The genetic effects of Late Quaternary climatic changes over a tropical latitudinal gradient: diversification of an Atlantic Forest passerine

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Abstract

The increase in biodiversity from high to low latitudes is a widely recognized biogeographical pattern. According to the latitudinal gradient hypothesis (LGH), this pattern was shaped by differential effects of Late Quaternary climatic changes across a latitudinal gradient. Here, we evaluate the effects of climatic changes across a tropical latitudinal gradient and its implications to diversification of an Atlantic Forest (AF) endemic passerine. We studied the intraspecific diversification and historical demography of *Sclerurus scansor*, based on mitochondrial (ND2, ND3 and cyt*b*) and nuclear (FIB7) gene sequences. Phylogenetic analyses recovered three well-supported clades associated with distinct latitudinal zones. Coalescent-based methods were applied to estimate divergence times and changes in effective population sizes. Estimates of divergence times indicate that intraspecific diversification took place during Middle–Late Pleistocene. Distinct demographic scenarios were identified, with the southern lineage exhibiting a clear signature of demographic expansion, while the central one remained more stable. The northern lineage, contrasting with LGH predictions, exhibited a clear sign of a recent bottleneck. Our results suggest that different AF regions reacted distinctly, even in opposite ways, under the same climatic period, producing simultaneously favourable scenarios for isolation and contact among populations.

Keywords: biogeography, historical demograhy, Neotropical Region, phylogeography, *Sclerurus scansor*

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Introduction

The marked increase in biodiversity from regions of high to low latitudes is well documented across distinct taxonomic levels and constitutes a widely recognized biogeographical pattern (Fischer 1960; Simpson 1964; Stelli *et al.* 1969; Willig *et al.* 2003). While its underlying processes have intrigued ecologists, systematists and biogeographers worldwide, few historically based hypotheses have been proposed to explain its origin (see Brown & Lomolino 1998). One such example is the notion that

the latitudinal biodiversity gradient may reflect the distinct influence of Pleistocene glacial–interglacial cycles in geographic space (Fischer 1960, Hewitt 2004).

Pleistocene climate change strongly affected environmental ranges throughout the world (Pielou 1991; Hewitt 2000). The effects of this dynamic are reflected in a diverse group of organisms, especially at higher latitudes, where postglacial expansion is usually considered primarily responsible for current genetic diversity patterns (Hewitt 1996, 1999, 2000, 2004). Like their temperate counterparts, tropical habitats also expanded to higher latitudes since the last glacial period (Behling 1995; Behling & Negrelle 2001; Bauermann 2003; Carnaval & Moritz 2008). Thus, it is expected that

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within-population genetic diversity, as well as the degree of genetic structure, should decrease from low to high latitudes, along a tropical latitudinal gradient (Miller *et al.* 2010).

The genetic effects of the glacial/interglacial periods across the latitudinal gradient have been studied at multiple geographic scales. Based on analysis of a diverse group of vertebrates, Lessa *et al.* (2003) observed different demographic histories across regions at different latitudes in North America and the Amazon. Taxa from North America exhibited clear signatures of recent population expansion, while species from the Amazon forest were apparently stable since the end of the Pleistocene. A similar scenario was observed by Pinho *et al.* (2007) in a more restricted geographic scale. An analysis of the effects of climatic changes in closely related taxa (species of *Podarcis* lizards) through the Iberian Peninsula and North Africa indicated higher stability at lower latitudes, evidenced by the greater genetic structure and diversity.

Carnaval *et al.* (2009) also showed similar findings, working within the tropics. In their study with Atlantic Forest (AF) amphibians, they reported higher levels of genetic diversity and structure at low (central AF) than at high latitudes (southern AF), corroborating paleoenvironmental inferences for this region (Carnaval & Moritz 2008).

The hypothesis about the effects of climatic cycles across the latitudinal gradient (latitudinal gradient hypothesis – LGH) makes explicit predictions, also applicable to the tropics: (i) populations from higher latitudes experienced more pronounced changes in their effective population sizes and therefore exhibit signatures of recent demographic expansion and a lower genetic structure; (ii) populations from lower latitudes experienced smaller or no changes in effective sizes, thus presenting high diversity and genetic structure.

Although the LGH makes explicit and testable predictions at the population level, few studies have explicitly tested it in a strictly tropical latitudinal gradient. Here, we investigate the influences of the differential dynamics of the environment throughout a tropical latitudinal gradient on the genetic diversification and historical demography of *Sclerurus scansor* (Ménétriès 1835). *Sclerurus scansor* is a polytypic species formed by two subspecies: *Sclerurus scansor scansor* (Ménétriès 1835) that ranges from the southern limits of the AF to the southern region of the state of Bahia and *Sclerurus scansor cearensis* (Snethlage 1924) that restricts to the interior of forest relicts of northeastern Brazil. This strictly forest passerine is endemic to the AF region (Stotz *et al.* 1996). Two reasons make it a powerful model for studying AF biogeography. First, *S. scansor* is highly sensitive to environmental changes, such as those caused by forest fragmentation (Stotz *et al.* 1996; Hansbauer *et al.* 2008a,b). Second, it is distributed throughout the AF, ranging from lat 3° to 32°S.

As stressed by Pinho *et al.* (2007), studies with closely related taxa make the inferences simpler and straightforward, because ecological requirements, dispersal ability and important quantitative information needed to obtain estimates of demographic parameters (e.g. mutational rate and generation time) can be assumed to be similar for the entire range, without adding significant noise to the analysis. Conversely, the analysis of a single biogeographical entity (the AF) to test the predictions associated with the LGH makes the inferences and interpretation of the results more robust, because its distribution is defined by the same general limits of environmental conditions (e.g. soil, temperature and precipitation).

