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Phylogeography of *Xiphorhynchus fuscus* (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in southern Atlantic forest

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Knowledge of the evolutionary processes that shaped a biota is important for both academic and conservation purposes. The objective of the present study is to analyse the mitochondrial genetic variation of *Xiphorhynchus fuscus* (Aves: Dendrocolaptidae) from the southern Atlantic forest in Brazil and Argentina, and to discuss whether the results support different hypotheses regarding the local intraspecific diversification of this species. We sequenced 575 bp of the control region of 114 specimens collected in the Brazilian states of Bahia, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina, and in the province of Misiones in Argentina. We studied the population genetic structure with analysis of molecular variance and the demographic history with multiple regression analysis, coalescence simulations, and demographic tests. *Xiphorhynchus fuscus* presented a significant population genetic structure ($\Phi_{st} = 0.57$). Three mitochondrial lineages were described, one associated with *Xiphorhynchus fuscus tenuirostris* and the others with *Xiphorhynchus fuscus fuscus*. The data did not support the primary influence of geographical barriers or rivers in the intraspecific diversification of *X. fuscus* in the southern Atlantic forest. Instead, the data supported the influence of isolation by geographical distance, recent vicariance events, and demographic expansions apparently related to Pleistocene and Holocene forest dynamics.

ADDITIONAL KEYWORDS: coalescence simulations – forest refuges – isolation by distance – mitochondrial control region – multiple regression – Neotropics – Pleistocene – riverine barriers – Valley of the Paraíba do Sul river.

The Atlantic forest is distributed along eastern Brazil, eastern Paraguay, and north-eastern Argentina (Gusmão Câmara, 2003). Its unique biota is probably the result of a complex evolutionary history; however, few studies have attempted to clarify the biogeographical processes that shaped it (Mustrangi & Patton, 1997; Costa *et al*., 2000; Geise, Smith & Patton, 2001; Pellegrino *et al*., 2005). The knowledge of these evolutionary processes is important for both academic and conservation purposes (Moritz, 2002). Among the several hypotheses on the diversification of rainforest biotas (Moritz *et al*., 2000), the evolution in palaeorefuges and the influence of geographical barriers are two of the most discussed ones.

In the Neotropics, the refuge theory was originally proposed to explain speciation during the Pleistocene mainly in the Amazon basin (Haffer, 1969; Vanzolini & Williams, 1970; Brown & Ab'Saber, 1979; Haffer & Prance, 2001). This theory proposes that during the glaciations the rainforests were reduced to refuges isolated by open areas, and that organisms isolated in these refuges could have diverged and originated new lineages. Then, in the next interglacial period, the forest expanded and the new clades would be in contact. This hypothesis requires the expansion and contrac-

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tion of open areas and the formation of forest refuges. Palinological (Ledru *et al.*, 1998; Behling & Negrelle, 2001; Behling, 2002) and other types of studies (Brown & Ab'Saber, 1979) propose that open areas dominated the Atlantic forest's landscape during the maximum of Late Pleistocene glaciations, suggesting that the refuge theory can be important to understand the biological diversification of the biome.

Pellegrino *et al*. (2005), based on a phylogeographical study of the gecko *Gymnodactylus darwinii* (Gekkonidae, Squamata), proposed that rivers play an important role in the diversification of Atlantic forest biota. Other authors proposed that the tectonic activity associated with the formation of geographical landmarks could be relevant to biodiversity modelling, as suggested by Silva & Straube (1996) who observed that the geographical range of some passerines was limited by a graben, the valley of the Paraíba do Sul river (VPSR). Those tectonic episodes could have been important for the biota in the southern Atlantic forest, where a complex relief exists with many mountain ranges and valleys, and where neotectonic activity was described (Petri & Fulfaro, 1983; Riccomini *et al*., 1989).

