T. melanops*. The specific questions were: (1) Is the major phylogeographic gap of *T. melanops* located between the Andean and the Atlantic forests?, (2) Are the *T. melanops*' populations product of vicariance, or is any of them product of peripatry?, (3) Are the climatic niches of the Andes and Atlantic Forest populations significantly different?, and (4) What do phylogenetic analyses and paleodistribution models indicate about the connection route between these rainforests?.

2. Material and methods

2.1. Taxon sampling and DNA amplification

We sampled 57 individuals of *T. melanops* from 25 localities distributed across the central Andean forests (localities: 1–3), the Atlantic Forest (localities: 4–6, 8–25) and from one locality in the southern Cerrado (locality: 7) (Fig. 1b). Based on Burns and Racicot (2009), we sampled one individual of *Coryphospingus cucullatus*, two of *Eucometis penicillata* and one *Lanio versicolor* to be included as outgroups. See Table A.1 for full information about the samples.

Genomic DNA from muscle and blood tissue samples was purified following a glass fiber-based extraction protocol (Ivanova et al., 2006). We studied Cytochrome *b* (Cyt *b*, 930 bp), as well as intron 9 of the Z-linked Low Density Lipoprotein Receptor gene (VLDL9R, 448 bp), and intron 11 of Glyceraldehyde-3-Phosphate Dehydrogenase (G3PDH, 330 bp). See primers and PCR conditions in Supplementary Material I. Sequences obtained in this study are deposited in GenBank under accession numbers MH748283–MH748448. Nuclear sequences with heterozygous indels were analyzed to resolve haplotypes in Codon Code Aligner 3.7 (Codon Code Corp., Dedham, MA). Also, we resolved the gametic phase of nuclear markers without indels by using PHASE (Stephens and Donnelly, 2003), as implemented in DnaSP (Librado and Rozas, 2009). We used the PHI test (Bruen et al., 2006), as implemented in SplitsTree4 (Huson and Bryant, 2006), to test for genetic recombination ($P > 0.15$ in all cases).

2.2. Phylogenetic analyses

We performed Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic analyses to study intraspecific relationships. We analyzed a concatenated data-set of Cyt *b*, VLDL9R and G3PDH (total alignment of 1708 bp). We analyzed heterozygous sites in nuclear markers as polymorphic characters. We used MrModelTest (Nylander, 2004) and the Akaike information criterion to select nucleotide substitution models (for Cyt *b*, model GTR + I; for VLDL9R, HKY and for G3PDH, GTR + I). The BI analyses were performed in MRBAYES 3.1 (Ronquist and Huelsenbeck, 2003), with two parallel runs using one 'cold' and three 'hot' chains, for a total of 20 million generations and sampling every 100 generations. Alignments were partitioned by DNA marker, analyzed under the best fit model for each partition, and allowing different rates across regions. We used a 25% burn-in and a 50% majority rule consensus tree was calculated to obtain the posterior probabilities for each node. We used GARLI 2.0 (Zwickl, 2006) to perform ML phylogenetic analyses, with a maximum search of 20 million generations. The best fit model of molecular evolution was considered for each marker, and nodal support was evaluated with non-parametric bootstrap (500 replicates). Resulting topologies were

visualized in FigTree 1.4 (available from <http://tree.bio.ed.ac.uk/software/figtree>). We also used Network 4.5.1.0 (Bandelt et al., 1999) to obtain median-joining haplotype networks.

2.3. Genetic structure, summary statistics, divergence time and gene flow

In order to evaluate whether the used sequences were representative of the genetic constitution of *T. melanops*, we estimated $P = [(k - 1)/(k + 1)]$, which represents the probability that a sample of size *k* and the whole population share the most recent common ancestor (Hein et al., 2005). Hence, *P* can be interpreted as the probability of the sample being representative of the genetic diversity of the population.

We employed BAPS 6.0 (Corander et al., 2008) to estimate the most likely number of genetically differentiated populations. For each marker we surveyed the probability of different number of genetic clusters ($K = 1$ to $K = 5$) under the model of "spatial clustering of individuals". In addition, to assess the population genetic structure and to estimate pairwise Φ_{ST} values for each marker we performed an Analysis of Molecular Variance (AMOVA). Also, we calculated nucleotide (π) and haplotype (*h*) diversities, the number of polymorphic sites (*s*), and performed neutrality tests (Tajima's *D* and Fu's *F_s*) in ARLEQUIN 3.5 (Excoffier and Lischer, 2010).

We estimated divergence time, effective size and gene flow between regions in Ima2 (Hey and Nielsen, 2004; Nielsen and Wakeley, 2001), by using all the genetic markers. General conditions of the analysis were: -b2000000 -d1000 -m3 -q45 -t4, with no less than 20000000 generations per run. We assumed a generation time of one year (Cabanne et al., 2008) and converted coalescent times to years according to the user's manual. The substitution rates used were: 1.05×10^{-2} substitutions/site/Million years (s/s/My) for Cyt *b* (Weir and Schluter, 2008), and 1.35×10^{-3} s/s/My and 1.62×10^{-3} s/s/My for G3PDH and VLDL9R, respectively (Ellegren, 2007). See the output file of Ima2 in Supplementary Material II.

2.4. Testing diversification mode and connection route by using ABC

We used an Approximate Bayesian Computation (ABC) approach (Beaumont et al., 2002) to explore the fit of the observed genetic data to two sets of hypothetical population models: (a) diversification mode models, and (b) models of connection route between the Andean-Atlantic forests. For these analyses, we evaluated simple models because in this kind of procedure complex scenarios must be avoided, since they could get a higher posterior probability without being plausible (Nielsen and Beaumont, 2009; Csilléry et al., 2010). Also, we performed inferences based on rejected scenarios rather than on accepted models.

First, in the diversification mode test (models: 1–4, Fig. 2a) we evaluated if the divergence between regions occurred by vicariance or by peripatry (dispersal and vicariance, *sensu* Coyne and Orr, 2004). For this analysis, the demographic units (populations) were defined as the Andean (localities 1–3) and Atlantic/Cerrado populations (localities 4–25), and the models evaluated were: (1) a single population; (2) a vicariance event in which the ancestral population was divided into two populations (Andean and Atlantic); (3) a peripatric event, with an initial dispersal from the Atlantic Forest to the Andes (in this model the Andean is the peripheral population, which experienced population growth); and (4) a peripatric event, with an initial dispersal from Andes to the Atlantic Forest (in this model the Atlantic is the peripheral population, which experienced population growth). We used 18 summary statistics to evaluate the fit of the observed data; we estimated for each marker (Cyt *b* and VLDL9R) *h*, π , *s* and *D* of each population (Table 1), and Φ_{ST} between the Andes and the Atlantic populations (See Results).