In this study, we test the LGH predictions for AF region based on the phylogeographic and demographic history of *S. scansor* and analyse the hypothesis proposed to explain the origin of current biogeographical patterns of the AF.

Methods

Samples and markers

Tissue samples were obtained from the following collections: (i) Laboratório de Genética e Evolução Molecular de Aves (LGEMA), São Paulo, Brazil; (ii) Field Museum of Natural History (FMNH), Chicago, USA; (iii) Louisiana State University Museum of Natural Sciences (LSUMNS), Baton Rouge, USA; and (iv) Laboratório de Biodiversidade e Evolução Molecular, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil (Appendix S1, Supporting information).

Analyses were based on two data sets. *Data set A*: NADH dehydrogenase subunit 2 (ND2) sequences (1041 bp) from 85 samples, extracted from muscle and blood tissues. This data set was used to analyse the phylogeographic structure of *Sclerurus scansor.* This analysis was carried out using *Sclerurus albigularis* (the sister species of *S. scansor*) and *Sclerurus mexicanus* as outgroups, based on a *Sclerurus* molecular phylogeny (d'Horta 2009). *Data set B*: Based on the initial exploratory analysis (data set A), we sampled a subset of individuals that include all main geographic regions. For 48 individuals, we sequenced 1025 bp of cytochrome *b* (cyt*b*) and 345 bp of NADH dehydrogenase subunit 3 (ND3), and for 44 individuals, we sequenced 954 bp of a nuclear locus and β-fibrinogen intron 7 (Fib7). Procedures of DNA extraction, purification and sequencing are described on Appendix S2 (Supporting information).

Data analyses, neutrality tests and recombination (data sets A and B)

Sequences of ND2 (data sets A and B), ND3, cyt *b* and Fib7 (data set B) were used to perform phylogeographic and demographic analyses. Sequences were compared and edited using the program CodonCode Aligner (CodonCode Corporation), and the alignment was performed with Clustal X (Thompson *et al.* 1997) and then manually checked.

Heterozygous nucleotide positions were identified by double peaks in the electropherograms, in the case of the nuclear locus. We used a Bayesian approach implemented in the program PHASE 2.0 (Stephens *et al.* 2001; Stephens & Donnelly 2003) to identify haplotypes of Fib7. Heterozygous indels were not found. To test the hypothesis of neutrality in mtDNA, the MacDonald and Kreitman test (MK *test* – MacDonald & Kreitman 1991) was applied, considering the sister species of *S. scansor*, *S. albigularis*, as outgroup (d'Horta 2009). This test was applied to the entire species distribution and also to each lineage. To evaluate the existence of recombination on Fib7, we applied the *phi* test, performed in the SPLIT-STREE4.10 program (Huson & Bryant 2006). We chose this test because of its sensitivity to recombination events and its power to distinguish recombination events from homoplasies (Bruen *et al.* 2006).

Haplotype identification and phylogenetic analyses (data sets A and B)

We used the NETWORK 4.5.0.0. software (http://www.fluxus-technology.com) to identify ND2 and FIB7 haplotypes and to obtain a network with the *median joining* method (Bandelt *et al.* 1999).

The phylogenetic analyses were performed for mtDNA (data sets A and B), using two methods, maximum-likelihood estimation (ML) and Bayesian analysis (BA). To select the simplest model of molecular evolution with the highest likelihood for the data, we applied a likelihood-ratio test (LRT) performed by the MODEL-TEST program (Posada & Crandall 1998). The selected model parameters were used for the ML analysis implemented on PHYML program 2.4.4 (Guindon & Gascuel 2003). The robustness of the ML tree nodes was determined by 500 bootstrap replications.

The BA, with Markov chain Monte Carlo (MCMC) sampling, was implemented with MrBayes 3.1 (Ronquist & Huelsenbeck 2003), considering the partitions of the data set applying the partitioned likelihood method (one partition per gene). Parameters were independently estimated for each data partition (nst = 6; rates = invgamma). Two independent analyses were performed, each with 10^6 generations, sampling every 1000 generations and with a burn-in of 20%, determined by the convergence time of the likelihood values. The posterior probability values of each node were calculated by combining all sampled trees in a consensus.

Population level analyses (data set B)

Broader population groups used for analysis of divergence times (IMa) and historical demography (LAMARC and neutrality tests) were defined based on geographic criteria and corroborated by the analysis of data set A (see Results).

Divergence times. Estimation of divergence times (*t*) between pairs of *S. scansor* lineages (southern, central and northern lineages; Fig. 3) were obtained applying the *isolation–migration* model (Nielsen & Wakeley 2001; Hey & Nielsen 2004), implemented by IMa program (ver. 17 December 2009). Based on a coalescent and MCMC method, IMa calculates the distribution of posterior probability values of parameters considered in the model (Nielsen & Wakeley 2001; Hey & Nielsen 2004). The effective population size was estimated for current populations ($\theta 1$ and $\theta 2$) and the ancestral population (θa). To obtain the divergence time estimates between lineages, we set migration rates (m_1 and m_2) to 0. To define the priors, initial runs were carried out, followed by five posterior runs, for each pair of populations, using distinct seed values, to verify the convergence of parameter estimates. For each analysis, a *burn-in* of 500 000 steps was applied, and they were finished with at least 50 000 000 steps in the chain after the *burn-in* stage and a minimum effective sample size value of 150. To obtain the divergence time in years, we considered $t = t/u$ (Hey & Nielsen 2004), where *t* is estimated by IMa and *u* is the geometric mean of mutational rates (per year) of mitochondrial genes (cyt *b*, ND2 and ND3) and Fib7. To calculate the *u* value, we assumed a divergence of 2.1% per million of years for mtDNA genes (Wier & Schluter 2007) and 0.72% for Fib7 (Axelsson *et al.* 2004). All analyses were performed using Hasegawa-Kishino-Yano (HKY – Hasegawa *et al.* 1985) evolutionary model.