Xiphorhynchus fuscus is an Atlantic forest endemic member of the Family Dendrocolaptidae (Aves) with a broad distribution. It occurs in eastern Brazil (from the state of Ceará to the state of Rio Grande do Sul), eastern Paraguay and north-eastern Argentina (Marantz *et al*., 2003). There are four subspecies defined by slight variations in colour and morphological measurements (Marantz *et al*., 2003). *Xiphorhynchus fuscus tenuirostris* and *Xiphorhynchus fuscus fuscus* inhabit the eastern and the southern range of the species, respectively (Fig. 1). The other subspecies inhabit the interior of the Brazilian state of

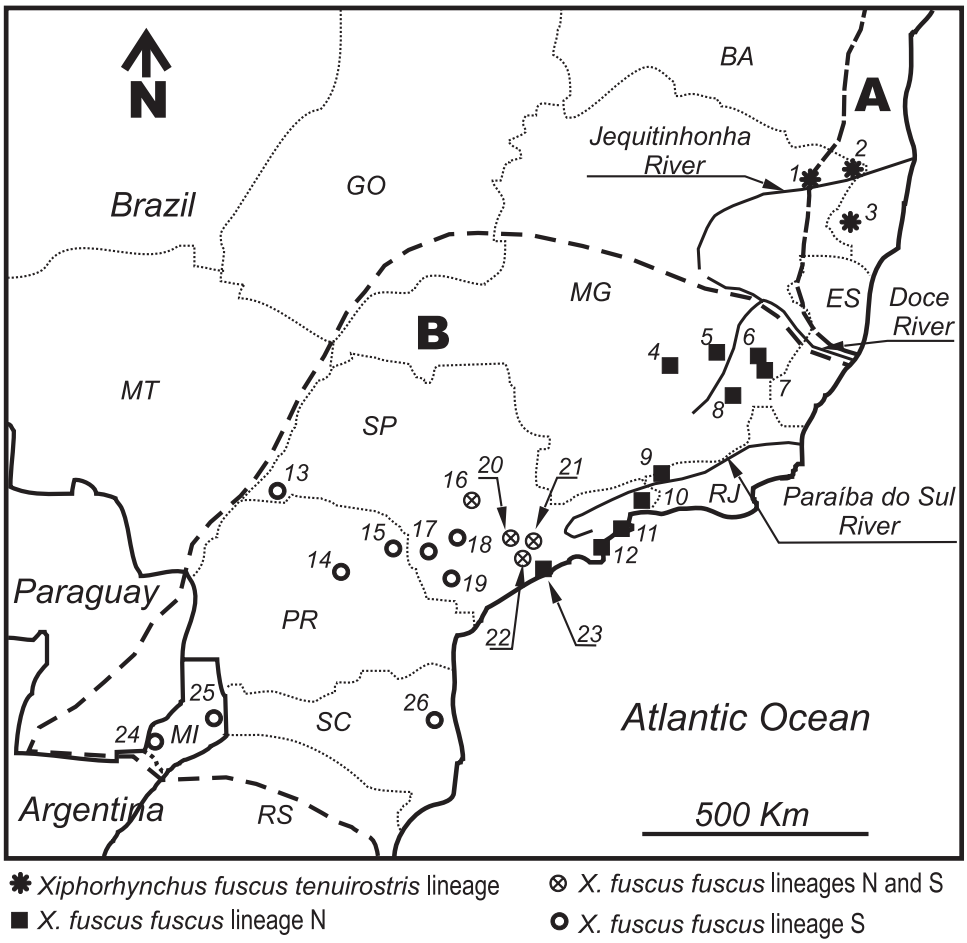

Figure 1. Study area, sampling localities, and distribution of *Xiphorhynchus fuscus* subspecies and mtDNA lineages. Locality numbers correspond to Table 1. BA, Bahia; ES, Espírito Santo; GO, Goiás; MG, Minas Gerais; MI, Misiones; MT, Mato Grosso do Sul; PR, Paraná; RJ, Rio de Janeiro; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo. Approximate distribution limits of the two subspecies indicated by broken lines (Zimmer, 1947; Marantz *et al*., 2003). A: *X. fuscus tenuirostris*, B: *X. fuscus fuscus*.