Second, to evaluate the connection route we designed four models (models: 5–8, Fig. 2b). We grouped sampling localities by geographic

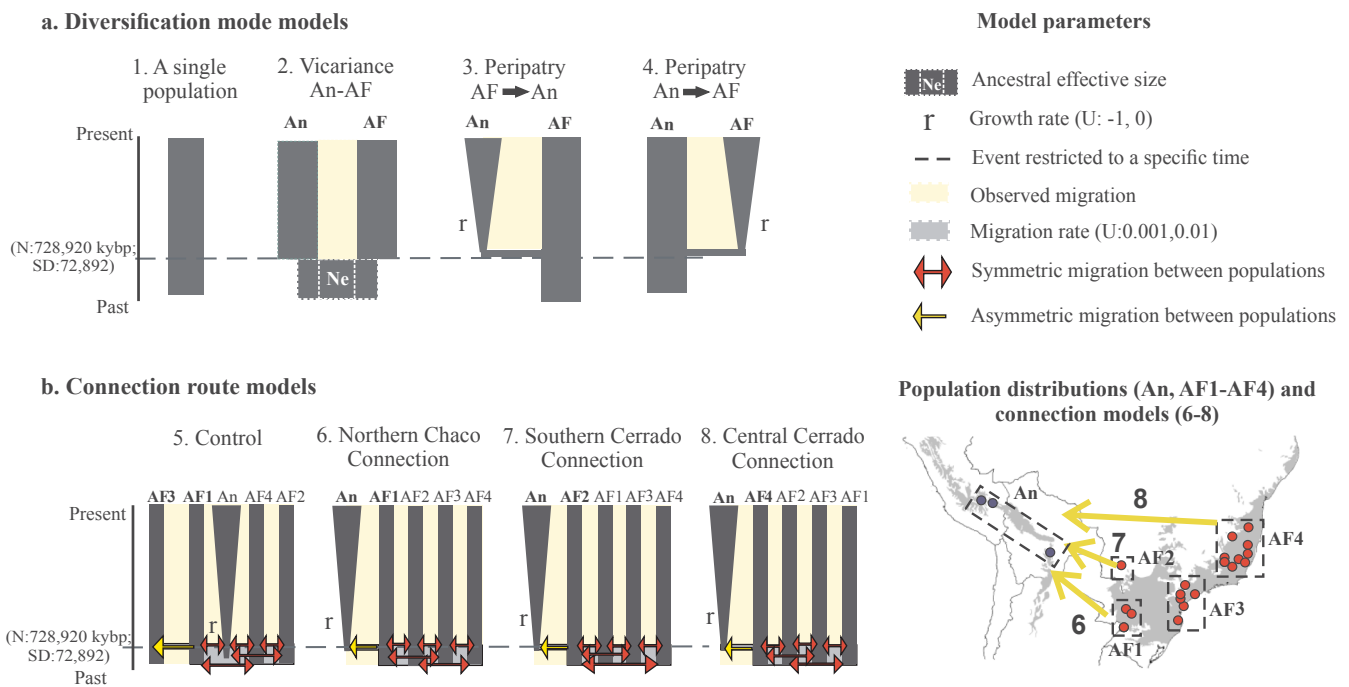


Fig. 2. Demographic models of *Trichothraupis melanops* tested by Approximate Bayesian Computation analyses. (a) Diversification mode models (1–4), and (b) connection route models (5–8) between the Andean and Atlantic forests. The time of demographic events (in thousands of years before present, kybp) was modeled as a normal (N) distribution with a specific standard deviation (SD), while migration rates were modeled as a uniform (U) distribution. Population acronyms correspond to: An, Andes; AF, Atlantic Forest; AF1, southern Atlantic forest; AF2, gallery forests at southern Cerrado; AF3, southeastern Atlantic Forest; AF4, central Atlantic Forest. See input files of the coalescent simulations in Supplementary material I.

proximity into five populations: Andean localities 1–3 (An), southern Atlantic Forest localities 4–6 (AF1), gallery forests at southern Cerrado locality 7 (AF2), southeastern Atlantic Forest localities 8–14 (AF3) and central Atlantic Forest localities 15–25 (AF4). Grouping by geographic proximity was the simplest way to evaluate an island model and to explore what samples from the Atlantic region contacted the Andean region. The models evaluated were: (5) a control model, for which we expected a non-significant fit of the observed data; (6) An-AF1 contact, which represents a connection between the Andes and the southernmost Atlantic Forest through northern Chaco; (7) An-AF2 contact, which represents a connection through the gallery forests of the southern Cerrado; and (8) An-AF4 contact, which represents a connection between the Andes and the central Atlantic Forest through the central Cerrado or southern edge of the Amazonia. We used 16 summary statistics to evaluate the fit of the observed data; we estimated for

each marker (Cyt *b* and VLDL9R) of each population, and Φ_{ST} between An and either AF1, AF2, and AF4. See values of summary statistics employed in this analysis in Table A.2.

For coalescent simulations we only modeled the most variable markers (Cyt *b* and VLDL9R). For each model and marker we performed 100000 coalescent simulations by using BayeSSC (Anderson et al., 2005). For the diversification mode models the current migrations and the effective size (N_e) of each population and marker were estimated in IMA2, and then used as input for the simulations. For the connection route models, the N_e of each population in the Atlantic region (AF1-AF4) was assumed to be 1/4 of the entire Atlantic population's N_e estimated in IMA2. See input files of BayeSSC in Supplementary Material I. Finally, to select the model with best fit to the observed data within each test (i.e., among diversification mode models and among connection route models) we implemented both the *mnlogistic* and *neuraltnet*

Table 1

Summary statistics of *Trichothraupis melanops* populations. Sample size in number of sequences (n), nucleotide (π) and haplotype (h) diversity, polymorphic sites (s), Tajima's D, Fu's Fs, population effective size in number of individuals (N_e) and confidence intervals (HPD95) are presented. Statistically significant values of D ($P < 0.05^*$) and Fs ($P < 0.02^{**}$) are in bold.