Population expansion/decline. Tajima's D (Tajima 1989), Fu's F_s test (Fu 1997) and R_2 (Ramos-Onsins & Rozas 2002) were used to test the scenarios of population expansion. The significance of the tests was determined based on 10 000 coalescent simulations, which assumed neutrality and equilibrium conditions. These analyses were performed with DnaSP 4.10.9 program (Rozas *et al.* 2003). These three test statistics were selected because of their power to detect population expansion scenarios in

specific sampling conditions and population expansion rate, time since the expansion, sample size and number of segregation sites (Ramos-Onsins & Rozas 2002).

In addition to the tests with summary statistics, we estimated genetic diversity levels (θ) and exponential population growth rates (g) using the LAMARC program (Kuhner 2006). LAMARC calculates the likelihood surface for θ and g, allowing one to find values that maximize the probability of originating data. These parameters (θ and g) were estimated for the three main phylogeographic lineages identified in *S. scansor*, fixing the migration rate to 0. For the LAMARC analyses, five replicates of 10 short chains each (500 genealogies sampled each 50 interactions and a *burn-in* of 1000 genealogies) and two long chains (20 000 genealogies sampled each 50 interactions and a *burn-in* of 1000 genealogies) were performed. Because of the failure of the *phi* test in rejecting the null hypothesis of nonrecombination, the parameter *r* (recombination) was not considered for the LAMARC analyses.

Results

Sequence variability

In data set A (1041 bp of ND2; *n* = 85), we identified 53 variable and 41 parsimony-informative sites. In data set B for the mtDNA (total of 2432 bp, been 345 bp of ND3, 1041 bp of ND2 and 1046 bp of cyt*b*; *n* = 48), we identified 93 (five for ND3, 49 for ND2 and 39 for cyt*b*) variable and 69 (three for ND3, 39 for ND2 and 27 for cyt*b*) parsimony-informative sites, and for the nuclear locus (Fib7) in 954 bp (*n* = 44), we found 10 (1.05%) variable sites, of which 9 (0.94%) were parsimony informative. The results of MK test were nonsignificant (*P* > 0.1) for

all analyses (both treating *Sclerurus scansor* as a single taxon and treating each phylogeographic lineage separately). The *phi* test did not reject the null hypothesis of nonrecombination for Fib7 (*P* > 0.1).

Twenty-five haplotypes were identified for the ND2 gene (data set A: 1041 bp, *n* = 85), while 30 were diagnosed for Fib7 (data set B: 954 bp, *n* = 44). The ND2 network showed a strong geographic association of haplotype groups (Figs 1a and 2). Three main groups were identified, representing the northern (SN), central (SC) and southern (SS) lineages. On the other hand, the Fib7 network showed extensive sharing of haplotypes between SC and SS. The SN lineage was the only one which did not share haplotypes with other groups (Fig. 1b).

Nucleotide diversity (π) and haplotype diversity (H) were low for all phylogeographic lineages (Table 1). Lineage SC showed the highest values of π and H for mtDNA (data set A and data set B; 0.0043 and 0.731 for ND2; 0.0035 and 0.908 for concatenated mtDNA, respectively). SS exhibited the lowest π and H values for mtDNA (0.00116 and 0.706 for ND2; and 0.00118 and 0.808 for mtDNA concatenated). SN (*cearensis*) exhibited intermediate values of π and H (0.002 and 0.630 for ND2; 0.0021 and 0.0846 for mtDNA concatenated). Among the three groups analysed for Fib7, SN exhibited the lowest π and *H* values (0.00061 and 0.499, respectively), while the SC the highest ones (0.0049 and 0.948), which can be explained by the wide sharing of haplotypes observed between the SS and SC lineages (Fig. 1b).

Evolutionary relationships, distribution and contact among phylogeographic lineages

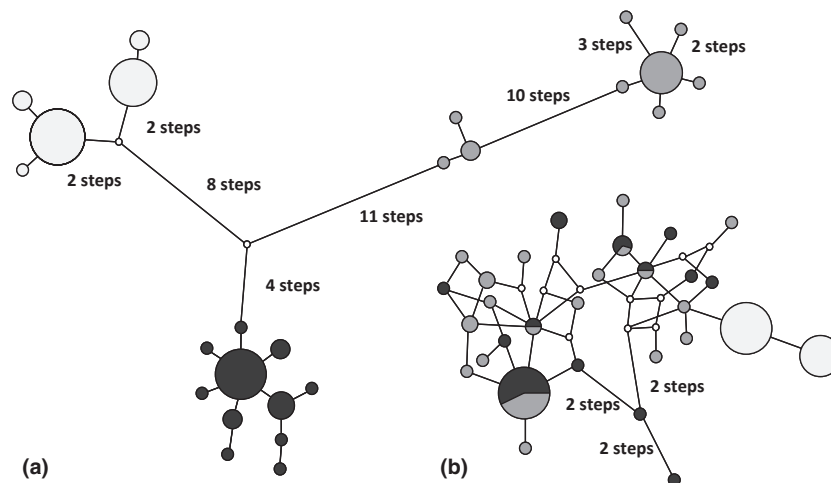Three well-supported clades were identified for *S. scansor*, based on mtDNA (data set B): (i) SS, which is