Table 1. Localities, sample sizes (*N*), haplotypes, and nucleotide diversity (π) in percentage

	Locality	*N*	Haplotypes[j]	π% (SD%)
1.	Minas Gerais (MG), Jequitinhonha, left bank of the Jequitinhonha river, 16°20′S, 41°00′W	5[a]	T2[1], T4[1], T6[1], T7[2]	0.5186 (0.2198)
2.	MG, Salto da Divisa, left bank of the Jequitinhonha river, 16°05′S, 40°02′W	6[a]	T1[4], T5[1], T7[1]	0.4606 (0.1785)
3.	Bahia, Porto Seguro, 17°22′S, 40°17′W	1[b]	T3[1]	–
4.	MG, Nova Lima, 19°59′S, 43°49′W	1[a]	N8[1]	–
5.	MG, Marliéira, 19°43′S, 42°44′W	1[a]	N9[1]	–
6.	MG, Caratinga, 20°50′S, 42°05′W	1[a]	N12[1]	–
7.	MG, Simonésia, 20°07′S, 42°00′W	1[a]	N13[1]	–
8.	MG, Araponga, 20°40′S, 42°31′W	3[a]	N2[1], N10[1], N11[1]	0.4710 (0.2374)
9.	Rio de Janeiro, Itatiaia National Park, 22°25′S, 44°36′W	10	N2[6], N6[1], N14[1], N15[2]	0.2274 (0.1239)
10.	São Paulo (SP), Bananal State Park, 22°41′S, 44°19′W	2[c]	N3[1], N6[1]	0.7109 (0.3368)
11.	SP, Picinguaba, 22°31′S, 44°50′W	4	N2[4]	0.0
12.	SP, Caraguatatuba, 23°37′S, 42°26′W	2	N2[1], N14[1]	0.1760 (0.1771)
13.	SP, Morro do Diabo State Park, 22°30′S, 52°18′W	1	S1[1]	–
14.	Paraná (PR), Ortigueira, 24°12′S, 50°55′W	1	S8[1]	–
15.	PR, Wenceslau Braz, 22°51′S, 49°47′W	7[d]	S1[5], S8[2]	0.1677 (0.1237)
16.	SP, Barreiro Rico, 22°38′S, 48°13′W	3[e]	N2[2], S8[1]	0.9479 (0.3195)
17.	SP, Itaberá State Park, 23°51′S, 49°08′W	7	S4[3], S6[3], S10[1]	0.2014 (0.1154)
18.	SP, Burí State Park, 23°39′S, 48°32′W	9[f]	S4[4], S7[1], S8[3], S9[1]	0.1760 (0.1098)
19.	SP, Caboclos, 24°28′S, 48°35′W	8	S4[5], S8[3]	0.0943 (0.0949)
20.	SP, São Roque, 23°34′S, 47°09′W	9	N1[3], N2[1], S4[5]	0.9199 (0.2815)
21.	SP, Morro Grande State Park, 23°42′S, 46°59′W	14	N1[5], N2[3], S5[1], S6[2], S7[3]	0.8830 (0.2589)
22.	SP, Juquitiba, 23°53′S, 47°00′W	7[g]	N1[1], N4[2], S4[1], S6[1], S7[1], S8[1]	1.0172 (0.3025)
23.	SP, Serra do Mar State Park, Station Curucutú, 23°58′S, 46°44′W	4[h]	N2[1], N4[1], N5[1], N7[1]	0.4424 (0.1858)
24.	Argentina, Misiones (MI), Campo San Juan State Park, 27°22′S, 55°39′W	3	S1[1], S2[2]	0.1173 (0.1065)
25.	MI, Yabotí Biosphere Reserve, 26°48′S, 53°55′W	3	S1[2], S3[1]	0.1173 (0.1181)
26.	Santa Catarina, Botuverá, 27°13′S, 49°03′W	1[i]	S1[1]	–

[a]Tissue samples deposited at the Universidade Federal de Minas Gerais; all the other samples are deposited at the Universidade de São Paulo.

Samples with museum voucher or specimen field (f) numbers: [b]Museu Paraense Emílio Goeldi fAA568; [c]Museu de Zoologia da Universidade de São Paulo (MZUSP) f76, f91; [d]MZUSP fITA257, fITA302, f ITA283; [e]MZUSP f31, f59, f60, [f]MUZUSP 76134, MZUSP 75588; [g]MZUSP fITA141, fITA171, fITA182, fITA152, fITA172, fITA157, 75565; [h]MZUSP f9; [i]MZUSP 75032.

[j]Numbers in superscript indicate the number of individuals with the corresponding haplotype.