Metric	Populations and markers					
	Andean			Atlantic		
	Cyt <i>b</i>	VLDL9R	G3PDH	Cyt <i>b</i>	VLDL9R	G3PDH
n	6	11	12	44	74	88
π	0.004	0.002	0.015	0.002	0.003	0.014
h	0.933	0.781	0.927	0.768	0.741	0.844
s	9	3	9	20	14	21
D	0.085	0.101	-0.221	-2.134*	-1.644*	-1.866*
Fs	-0.746	-1.929**	-1.815	-15.19**	-7.02**	-12.35**
N_e	877341 (HPD95: 475522–1563324)			3030968 (HPD95: 1996416–4795794)		

algorithms (Beaumont, 2010) in the abc package for R (Csilléry et al., 2012), using three tolerance rate values (0.001, 0.0005, and 0.0003). We only used *mnlogistic* for final model selection because it was the only algorithm that provided stable results across tolerance rates. For model comparisons we evaluated Bayes Factors (posterior probability ratios) according to Kass and Raftery (1995).

2.5. Ecological niche models and niche comparison between regions

To study the Andean-Atlantic forests connection we modeled distribution maps of *T. melanops* using MAXENT (Phillips et al., 2006). We selected MAXENT because it performs well relative to other modeling approaches (Elith et al., 2006). We compiled occurrence records from our own field work, from Centro de Coleções Taxonômicas (Universidade Federal de Minas Gerais, Brazil), Museo Argentino de Ciencias Naturales (Buenos Aires), and from the Global Biodiversity Information Facility (GBIF; www.gbif.org). To reduce spatial autocorrelation, we randomly removed occurrence records that were less than 5 km apart from each other, which resulted in a final data-set of 112 presence records. See MAXENT input file in [Supplementary Material I](#).

For MAXENT analyses we used the 19 climate variables available at WordClim 1.4 (Hijmans et al., 2005) with a resolution of 2.5 arc min. For the final analysis, we selected five bioclimatic variables (BIO 1, BIO 4, BIO 7, BIO 13 and BIO 17) by first rejecting highly correlated variables (Peterson et al., 2011), and then by selecting relevant variables by a rationale of permutation importance > 5%. The general conditions for analyses were: random test points = 25; replicates = 10; replicate type: subsample; maximum iterations = 5000. We validated models by evaluating AUC values (Pearson et al., 2007). Then, we projected the niche model to three past periods: (1) Mid-Holocene (6000 years before present –ybp–), with two climate models: CCSM4 (Community Climate System Model) and MIROC (MIROC-ESM, <http://www.worldclim.org/paleo-climate>); (2) Last Glacial Maximum (LGM, 21000 ybp), past climate models: CCSM3 (Community Climate System Model, <http://www.cesm.ucar.edu>) and MIROC (Model of Interdisciplinary Research on Climate, available at: <http://www.ccsr.utokyo.ac.jp/kyosei/hasumi/MIROC/tech-repo.pdf>); and (3) Last Interglacial (LIG, 120000 ybp; (Otto-Bliesner et al., 2006). We obtained binary maps by using a threshold of equal training sensitivity and specificity.

We assessed niche divergence between the Andean and the Atlantic populations by using the PCA-environmental ordination test (henceforth, PCA-env) (Broennimann et al., 2012) and the Background test (Warren et al., 2008). These tests measure climatic niche overlap (Schoener's D; Schoener, 1970) and evaluate its significance by comparing the observed value (Dobs) to a null distribution of D (Dnull) obtained by permutation. The metric D varies from 0 (complete dissimilarity) to 1 (fully overlapping niches). Niche divergence is supported when Dobs < Dnull (Callen and Miller, 2015). For these analyses the occurrence records were separated by regions (Andes and Atlantic regions) and eight environmental variables were selected (BIO 1, BIO 3, BIO 4, BIO 5, BIO 6, BIO 12, BIO 16 and BIO 17) based on their contribution in the rationale permutation importance (> 5%) estimated from the ecological niche modeling performed for each population. For these tests, environmental variables were used at a 30 s resolution and different convex polygons were assessed as background areas: (1) background 1: polygon with zero buffer area (i.e., a polygon that circumscribed the occurrences of each population), (2) background 2: polygon with 20 km of buffer area, and (3) background 3: polygon with 50 km of buffer area. The number of background random points was equivalent to the sample size available in each region, and the values of

Dnull were obtained from 100 pseudoreplicates. The PCA-env test was performed with the package ecospat for R and following the methodology proposed by Broennimann et al (2012), and the Background test was performed in the ENMTools 1.3 (Nakazato et al., 2010) following the method described in Warren et al. (2008). Because results of both tests were similar across backgrounds, we only report results with background 3.

3. Results

3.1. Intraspecific phylogeny and population genetic analyses

The BI and ML analyses recovered *T. melanops* as monophyletic. Also, these analyses indicated that the Andean samples formed a well-supported monophyletic clade, characterized by presenting many apomorphies (apomorphic population, *sensu* Frey, 1993), while samples from the Atlantic Forest and gallery forests in the Cerrado were plesiomorphic (*sensu* Frey, 1993) (Fig. 3a). The phylogenetic tree was in full agreement with the Cyt *b* network (Fig. S1), where the ancestral haplotype was related the Atlantic region (Atlantic forest plus gallery forests in Cerrado) and the Andean haplotypes grouped in a cluster separated by at least seven substitutions from the closest Atlantic sequence. The networks of the nuclear markers did not show any genetic structure between regions (Fig. S1).

Before performing population genetic analyses, we evaluated P and validated that the genetic sample employed in this study was suitable to estimate population and genealogical parameters of *T. melanops*. Regarding the full sample, P for Cyt *b* was 0.96, for VLDL9R was 0.97 and for G3PDH was 0.98. For the Andean sample, P for Cyt *b* was 0.72, for VLDL9R was 0.83 and for G3PDH was 0.84; and for the Atlantic sample, P for Cyt *b* was 0.9, for VLDL9R 0.97 and for G3PDH was 0.98.