Fig. 1 Median joining networks based on (a) 1041 bp of ND2 (data set A) and (b) 884 bp of Fib7 (data set B) of *Sclerurus scansor*. Lineage distribution, represented in black (SS), gray (SC) and light gray (SN) circles.

distributed from the southern limit of the species' occurrence to the central portion of São Paulo state; (ii) SC, which ranges from the central portion of São Paulo state, where it occurs in sympatry with SS, until southern Bahia; (iii) SN, which occurs associated with forest enclaves from the interior of the Caatinga, apparently totally isolated from the SS and SC (Fig. 2). The relationships among populations suggest a first split between SN (*cearensis*) and the two southernmost clades (SS and SC) followed by the split between SS and SC (bootstrap of 77 and posterior probability of 100) (Fig. 3).

For two lineages, SC and SN, two phylogeographic subgroups were identified. In SN, we found two mitochondrial lineages that correspond to the two haplotype clusters in the network (Fig. 1a), which comprise the samples from the western SN range (Serra de Ibiapaba, Serra das Almas and Chapada do Araripe) and the samples from eastern SN range (Serra de Maranguape, Serra de Baturité and Serra do Machado) (Figs 2 and 4). These results suggest that the contact within the eastern as well as within western forest relicts was more frequent and recent than between these two groups. In SC, two lineages were also identified, one comprising the samples lying north and the other, those lying south of the Doce river region (Figs 2 and 4).

Among the main phylogeographic lineages of *S. scansor*, contact is observed only between the SS and SC. Of the total of 18 localities sampled SC and SS, only three

(those in the region of upper Tietê and Paraíba do Sul rivers, see discussion) showed evidence of sympatry between these lineages. The current distribution of *S. scansor scansor* (SS and SC), as well as *Sclerurus scansor cearensis* (SN), suggests a complete genetic isolation.

Population analyses

Our analysis of data set A showed a strong congruence between geographic regions and clade membership. The only exception to this patter was found for three localities in the region of contact between SS and SC, where haplotypes from different lineages are found in sympatry. We argue that this contact zone arises as a consequence of secondary contact (see Discussion) and therefore that treating lineages as populations is justified. Our estimates of population expansion and divergence times were thus applied on these lineages and assumed zero migration rates.

Divergence times. The analysis of divergence times between intraspecific lineages resulted in values that point to a relatively recent process of intraspecific diversification, with events of cladogenesis concentrated on Middle and Late Pleistocene (Fig. 4 and Table 2). Divergence times estimated by IMa (data set B – mitochondrial and nuclear markers), as well as the topology based on mitochondrial genes, suggest a first event of cladogenesis originating SN (*cearensis*) and the SC + SS
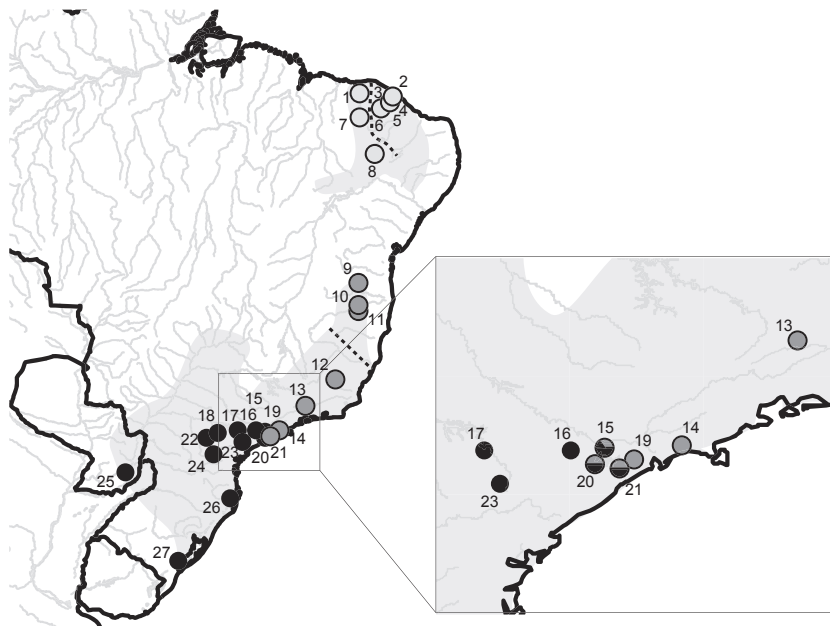

Fig. 2 Sample localities of *Sclerurus scansor*. Different circle colours represent the three main mitochondrial lineages. In black SS, gray SC and bright gray SN. The inset map depicts the zone of sympatry between SS and SC. Black/gray circles show the localities where SS and SC lineages are reported. Dotted lines mark the position of the brakes among internal phylogeographic units in SC and SN.