Bahia (*Xiphorhynchus fuscus brevirostris*) and the north-eastern part of the country (*Xiphorhynchus fuscus atlanticus*). The distribution limits of these subspecies are not well known. Given its geographical distribution and deep-forest dependency, *X. fuscus* is a good model to study the diversification of the Atlantic forest biota.

The objective of the present study is to analyse the mitochondrial DNA variation of populations of *X. fuscus* from the southern part of the Atlantic forest and to discuss whether the results support different hypotheses regarding the local intraspecific genetic diversification of this bird.

MATERIAL AND METHODS

STUDY AREA AND SAMPLES

Samples (blood or muscle, $N = 114$) were collected between 2000 and 2004 in the Brazilian states of Bahia, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina, and in the province of Misiones in Argentina (Fig. 1, Table 1). The collection localities are covered by dense ombrophilus, mixed or semideciduous forest, which are the main forest types of the Atlantic forest (Veloso, 1991). The relief in the study area is complex, especially in the eastern portion due to the presence of the Serra do Mar and the

Serra da Mantiqueira coastal ridges, which can surpass 2000 m a.s.l.

Blood was collected (approximately 0.1 mL) with insulin syringes from the largest vein in the right cervical region. Muscle was obtained from specimens which were deposited at the Museu de Zoologia da Universidade de São Paulo. Each bird was captured with mist nets, photographed and marked with an aluminium ring. All tissue samples are deposited at the Laboratório de Genética e Evolução Molecular de Aves (Instituto de Biociências, Universidade de São Paulo, Brazil), or at the Laboratório de Biodiversidade e Evolução Molecular (Instituto de Ciências Biomédicas, Universidade Federal de Minas Gerais, Brazil).

Molecular methods

Total DNA was obtained from blood or muscle samples by a conventional proteinase K–SDS digestion, organic extraction with phenol–chloroform, and ethanol precipitation (Bruford *et al*., 1992). To diminish the possibility of amplifying unexpected copies of nuclear mtDNA inserts, we amplified an approximately 1600-bp fragment with the primer pair LXfB2 (5′-TCAATTCCAAACAAACTAGGAGG-3′, present study)/HPRO (5′-GCTTTGGGAGTTGGAGATAA AGG-3′, present study). This amplicon contained the complete control region (total size of 1275 bp, determined in the present study). The PCR reaction (10 µl) contained 20–40 ng of total DNA, 1X of *Taq* buffer (Pharmacia Biotech), 200 µM of each dNTP, 1 µM of each primer, and 0.1 U of *Taq* polimerase (Pharmacia Biotech). Amplifications were performed with an initial step at 95 °C for 4 min and 37 cycles of 45 s at 94 °C, 45 s at 53.5 °C, and 110 s at 72 °C, followed by a final extension of 10 min at 72 °C. PCR products were purified with shrimp alkaline phosphatase and exonuclease I. Sequencing reactions were performed with Big Dye Terminator Kit, version 3.0 (Applied Biosystems Inc.) using the amplification and the internal primers (HXfRC1 5′-GGGGAAAATAAACGTTTATTA AGTG-3′, HXfRC2 5′-CAAGATGGACATGTTCGAC ACCG-3′, present study, and L537 5′-CCTCTGGTTC CTCGGTCAG-3′; Sorenson *et al.*, 1999). The sequencing reactions were precipitated with isopropanol 75% and etanol 70%, and analysed in an automatic sequencer ABI Prism 377 (Applied Biosystems Inc.).

Analytical methods

The complete control region (1275 bp) and flanking genes ($tRNA^{Thr}$ 70 bp, $tRNA^{Pro}$ 70 bp) from one *X. f. fuscus* sample (tissue IBUSP P652) were sequenced. The analysis of 1048 bp of the control region (positions 57–1104) from 20 individuals revealed that the most variable portion encompasses 575 bp (positions 57–631); thus, this segment was used in the phylogeographical analyses. Identification and simulation of the secondary structure of the tRNA sequences were carried out with the program tRNAscan-SE 1.21 (http://www.genetics.wustl.edu/eddy/tRNAscan-SE/Lowe & Eddy, 1997). All sequences are deposited in GenBank under the access numbers AY948386–AY948394, AY948396–AY948415, and DQ144622–DQ144636.