BAPS analyses with Cyt *b* and G3PDH identified two genetic clusters, while the analysis with VLDL9R revealed a single cluster (Fig. 3b). Only the results with Cyt *b* presented geographic coherence, with one cluster associated to the Andean samples and the other associated to the Atlantic region (samples from the Atlantic Forest and gallery forests in the Cerrado). Genetic differentiation between the Andean and the Atlantic region was corroborated by the AMOVA, which indicated that the highest proportion of the total genetic variation of Cyt *b* was allocated between these regions (Cyt *b* Φ_{ST} = 0.822, $p < 0.01$), whereas the other markers presented a lower proportion of variation between regions (VLDL9R Φ_{ST} = 0.181, $p < 0.01$ and G3PDH Φ_{ST} = 0.180, $p < 0.01$).

Summary statistics (Table 1) indicated that the Andean and the Atlantic populations of *T. melanops* presented similar levels of genetic diversity in the nuclear markers. In contrast, the Andean population has slightly higher genetic diversity of Cyt *b* than the Atlantic population. For the Atlantic population, the neutrality tests (Tajima's D and Fu's Fs) indicated a demographic expansion. For the Andean population, Tajima's Ds were not significant, while Fu's Fs were negative but not significant for all markers, indicating demographic stability or a shallow demographic expansion. Finally, the estimate of N_e of the Atlantic population was three orders of magnitude greater than the Andean N_e .

The estimated divergence time suggests a Mid-Pleistocene split between the Andean and Atlantic populations (0.728 Million of years -My-, HPD95 = 0.378–1.610 My), and gene flow estimates indicated that both populations are isolated (migration from Andes to Atlantic Forest = 0.17 individuals per generation (ind/gen), HPDH95 = 0–0.6 ind/gen; and migration from Atlantic Forest to the Andes = 0.02 ind/gen, HPD95 = 0–3.9 ind/gen).

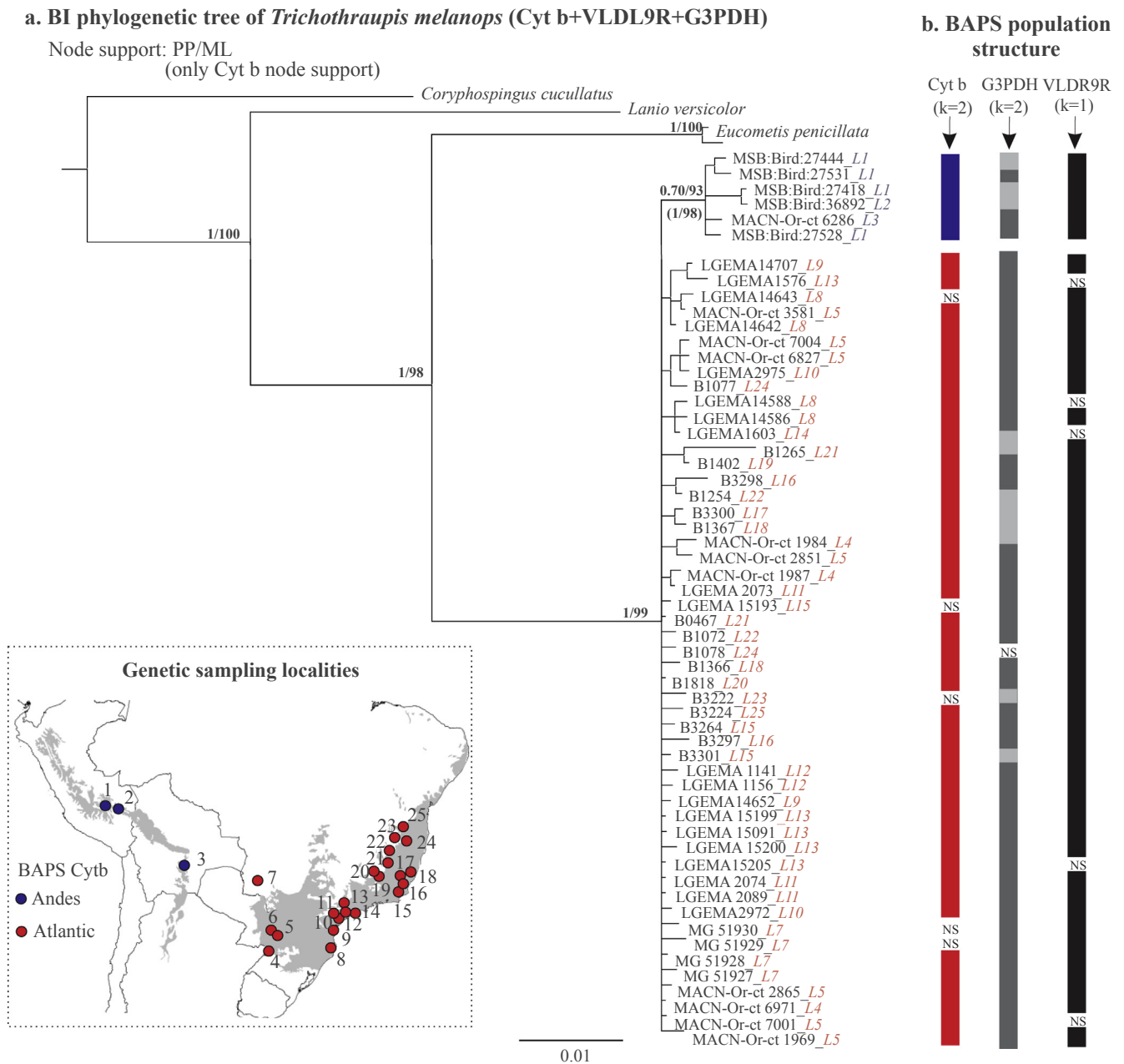


Fig. 3. Intraspecific phylogeny and BAPS population structure in *Trichothraupis melanops*. (a) Bayesian Inference (BI) phylogenetic tree obtained with concatenated sequences of Cyt b, VLDR9R, and G3PDH (total 1708 bp). Node supports are indicated as posterior probability (PP) and maximum likelihood bootstrap score (ML, 500 replicates); supports between parentheses correspond to those obtained with Cyt b sequences. Sample labels represent tissue number and sampling locality (L) number as is shown in the map (see also Fig. 1b). See Table A.1 for further details on samples and localities. (b) Bayesian analysis of population genetic structure (BAPS) based on the Cyt b, VLDR9R, and G3PDH sequences. Colors indicate different genetic clusters, and samples without data for the BAPS analyses are denoted with NS.