Table 1 Diversity values and the results of statistical tests to ND2; concatenated mtDNA (cytb, ND2 and ND3); and Fib 7

| Lineages | Taxa | Gene | N | Diversity | | | Tests | |
				π	H	D	F_s	R_2
Southern	*Sclerurus scansor scansor*	ND2	30	0.00116	0.706	−1.6441 ns	−7.300 **	0.0571**
		Mit	15	0.00118	0.808	−0.7652 ns	−1.267 ns	0.1120 ns
		Fib7	14	0.00350	0.791	−0.3216 ns	−6.528*	0.1159 ns
Central	*S. scansor scansor*	ND2	19	0.00433	0.731	−0.5047 ns	−0.588 ns	0.1090 ns
		Mit	16	0.00350	0.908	−0.3645 ns	−1.350 ns	0.1149 ns
		Fib7	13	0.00490	0.948	−0.5230 ns	−13.366 **	0.1002 ns
Northern	*Sclerurus scansor cearensis*	ND2	36	0.00205	0.630	0.6128 ns	1.198 ns	0.1456 ns
		Mit	17	0.00210	0.846	0.4534 ns	0.016 ns	0.1464 ns
		Fib7	17	0.00061	0.499	1.5592 ns	1.711 ns	0.2496 ns
Total	*Sclerurus scansor*	ND2	85	0.01241	0.871	0.5944 ns	−0.543 ns	0.1154 ns
		Mit	48	0.01042	0.941	1.2173 ns	0.904 ns	0.1472 ns
		Fib7	44	0.00403	0.875	−0.4057 ns	−22.043**	0.0836 ns

N, number of sequences; π, nucleotide diversity; H, haplotypic diversity; D, Tajima's D (1989); F_s, Fu (1997); R_2, (Ramos-Onsins & Rozas 2002).
ns, nonsignificant ($P > 0.05$); *Significant ($P < 0.05$); **Significant ($P < 0.01$).

(*scansor*) lineages. A second event, near the end of the Pleistocene, originated the SS and SC (Table 2). The divergence time between SS and SC was estimated to be approximately 0.06 Ma (CI 90%: 0.04–0.09 Ma). Divergence times between SS and SN, and SC and SN were estimated to be approximately 0.54 (CI 90%: 0.32–0.76 Ma) and 0.24 Ma (CI 90%: 0.06–0.39 Ma), respectively.

Historical demography. The results of Tajima's D test were nonsignificant for all populations, thus not rejecting the hypothesis that they are in equilibrium. F_s, on the other hand, showed that for SS, both ND2 (30 individuals) and Fib7 (12 individuals) had negative and significant values, supporting the hypothesis of population expansion. The R_2 values for this same lineage also point to the same scenario (when ND2 was used). The data for the SC lineage showed that only the F_s for Fib7 resulted in significant values. Conversely, none of the tests applied to the SN lineage resulted in significant values, indicating demographic stability (Table 1). Although Tajima's D is relatively sensitive to demographic expansion, the simulations performed by Ramos-Onsins & Rozas (2002) showed that it tends to have less power than F_s (Fu 1997) and R_2 (Ramos-Onsins & Rozas 2002) in scenarios where time since expansion is high and/or the sample size and/or the growth rate are low.

The demographic analyses for the three main lineages of *S. scansor*, based on genealogy sampling using LAMARC, are partially congruent with the results obtained using the tests based on summary statistics. Concerning diversity levels, SS presented the lowest values, much lower than those observed for SC and SN, compatible with the scenario of deep changes in forest distribution at higher latitude regions. On the other hand, the estimates of exponential population expansion rate (g) showed markedly different results for the three lineages. For SS, the values indicate recent population expansion, with a point value of g = 4314.370 and the lower and upper limits of the confidence interval indicating positive values (CI 95%: 3711.105–4698.091). Although the SC lineage exhibits positive g values (g = 613.0905; CI 95%: 180.2021–951.3234), they are much closer to 0 than those obtained for SS (Fig. 5). Because of the tendency of g to be shifted towards positive values, especially when one or few markers are used (Kuhner *et al.* 1998; Kuhner 2006), it is not possible to completely discard the scenario of stability for SC. On the other hand, SN (*S. s. cearensis*) exhibits g values that indicate a completely different demographic condition than the observed for the other lineages. The values for the SN (g = −1793.549, CI 95%: −4472.767 to −184.0114) are compatible with a scenario of a population bottleneck (Fig. 5), mainly if we consider the tendency for LAMARC to overestimate g values.