Sequences were aligned with the program Clustal X (Thompson *et al*., 1997). The likelihood ratio test as implemented in the software Modeltest, version 3.7 (Posada & Crandall, 1998), was used to select the best fit evolutionary model [HKY85 with the proportion of invariable sites (I) of 0.8404 and a discrete gamma distribution ($\alpha = 0.6467$); Hasegawa *et al.*, 1985]. We reconstructed the phylogenies by Neighbour-joining (NJ; Saitou & Nei, 1987) with the software PAUP*, version 4.0b10 (Swofford, 2001) and by maximum likelihood (ML) with PHYML Online (Guindon & Gascuel, 2003; Guindon *et al*., 2005). The starting tree for the ML analysis was obtained with PHYML Online. For both analyses, the best fit evolutionary model was used. A sequence of *Xiphorhynchus pardalotus* (tissue IBUSP P264) was used to root the resulting trees. The support of the nodes was evaluated by nonparametric bootstrap (100 replicates; Felsenstein, 1985).

In all further population analyses, we used the Tamura & Nei (1993) model of evolution, which is the closest one to the HKY85 model available in the software we used. The haplotype network was constructed in accordance with Templeton, Crandall & Sing (1992) with the program TCS, version 1.13 (Clement, Posada & Crandall, 2000). The net divergence between sets of sequences was obtained in MEGA, version 3.0 (Kumar, Tamura & Nei, 2004) with the formula $\delta = \delta_{xy} - [0.5(\delta_x + \delta_y)]$, where δ_{xy} is the mean divergence between all the sequences, and δ_x and δ_y are the mean divergences within each group of sequences x and y, respectively (Wilson *et al*., 1985). The mutation rate used to estimate the divergence time between the lineages was $\mu = 8.72 \times 10^{-8}$ changes per nucleotide, as used by Milot, Gibbs & Hobson (2000) for the analysis of the first domain of the mtDNA control region of the passerine *Dendroica petechia*. We assumed a generation time of 1 year (Klicka & Zink, 1999).

To test whether the *X. f. fuscus* gene genealogy is the result of the neutral coalescence process within a panmictic population, we followed Knowles (2001) and applied a gene-tree population-tree approach using the program MESQUITE 1.02 (Maddison & Maddison, 2004). In this approach, gene trees (300 replicates) were simulated by neutral coalescence under a null model of a unique panmictic *X. f. fuscus* population whose effective population size (N_e) was invariable until total coalescence. Four arbitrary N_e were tested: 100 000, 50 000, 25 000, 12 000, and 6000. The

discordance between these gene trees and the alternative model of two isolated populations (one in the north and the other in the south of the Valley of the Paraíba do Sul river, VPSR; Fig. 1) was measured using statistic *s* (Slatkin & Maddison, 1989). The discordance between the reconstructed gene tree (NJ tree of *X. f. fuscus* sequences) and the two populations' model was also evaluated with the statistic *s* and this value was compared with the expected distribution of *s*-values obtained from the neutral simulations. If the *s*-value of the reconstructed gene tree is significantly lower than the values from the simulated gene trees ($\alpha = 0.05$), the null model is rejected. We used the nucleotide diversity (π, Nei & Kumar, 2000) calculated in Arlequin, version 2.0 (Schneider, Roessli & Excoffier, 2000) to approximate theta (Θ) and to calculate the empirical N_e according to the relationship $\pi = \Theta = 2N_e\mu$ (Watterson, 1975; Nei & Kumar, 2000).

We estimated the nucleotide diversity for each locality where two or more individuals were available using Arlequin 2.0. The analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was used to study the population genetic structure as implemented in Arlequin, version 2.0. We performed global (all the localities) and regional analyses (subgroups of localities). The Φ_{ST} was obtained using corrected distances and its statistical significance was estimated by a nonparametric permutation test (Excoffier *et al*., 1992).