3.2. Test of demographic models by ABC

First, the test of diversification mode (models 1–4, Fig. 2a) selected as the best fit across tolerance rates a model with two populations, with peripatric diversification and with dispersal from the Atlantic Forest to the Andes during the Mid-Pleistocene (Table 2a, model 3). Second, the test of the connection route between these rainforests (models 5–8, Fig. 2b) selected as best fit across tolerance rates the connection model through the northern Chaco (Table 2b, model 6). The connection model through southern Cerrado was also plausible when less stringent tolerance rates were tested (model 7, BF = 1.68 and 2.58). The test

presented substantial evidence against the Control model (Table 2b, model 5, BF > 50) and against the central Cerrado connection model (Table 2b, Model 8, BF > 6). Thus, these results are most compatible with a corridor that linked the Andes and the southern Atlantic Forest through the Cerrado-Chaco transition.

3.3. Paleodistribution and niche divergence between the Andean and the Atlantic populations

The model of distribution of *T. melanops* performed better than a random model (AUC = 0.87, Fig. 4a). The distribution for the Mid-

Table 2

Selection of demographic scenarios of *Trichothraupis melanops* by using Approximate Bayesian Computation. (a) Diversification mode models (1–4). (b) Connection route models (5–8) between the Andean (An) and the Atlantic forests (AF). See details of the models and populations in Fig. 2. P, posterior probability obtained in abc for R based on the algorithm *mnlogistic*. T, tolerance rate, and BF, Bayes Factor. Best-fit models are in bold.

Scenarios	Model	P (T = 0.001)	BF	P (T = 0.0005)	BF	P (T = 0.0003)	BF
a. Diversification mode	1. Single population	0.0672	13.61	0.3804	1.56	0.0567	16.62
	2. Vicariance	0.0150	60.97	0.0256	23.18	0.0007	> 50
	3. Peripatry AF → An	0.9146	–	0.5935	–	0.9426	–
	4. Peripatry An → AF	0.0032	> 50	0.0005	> 50	0	> 50
b. Andes/Atlantic forest connection route	5. Control	< 0.0001	> 50	0	> 50	0	> 50
	6. Northern Chaco connection	0.5690	–	0.6618	–	1	–
	7. Southern Cerrado connection	0.3378	1.68	0.2560	2.58	0	> 50
	8. Central Cerrado connection	0.0932	6.10	0.0822	8.05	0	> 50

Holocene and LIG were relatively similar to the current distribution (Fig. 4b and d). The model for the LGM indicated expansion of suitable habitats for the species at the southern Cerrado and northern Chaco, which suggested a connection between the Andean and the Atlantic forests through these regions and period. A connection through central Cerrado was also plausible during the LGM, but seemed to be less likely because a path of habitat suitability was not continuous between regions.

The niche divergence analyses (PCA-env and the Background tests, Fig. 5 and Fig. S2, respectively) supported niche conservatism between the Andean and Atlantic populations of the focal species. Both tests indicated low climatic niche overlap between the Andean and Atlantic populations, with Dobs equal to 0.14 (PCA-env test) and 0.45

(Background test). In the comparisons from the Atlantic Forest to the Andes, both tests resulted in marginally significant Dobs values, supporting niche conservation (Dobs > Dnull values, Fig. 5 and Fig. S2). In the comparison Andes to the Atlantic Forest, results of both tests were not significant (Dobs = Dnull values, Fig. 5 and Fig. S2). Thus, these tests do not suggest a link between the observed genetic differences and climatic niche differences between regions.

4. Discussion

Our findings with *T. melanops* support a connection scenario between the central Andes and the southern Atlantic Forest via the Cerrado-Chaco transition, during glacial periods, as well as a peripatric