Discussion

Hypothesis of vicariant events

Many vicariant hypotheses have been proposed to explain the origin of the recurrent patterns of distribution of lineages/taxa in the Neotropical forests. These hypothesis are based on the role of physiographic (e.g.
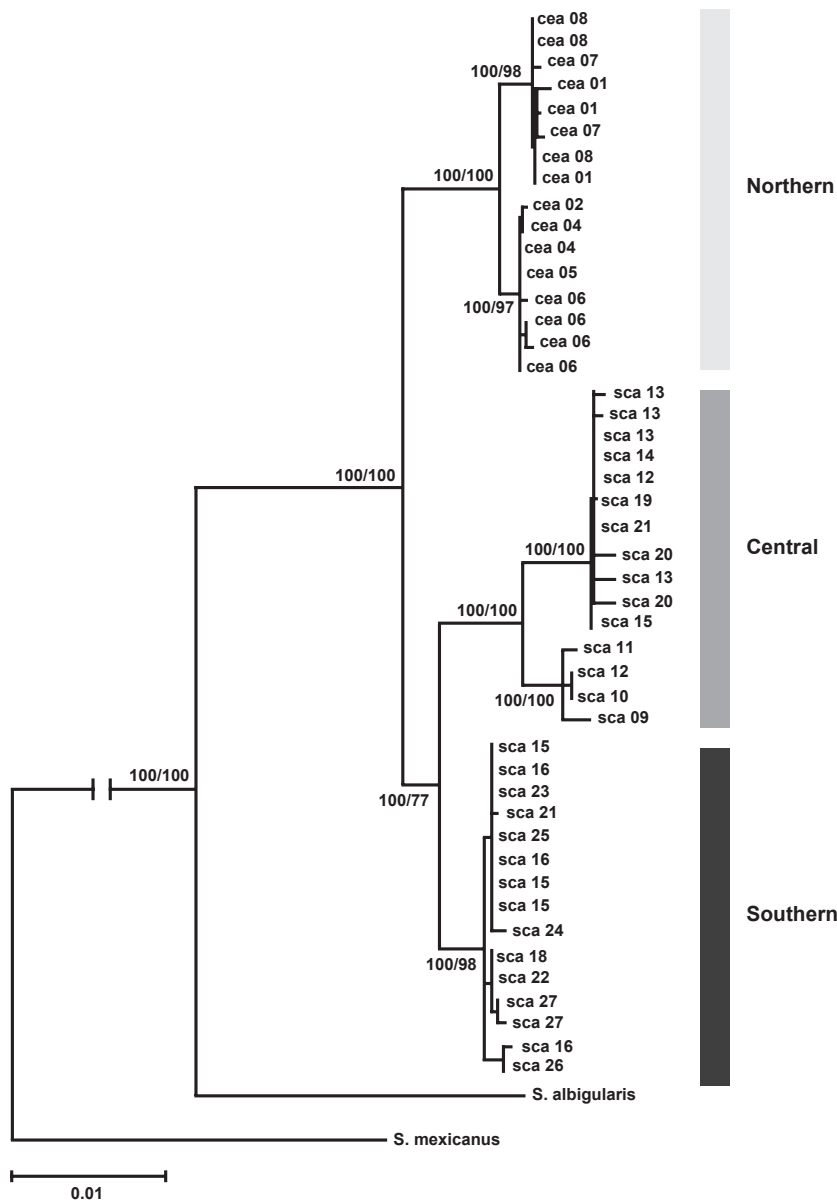
Fig. 3 Maximum-likelihood (ML) tree based on mitochondrial genes (2432 bp of ND2, ND3 and cytb – data set B). Numbers at each node represent the posterior probability from the Bayesian analysis and the bootstrap values (based on 500 replicates) from ML analysis. Number of locality sampled on the terminals (see Appendix S1, Supporting information).

Wallace 1852; Sick 1967; Ayres & Clutton-Brock 1992; Marroig & Cerqueira 1997; Nores 1999; Patton *et al.* 2000) or ecological barriers (e.g. Haffer 1969; Vanzolini & Williams 1970; Brown & Ab'Saber 1979; Colinvaux 1998) in reducing gene flow among populations and promoting divergence in allopatry. Some of these hypotheses have been considered to explain the origin of the AF biodiversity patterns (rivers as barriers – Pellegrino *et al.* 2005; refuges – Brown 1987; Cabanne *et al.* 2007; Lara *et al.* 2005; and neotectonism – Silva & Straube 1996; Pessoa 2007).

The geographic pattern of intrapopulation genetic variation observed for *Sclerurus scansor* is compatible with that proposed by the refuge hypothesis (Haffer 1969; Vanzolini & Williams 1970). First, phylogeographic lineages within *S. scansor* likely originated via vicariant events. The SN lineage is represented by an allopathic population. Available evidence strongly indicates that the origin of contact zone between SS and SC is secondary, suggesting that the lineages that occupy these two regions also originated via a vicariant event. The contact zone between SC and SS is spatially congruent with
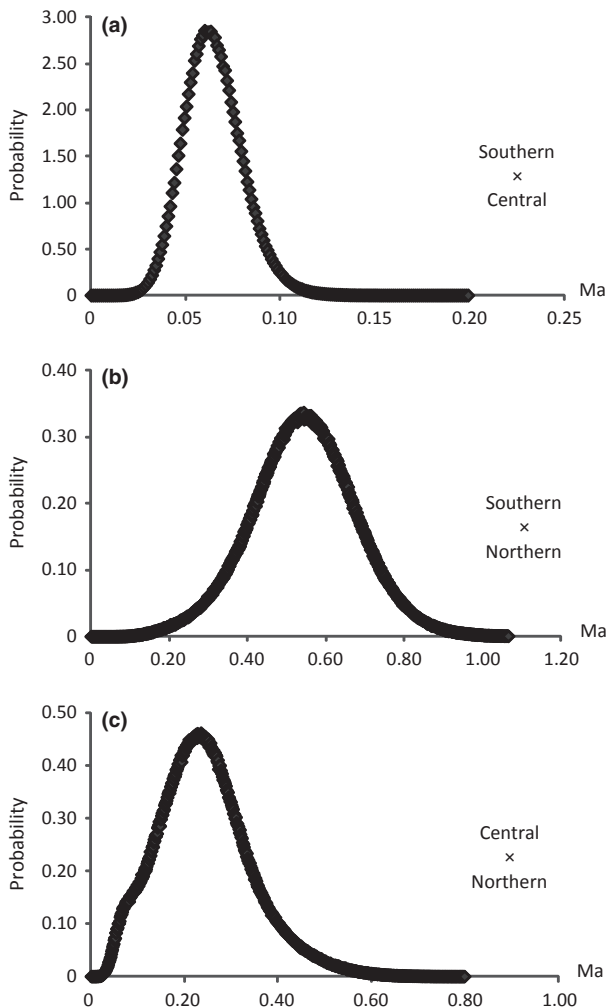
Fig. 4 Probability distributions of divergence times (million years ago), estimated by IMa, between lineages pairs of *Sclerurus scansor*, based on mitochondrial genes and Fib7 (data set B).

those documented between phylogeographic lineages of amphibians (Carnaval *et al.* 2009), birds (*Xiphorhynchus fuscus*, Cabanne *et al.* 2007; and *Conopophaga lineata*, Pessoa 2007), as well as between morphologically differentiated populations of birds (*Lepidocolaptes squammatus*, Silva & Straube 1996; and *Heliobletus contaminatus*, Silva & Stotz 1992). This pattern of a common contact region can be interpreted as evidence of a common vicariant event (Cracraft 1985; Cracraft & Prum 1988).