We performed a partial regression analysis (Smouse, Long & Sokal, 1986) to test the effect of two independent variables (linear geographical distance among the pairs of localities and their geographical location) on the average corrected genetic distances among individuals from pairs of locations. Genetic distances were estimated with the program MEGA, version 3.0. The matrix of geographical location was constructed as a dummy variable (Quinn & Keough, 2002) that indicated whether each pair of localities were within the same main geographical region (north-western Minas Gerais plus southern Bahia, south-eastern Minas Gerais plus southern Rio de Janeiro, and south from the VPSR). The magnitude of the partial regression coefficients indicates the relative importance of the different independent variables. If genetic distances reflect simple isolation by distance, geographical distance would be the best predictor. If vicariance were the most important process, the geographical location matrix would be the best predictor of genetic distances. Each matrix was transformed to a mean of zero and a variance of one before the analyses (Smouse *et al*., 1986). The partial regression analyses were performed in the program Fstat, version 2.9.3.2 (Goudet, 2002), using 10 000 permutations to obtain the probability values.

Demographic expansion was inferred by calculating F_s (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) using DnaSP, version 4 (Rozas & Sánchez-Del Barrio, 2003). Significantly negative F_s and low values of R_2 indicate demographic expansion. For these calculations, the three major lineages recovered in the genealogy were defined as populations, following Cheviron, Hackett & Caparella (2005). Significance was determined based on 1000 coalescent simulations under a model of population stability using empirical sample sizes and estimates of Θ. The parameters of exponential population growth (Θ_0, Θ_1, and τ), according to the model of Rogers & Harpending (1992), were estimated in the program Arlequin, version 2.0. The expansion time was estimated according to $t = \tau/2u$ using lower and upper values of 95% confidence interval of the parameter τ, where u is the mutation rate per generation per haplotype (Rogers & Harpending, 1992).

RESULTS

Characteristics of the sequences

The sequences obtained revealed evidence of being of mitochondrial origin: (1) the empirical base composition of the sequences used in the phylogeographical study (A, 27.7%; C, 25%; G, 16.3%; and T, 31%) is compatible with the expected one for avian mitochondrial DNA (Baker & Marshall, 1997); (2) the amplicon (1600 bp) contained the complete control region (1275 bp), flanked by a $tRNA^{Thr}$ at the 5′ end (70 bp) and a $tRNA^{Pro}$ at the 3′ end (70 bp), and this gene arrangement is the expected one for Suboscines (Mindell, Sorenson & Dimcheff, 1998); (3) the inferred secondary structure and the presence of expected anticodons (UGU and UGG) suggested that these tRNA genes are functional (data not shown); and (4) the amplicon analysed is longer than the average size of translocated copies of mitochondrial genes in an avian nuclear genome (Pereira & Baker, 2004). The 575 bp of the control region of 114 individuals of *X. fuscus* presented 32 haplotypes (Table 1, Figs 2, 3), with 31 polymorphic sites (5.39% of all positions), 30 transitions and two transversions, and no indels.

Genealogy, divergence, and geographical distribution of *X. fuscus* mitochondrial lineages

The haplotype network presented three major clades (Fig. 2): one associated with *X. f. tenuirostris* and the others with *X. f. fuscus* [northern lineage (N) and southern lineage (S)]. The *X. f. tenuirostris* lineage occurred in samples from north-eastern Minas Gerais and southern Bahia. Haplotypes of the *X. f. fuscus* northern lineage only occurred in south-eastern Minas Gerais, southern Rio de Janeiro and north-eastern São