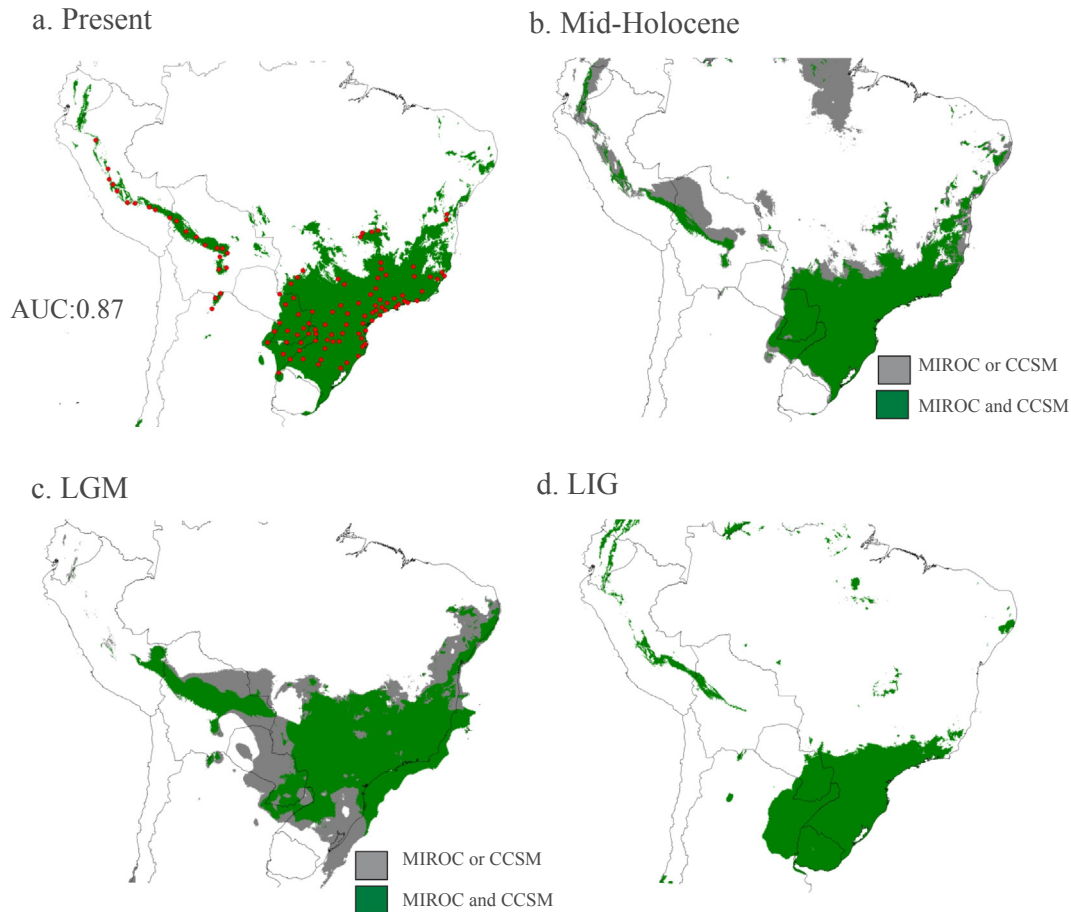


Fig. 4. Models of geographic distribution of *Trichothraupis melanops* obtained in MAXENT. (a) Present; (b) Mid-Holocene (6000 ybp); (c) Last Glacial Maximum (LGM, 21000 ybp); and (d) Last Inter-Glacial period (LIG, 130000 ybp). Species presence records are indicated in red. In (b) and (c), areas of overlap between climate models (MIROC and CCSM) are in green, while suitable areas determined by a single climate model are in gray (MIROC or CCSM).

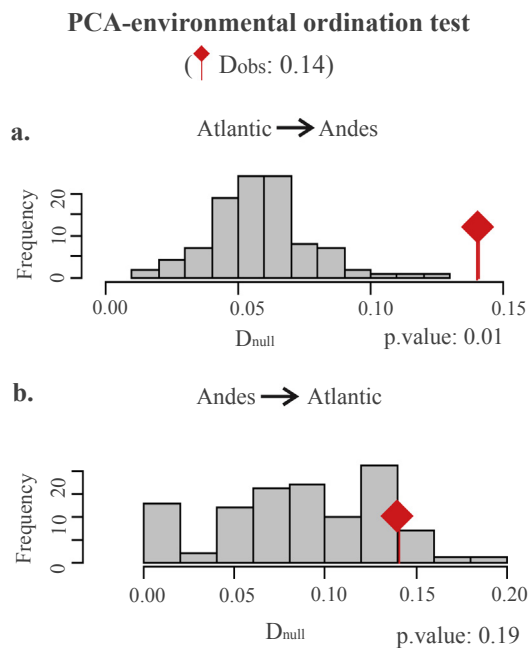


Fig. 5. Niche divergence test between the Andean and Atlantic populations of *Trichothraupis melanops* performed by the PCA-environmental ordination method. (a) Comparison of niche similarity from the Atlantic Forest to the Andes. (b) Comparison of niche similarity from the Andes to the Atlantic Forest.

scenario of divergence with an initial dispersal from the Atlantic Forest to the Andean forest. Also, our findings support that these two biomes have acted as a refugia system promoting divergence.

4.1. Biogeographical connection between the Andean and Atlantic forests

Our results supported an Andean-Atlantic forest connection during the glacial periods and through the transition region between the Cerrado and Chaco. Our results also suggest that the connection involved populations from the southern Atlantic Forest and the central Andes. First, the ABC analysis (Fig. 2b) indicated that the connection through the northern Chaco (model 6) was the best-fit model, followed by the connection through the southern Cerrado (model 7; Table 2b). These results supported a scenario of contact between these rainforests through the Cerrado-Chaco transition, linking the Andes with the southern Atlantic Forest, and not through the central Cerrado or the southern edge of Amazonia (model 8, Table 2b). Second, a connection through the Cerrado-Chaco transition is supported by the paleodistribution models, which suggested that at maxima of glacial periods the southern Cerrado and northern Chaco presented suitable habitats for this species (Fig. 4c), in full agreement with our ABC results.

A forest corridor through the transition between the Cerrado and Chaco has been also suggested by other studies. For instance, Batalha-Filho et al. (2013) assessed biogeographical connections between the Amazonia and the Atlantic Forest based on phylogenetic and distributional data of avifauna, and suggest that one of the connection routes between these rainforests could have occurred through the southern Cerrado and the transition area towards the Chaco. Also, Ledo and Colli (2017) supported this connection route as the most important for biotic exchange

between the Amazon and the Atlantic rainforests, suggesting that it may have been established many times in the past. However, it is worth noting that even though Ledo and Colli (2017) stated to have described a route between the Amazonia and the Atlantic Forest, because they equate high Andean locations with lowland Amazonian locations, their result actually described a link between Andean montane forests and the Atlantic Forest, rather than a link between the Amazon and Atlantic forests. Therefore, based on our results and by previous studies, a forest corridor through the Cerrado-Chaco transition would have been important for most forest taxa, but perhaps this corridor could have been more important for mid-elevation and mountain taxa (from the Andes and or from the Atlantic Forest) than for lowland taxa (e.g., Amazon taxa).

The consilience of our results with the few other studies available for this biogeographic system is remarkable, but it should be noted that there is also evidence for other routes and climatic periods of connection, suggesting that the isolation-connection dynamics between the Andean and Atlantic forests were complex and that the single contact route postulated by Nores (1992) and by Silva (1994) is not likely. For instance, some taxa such as the passerine *Arremon flavirostris* supported a double connection between these biomes, through the Cerrado and the Chaco in alternating climatic periods (Trujillo-Arias et al., 2017), whereas others, such as the tree *Eugenia uniflora* (Myrtaceae) (Turchetto-Zolet et al., 2016) and *T. melanops* supported a single connection route, through the Chaco in different climatic periods and through the Cerrado-Chaco transition during glacial maxima, respectively. Thus, even though the number of taxa studied is low, the evidence obtained so far suggests that the connections between these rainforests occurred through different regions and under different climatic conditions, likely involving in each connection event a different set of species with subtly different ecological requirements and life histories.

4.2. Peripatric divergence of the Andean population

The ABC result indicated that the Andean population of *T. melanops* could have been originated by a dispersal and colonization event, from the Atlantic Forest and during the Mid-Pleistocene (model 3, Table 2a). A peripatric diversification model is also consistent with: (1) the placement of the Andean population in the overall phylogeny, characterized by a high number of automorphies (Fig. 3a) and supporting a peripheral position (Frey, 1993); (2) a higher effective size in the Atlantic population than in the Andean population (Table 1), which supports for the former a core position and a higher probability to be source of migrants; and (3) more intense demographic changes in the Atlantic population than in the Andean population (Table 1), which would indicate stronger range fluctuations in the Atlantic realm that would in turn derive in higher opportunities of dispersal from this biome. In addition, the core position of the Atlantic population is also supported by the haplotype networks (Fig. S1).