Second, the *S. scansor* phylogeographic lineages presented, in different degrees, signatures of changes in their effective population size, which is expected in a refuge scenario. Finally, the estimates of the divergence time between phylogeographic lineages point to events during Middle and Late Pleistocene, a period for which there are extensive records documenting changes in forest distribution associated with climatic cycles

(Riccomini 1989; Behling 1995, 1997, 2002; Ledru *et al.* 1996, 2005; Behling & Lichte 1997; Behling & Negrelle 2001; Cruz *et al.* 2005; Carnaval & Moritz 2008). We also noticed that the geographic distribution of the lineages is congruent with what is expected according to the mountain refuges hypothesis (Moreau 1966; Lara *et al.* 2005). Based on the mountain refuge hypothesis, during drier periods, forest formations were more likely to occur in mountain areas, because of the higher pluviometric level resulting from the orographic effect. Such phenomenon is currently observed in the northeastern region of Brazil, where the occurrence of humid forests is strictly associated with areas of mountain ridges.

The hypothesis of rivers as primary barriers (Pellegrino *et al.* 2005) is not supported owing to the very recent origin of the phylogeographic lineages observed for *S. scansor*, as well as for other avian species (Pessoa 2007; Cabanne *et al.* 2008). However, these rivers can play an important role as a secondary barrier to dispersion, decreasing the migration between populations, or even completely isolating them. Silva & Straube (1996) propose that the tectonic activity associated with the Paraíba valley would have caused the isolation of the populations distributed in the north and south of this region. Although the location of the phylogeographic rupture between the SS and SC is relatively congruent with this notion, the divergence time between them is much more recent (Middle/Late Pleistocene, Table 2) than the periods of higher tectonic activity in the region, dated between the Miocene–Pliocene (about 15 Ma) and the Lower Pleistocene (about 1 Ma) (Petri & Fúlfaro 1983). Although there is evidence of recent tectonic movements during the Pleistocene and Holocene (Riccomini 1989), it would be necessary to admit that these movements caused landscape changes capable of imposing restrictions to gene flow between lineages SS and SC for a long period of time. On the other hand, analysing the deposition of Cenozoic sediments that fill the basins of the Continental Rift of southeastern Brazil, Riccomini (1989) recognized the effects of climatic cycles on erosion dynamics: increased erosion happened during drier climates, when vegetation cover was less dense (savannas), and decreased erosion during wetter climates, when vegetation covering was more dense (forest), which is compatible with refugia predictions.

Latitudinal gradient and demographic history

The importance of the differential effects of glaciations across latitudinal gradients, as well as their influence on the determination of current biodiversity patterns, is among the major subjects of Biogeography (Hewitt 1996, 2001; Brown & Lomolino 1998; Willig *et al.* 2003;

Table 2 Estimates of divergence times (million years ago) between pairs of phylogeographic lineages of *Sclerurus scansor*

	Divergence time (Ma)		
Lineages	Minimum (CI 90%)	Point estimates	Maximum (CI 90%)
SS vs. SC	0.04	0.06	0.09
SS vs. SN	0.32	0.54	0.76
SC vs. SN	0.06	0.24	0.39

CI, confidence interval.

Wier & Schluter 2007; Marshall *et al.* 2008). The latitudinal gradient has an important influence on the intensity of vicariant events, genetic diversity and demography, as demonstrated by Pinho *et al.* (2007) and Lessa *et al.* (2003). The description of patterns of genetic diversity and demographic history of populations across a tropical latitudinal gradient helps us to understand the differential effects of glacial cycles over the landscape and its importance in the origin of biodiversity patterns observed in these regions.

The geographic pattern of genetic diversity exhibited by lineages of *S. scansor* contrasts with the expectations of the LGH for the tropics. Although SS presents the lowest values of nucleotide and haplotype diversity for mtDNA, the values for SC, and not for SN, were the highest (Table 1). In the same way, the diversity values

of Fib7 were distinct from the expected for the SN. The values of nucleotide and haplotype diversity for SN were lower than those of the other lineages. SS showed intermediate values for Fib7, while the SC exhibits the highest (Table 1).

The results from demographic analyses support the same scenario. First of all, estimates of exponential growth rate (*g*) show a clear pattern of nonoverlapping confidence intervals (95%) among lineages (Fig. 5), which refutes the hypothesis of a similar demographic history related to the different latitudinal zones of AF.

The SS lineage, distributed between latitudes 23° and 32°S, shows a clear positive value of *g* (Fig. 5). The results from the present study, as well as from amphibians (Carnaval *et al.* 2009) and other birds (Cabanne *et al.* 2007; Pessoa 2007), support a model of recent population expansion associated with strictly forest taxa in higher AF latitude zones.