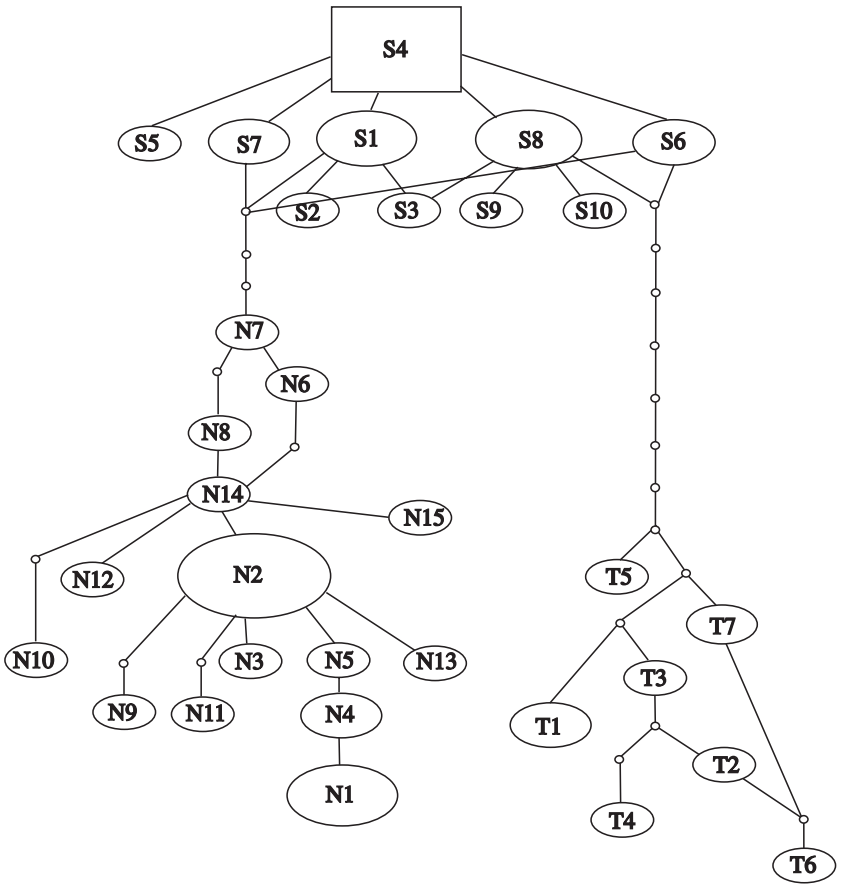

Figure 2. Haplotype network of *Xiphorhynchus fuscus* based on 575 bp of the control region. The size of each figure is proportional to the frequency of the corresponding haplotype in the total sample. Haplotype identification: T1 to T7, *Xiphorhynchus fuscus tenuirostris* lineage; N1 to N15, *X. f. fuscus* northern lineage; S1 to S10, *X. f. fuscus* southern lineage.

Paulo; whereas sequences of the southern lineage occurred in all localities in southern and central São Paulo, Paraná, Santa Catarina, and Misiones. Based on coalescence principles, the TCS algorithm (Crandall & Templeton, 1996) selected the haplotype S4 (*X. f. fuscus* lineage S) as the most likely ancestral one. The phylogenetic analysis by NJ and ML resulted in similar topologies and recovered the same three main lineages. Thus, we only present the NJ tree with the bootstrap supports obtained by both methods (Fig. 3).

Mean ± SD Tamura & Nei (1993) distance between *X. pardalotus* and *X. fuscus* was 12.366 ± 1.481%. The distance between *X. f. fuscus* and *X. f. tenuirostris* was 2.368 ± 0.561%, and between *X. fuscus* lineages N and S was 1.493 ± 0.400%. The net divergence between the two *X. f. fuscus* lineages was 1.228 ± 0.432%, which corresponds to 73 500 ± 24 500 years of divergence. The low number of samples of *X. f. tenuirostris* did not allow us to estimate the net divergence between this clade and *X. f. fuscus*; thus, the estimated divergence time between these clades (135 000 ± 32 000 years) was based on the mean distance between groups (2.368 ± 0.561%).

Coalescence simulations

Sequences within *X. f. fuscus* grouped in two main lineages that were strongly associated with specific geographical regions, suggesting that each lineage possibly evolved in allopatry. Given that the divergence between lineages is low and that the stochastic nature of the coalescence process can produce many different genealogies, we applied a gene-tree population-tree analysis to test whether a single historical population could have originated these results. The *s*-value (nine) of the reconstructed genealogy was significantly lower than the values obtained by the simulated gene trees, regardless of the N_e used ($P < 0.01$); thus, the null model of a single population was rejected. Also, the empirical N_e was 52 000 and is compatible with the range of N_e (6000–100 000) used in the simulations.