Peripatric diversification in *T. melanops* may have been promoted by forest corridors formed during glacial periods and by posterior isolation produced by retraction of those corridors during interglacial periods (Fig. 4), which would have enabled colonization from the Atlantic region and posterior peripheral isolation in the Andes. A model of peripatric diversification driven by range shifts in response to Quaternary climate changes

could be the preponderant diversification mode between these biomes. The few phylogeographic studies available for this system seem to support this idea. For instance, Rocha et al., (2014) suggest that the divergence between the parrots *Amazona pretrei* (Atlantic Forest) and *A. tucumana* (Andean region) occurred during the Pleistocene, most likely after the establishment of a small population in the Southern Andean forest by dispersal of a few founders from the southern Atlantic Forest. Studies of other taxa, such as the opilion *Discocyrtus dilatatus* (Gonyleptidae), also suggested a peripheral isolation of the Andean individuals after dispersal from the southern Atlantic Forest populations (Vergara et al., 2017). In addition, asymmetric dispersal from the Atlantic range is in agreement with previous studies suggesting that, during several periods of the Pleistocene, the Atlantic Forest have expanded their distribution range into the Cerrado and towards the Andes, while the Andean forest might have been more stable during the Pleistocene (Cabanne et al., 2016; Ledo and Colli, 2017; Silva, 1996; Sobral-Souza et al., 2015).

Finally, the results of the ABC test regarding the diversification mode (Fig. 2a, Table 2a) taken together with the niche divergence analyses (Fig. 5), suggest that the intraspecific diversification of *T. melanops* occurred under climatic niche conservatism. This result suggests that factors other than those related to climatic niche divergence are playing an important role in the diversification of the species, in accordance with many other studies of species with allopatric populations (e.g., Boucher et al., 2016; Dowell and Hekkala, 2016; McCormack et al., 2009; Peterson, 1999). This pattern of divergence with niche conservatism could be explained because species tend to maintain their ancestral ecological niche, and therefore to fail to adapt to the new environmental conditions of the barrier, which becomes a key factor in starting isolating populations and creating new lineages (Wiens and Graham, 2005; Wiens, 2004). Thus, other processes may contribute to the divergence of allopatric lineages, for instance, genetic drift, sexual or social selection. Genetic drift could be the main factor driving diversification of *T. melanops* because of the peripheral position and small effective size of the Andean population (Table 1, Fig. 3 and Fig. S1), as well as because of the recurrent shifts in its distribution range (Fig. 4), which could favor a faster fixation of new genetic variants, and thus the differentiation of the Andean from the Atlantic population.

4.3. Andean and Atlantic forests as a refugia system

Our study supports that the Andean and the Atlantic forests act as a refugia system, and therefore that their current isolation and past geographic dynamics have been important for the evolution of rainforest-associated taxa. The phylogeographic gap within *T. melanops* located between the Andean and Atlantic forests (Fig. 3) suggests that the connection dynamics of these rainforests has played an important role on the species diversification. This phylogeographic gap has been also reported for other taxa restricted to both biomes, including birds (Lavinia, 2016; Ribas and Miyaki, 2007; Trujillo-Arias et al., 2017) and frogs (Faivovich et al., 2005, 2004; Pavan et al., 2014; Percequillo et al., 2011). Even, it has been also found a gap at phenotypic level across several bird taxa, which has led to describe sister lineages associated with each biome, either at subspecies (e.g., *Pipraeidea melanonota melanonota* and *P. m. venezuelensis*, Ridgely and Tudor, 2009) or at species level (e.g., *Amazona pretrei* and *A. tucumana*, Rocha et al., 2014; *Arremon flavirostris* and *A. flavirostris dorbignii*, Buainain et al., 2016; Trujillo-Arias et al., 2017).

Our results, taken together with the cyclical nature of glaciations, suggest that the connection between the Andean and the Atlantic

forests have been recurrent across the Pleistocene. First, the paleodistribution models of *T. melanops* (Fig. 4), and of other species shared between both biomes (Trujillo-Arias et al., 2017; Turchetto-Zolet et al., 2016), as well as of the biomes themselves (Ledo and Colli, 2017; Sobral-Souza et al., 2015), indicate that climatic fluctuations during different periods have enabled connections between these rainforests, and considering that at least the last five glacial cycles have been similar in time interval and intensity (Lisiecki and Raymo, 2005), these connections could have been recurrent during the Pleistocene. Secondly, the heterogeneity in the temporal divergence across taxa with populations restricted to each forest also supports multiple biogeographic contacts between these regions. For instance, the divergence between these rainforests in a sample of three birds varied from 1.3 My (*Amazona pretrei/A. tucumana*, Rocha et al., 2014) to about 0.72 My and 0.15 My (*T. melanops* and *Pipraeidea melanonota*, this study and Lavinia, 2016, respectively). However, it should be noted that although the recurrent connections between these biomes could have led to periodic re-establishment of gene flow between regions after the initial separation, the historical gene flow between these rainforests has not been high enough to preclude divergence.

Finally, a definitive analysis of the phenotypic variation of *T. melanops* is necessary to determine the taxonomic status of its populations. Our results indicate that the Andean and Atlantic populations of *T. melanops* are genetically isolated (i.e., migration between populations $M < 1$ individuals per generation), and therefore, its current classification as a monotypic species might not be adequate to reflect its evolutionary history.

5. Conclusions

Our results with *T. melanops*, taken together with other studies, indicated that the Pleistocene climatic cycles modified geographic ranges of forest organisms and that they have been an important factor to drive biological diversification between the Andean and Atlantic forests. Also, these studies suggest that the link between these biomes occurred mostly through the Cerrado and or the Cerrado-Chaco transition, and that a single connection route, as postulated previously, is not likely. Finally, our findings suggest that the connection dynamic between these biomes promoted peripatric differentiation in some forest taxa of the Andean and Atlantic forests.

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Appendix A

See Tables A.1 and A.2.