For the SC, which occurs between 24° and 14°S, a positive *g* was also found. Although the confidence interval does not include 0, owing to the tendency of overestimation of *g* values by LAMARC (Kuhner *et al.* 1998; Kuhner 2006), it is not possible to completely refute the hypothesis of recent stability of the effective size for this population. The *g* values as well as the highest genetic diversity for the SC lineages are in accordance with the results of Carnaval *et al.* (2009), showing a higher stability of forest distribution in the central portion of AF. Either way, the results obtained
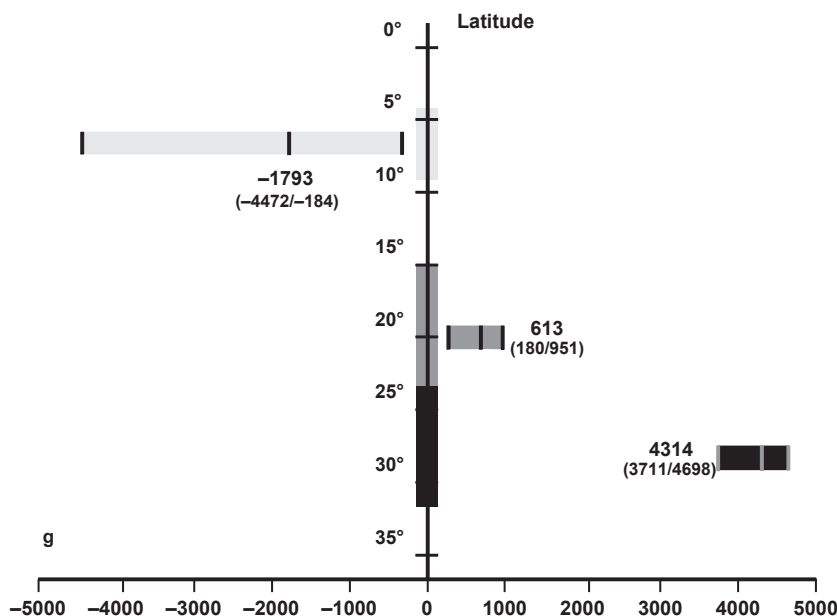

Fig. 5 Estimated values of population growth rate (*g*) (CI 95%) of the three main lineages of *Sclerurus scansor* (SS, SC and SN), based on mitochondrial genes and Fib7 (Data set B). Over the latitudinal axis, the amplitude of lineage ranges is shown. Horizontal bars represent the distribution of *g* values estimated for each lineage. Lineages are represented in black (SS), gray (SC) and bright gray (SN).

for the SS and SC lineages corroborate the general predictions of the LGH, that is, of higher demographic instability associated with higher latitude regions, where forest distribution suffered deep changes in function of glacial cycles.

In contrast, the SN lineage, associated with the lowest latitude zone (between 3° and 10°S), exhibits a signature of a population bottleneck. The recent changes in the effective size of the SN seem to have occurred in a markedly distinct way from that observed for the other populations. As the *g* values tend to be overestimated by LAMARC (Kuhner *et al.* 1998; Kuhner 2006), the scenario of population bottleneck seems to be reliable. These results contrast with the expected by the LGH to the tropics, where the highest stability on the effective size of the populations would be associated with the lower latitude regions. The observed scenario is congruent with the strict forest dependency of this species, and with its fragmented distribution, associated with humid forests from the interior of the northeastern Brazilian region ('brejos de altitude'). The 'brejos de altitude' are forest relicts associated with mountain ridges, amidst the semi-arid area of the Caatinga, where the precipitation levels are significantly higher (over 1200 mm/year; Andrade-Lima 1966, 1982) than the reported for the adjacent areas (240–900 mm, IBGE 1985, Lins 1989). The pluviometric regime of this region seems to respond in a distinct way to the observed for other regions of the AF (Auler & Smart 2001; Auler *et al.* 2004; Wang *et al.* 2004), which is compatible with the results for the SN (*Sclerurus scansor cearensis*) that suggest a contraction of forest distribution in a period during which southern forest experienced a wide expansion. A similar phenomenon is documented by the demographic history of other forest organisms (Carnaval & Bates 2007).

The general results of demographic analyses are congruent with the scenario inferred by Carnaval & Moritz (2008) based on modelling of AF paleo-distribution during the Quaternary, where southern AF was the most unstable area and the central region the most stable. Our results are also congruent with the general results obtained by Miller *et al.* (2010), who observed that central populations of tropical bird are more diverse than those from the edge of their ranges, indicating a history of higher instability in the edge than in the centre of their distributions.

The recent history of the *S. scansor* lineages indicates that the AF region, and the organisms strictly dependent on it, suffered deep changes in their distribution at higher as well as at lower latitudes, differing from the expected by the LGH for this region. Additionally, forests from lower and higher latitudes could have reacted even in opposite ways under the same climatic period.

While the AF at higher latitudes expanded, its distribution on lower latitudes became smaller and fragmented.

Conclusions

Because of the complexity of the physiographic and ecological features of the AF, and the intrinsic complexity of the ecophysiology of the AF organisms, it seems too simplistic to imagine that a single diversification mechanism can explain the origin of the current biogeographical patterns exhibited by AF species. However, this study of phylogeography and historical demography of *S. scansor* strongly supports the important role that Late Quaternary climatic changes had to the origin of the current biogeographical patterns.

The expected effects of the climatic cycles over the AF latitudinal gradient, on the other hand, were not confirmed by our results. The intraspecific evolutionary history of *S. scansor* indicates that different regions of AF can react in opposite ways under the same climatic period, generating asynchronous pulses of expansion (and coalescence) and retraction (and fragmentation) of habitats, producing at the same time favourable scenarios for isolation and contact between populations.

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Localities of analyzed samples, tissue number, vouchers, and GenBank accession numbers.

Appendix S2 Methods.

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