Table A.1

Samples used for the phylogenetic and phylogeographic analyses of *Trichothraupis 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 Emílio 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.

Species	Locality number	Locality	Sample size	Tissue ID	Sex
<i>Trichothraupis melanops</i>	1	San Pedro, Paucartambo, Cusco, Perú	4	MSB:Bird:27418	Male
<i>T. melanops</i>				MSB:Bird:27444	Male
<i>T. melanops</i>				MSB:Bird:27528	Female
<i>T. melanops</i>				MSB:Bird:27531	Male
<i>T. melanops</i>	2	Quespicanchis, Cusco, Perú	1	MSB:Bird:36892	Male
<i>T. melanops</i>	3	Pederal, Provincia Tomina, Chuquisaca, Bolivia	1	MACN-Or-ct 6286	Male
<i>T. melanops</i>	4	CIAR, Oberá, Misiones, Argentina	3	MACN-Or-ct 6971	Female
<i>T. melanops</i>				MACN-Or-ct 1984	Male
<i>T. melanops</i>				MACN-Or-ct 1987	Male
<i>T. melanops</i>	5	N.P. Iguazú, Iguazú, Misiones, Argentina	6	MACN-Or-ct 2851	Male
<i>T. melanops</i>				MACN-Or-ct 2865	Female
<i>T. melanops</i>				MACN-Or-ct 3581	Female
<i>T. melanops</i>				MACN-Or-ct 6827	Female
<i>T. melanops</i>				MACN-Or-ct 7001	Male
<i>T. melanops</i>				MACN-Or-ct 7004	Male
<i>T. melanops</i>	6	Paraje María Soledad, General Belgrano, Misiones, Argentina	1	MACN-Or-ct 1969	Female
<i>T. melanops</i>	7	Bonito, Mato Grosso do Sul, Brazil	4	MG51927	Female
<i>T. melanops</i>				MG51928	Female
<i>T. melanops</i>				MG51929	Male
<i>T. melanops</i>				MG51930	Male
<i>T. melanops</i>	8	Nova Trento, Santa Catarina, Brazil	4	LGEMA 14586	Female
<i>T. melanops</i>				LGEMA 14588	Female
<i>T. melanops</i>				LGEMA 14642	Male
<i>T. melanops</i>				LGEMA 14643	Female
<i>T. melanops</i>	9	Guaraqueçaba, Utinga, Paraná, Brazil	2	LGEMA 14652	Male
<i>T. melanops</i>				LGEMA 14707	Male
<i>T. melanops</i>	10	Buri, São Paulo, Brazil	2	LGEMA 2972	Male
<i>T. melanops</i>				LGEMA 2975	Female
<i>T. melanops</i>	11	Fazenda Barreiro Rico, Anhembi, São Paulo, Brazil	3	LGEMA 2073	Male
<i>T. melanops</i>				LGEMA 2074	Male
<i>T. melanops</i>				LGEMA 2089	Female
<i>T. melanops</i>	12	Juquitiba, São Paulo, Brazil	2	LGEMA 1141	Male
<i>T. melanops</i>				LGEMA 1156	Female
<i>T. melanops</i>	13	P. E. Serra do Mar, Núcleo Curucutú, São Paulo, Brazil	6	LGEMA 15091	Male
<i>T. melanops</i>				LGEMA 15199	Female
<i>T. melanops</i>				LGEMA 15200	Male
<i>T. melanops</i>				LGEMA 1576	Female
<i>T. melanops</i>				LGEMA 15193	Male
<i>T. melanops</i>				LGEMA 15205	Male
<i>T. melanops</i>	14	PETAR, Núcleo Caboclos, São Paulo, Brazil	1	LGEMA 1603	Male
<i>T. melanops</i>	15	Alfredo Chaves, Espírito Santo, Brazil	2	B3301	Male
<i>T. melanops</i>				B3264	Male
<i>T. melanops</i>	16	Conceição do Castelo, Espírito Santo, Brazil	2	B3297	Male
<i>T. melanops</i>				B3298	Female
<i>T. melanops</i>	17	Domingos Martins, Espírito Santo, Brazil	1	B3300	Female
<i>T. melanops</i>	18	Santa Teresa, Espírito Santo, Brazil	2	B1366	Female
<i>T. melanops</i>				B1367	Female
<i>T. melanops</i>	19	Santa Bárbara, Minas Gerais, Brazil	1	B1402	Male
<i>T. melanops</i>	20	Viçosa, Minas Gerais, Brazil	1	B1818	Male
<i>T. melanops</i>	21	Simonésia, Minas Gerais, Brazil	2	B047	Male
<i>T. melanops</i>				B1265	Male
<i>T. melanops</i>	22	Caratinga, Minas Gerais, Brazil	2	B1072	Male
<i>T. melanops</i>				B1254	Female
<i>T. melanops</i>	23	Turmalina, Minas Gerais, Brazil	1	B3222	Female
<i>T. melanops</i>	24	Jequitinhonha, Minas Gerais, Brazil	2	B1077	Female
<i>T. melanops</i>				B1078	?
<i>T. melanops</i>	25	Leme do Prado, Minas Gerais, Brazil	1	B3224	Male
<i>Coryphospingus cucullatus</i>				MACN-Or-ct 5022	
<i>Eucometis penicillata</i>				MACN-Or-ct 5950	
<i>Eucometis penicillata</i>				MACN-Or-ct 6032	
<i>Lanio versicolor</i>				MACN-Or-ct 6048	

Table A.2

Summary statistics of *Trichothraupis melanops* employed in the connection route analysis using ABC (models 5–8). The acronyms correspond to: s, polymorphic sites; Φ_{ST} , pairwise genetic divergence between populations; An, Andes; AF1, southern Atlantic Forest; AF2, gallery forests at southern Cerrado; AF3, Southeastern Atlantic Forest; AF4, Central Atlantic forest. See details of populations in Fig. 2b.

Markers	Summary statistics							
	s (An)	s (AF1)	s (AF2)	s (AF3)	s (AF4)	Φ_{ST} An- AF4	Φ_{ST} An- AF1	Φ_{ST} An- AF2
Cyt b	9	3	1	9	10	0.809	0.803	0.6938
VLDL9R	3	3	2	9	6	0.2074	0.289	0.176

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ympev.2018.08.005>.

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