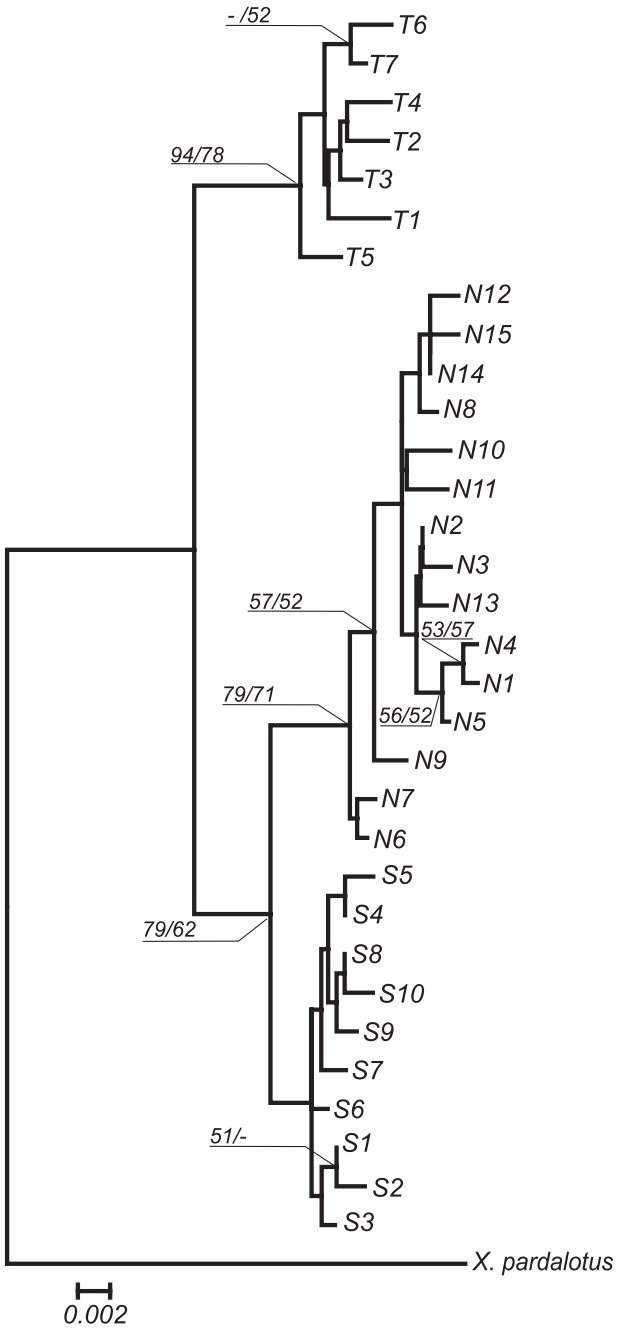

Figure 3. Neighbour-joining (NJ) tree based on 575 bp of the control region of *Xiphorhynchus fuscus*. The numbers at nodes show NJ and maximum likelihood bootstrap values above 50%, respectively. *Xiphorhynchus pardalotus* was used to root the tree. Haplotype identification: T1 to T7, *Xiphorhynchus fuscus tenuirostris* lineage; N1 to N15, *X. f. fuscus* northern lineage; S1 to S10, *X. f. fuscus* southern lineage.

Population genetic structure

A global AMOVA indicated that 57.7% ($\Phi_{ST} = 0.577$, $P < 0.01$) of the genetic variation in the samples of *X. fuscus* is allocated among the geographical regions where the main lineages occur (north-eastern Minas Gerais plus southern Bahia, south-eastern Minas Gerais plus southern Rio de Janeiro, and south of the VPSR). A regional AMOVA without the samples from the *X. f. tenuirostris* lineage (north-eastern Minas Gerais and southern Bahia) was performed subdividing each main region in two subregions. The subregions of the first region are Minas Gerais and Rio de Janeiro, whereas the subregions of the second region are São Paulo and south of São Paulo. This analysis indicated that 30.84% ($P < 0.01$) of the genetic diversity within *X. f. fuscus* was found among regions, that 12.93% ($P < 0.01$) occurred among the subregions within regions, and that 56.23% ($P < 0.01$) was allocated within subregions. The Φ_{ST} of this analysis was 0.43.

Relationships between genetic distances and predictor variables

The partial regression analysis indicated that 40.5% of the variation in genetic distances can be predicted by the linear geographical distance among pairs of localities (partial regression coefficient = 0.585, $P < 0.01$) and the geographical location (partial regression coefficient = 0.251, $P < 0.01$).

Historical demography

The F_s value for *X. f. tenuirostris* is nonsignificant, suggesting that there was demographic stability (Table 2). The F_s values are significant and negative for both *X. f. fuscus* lineages, suggesting the presence of past demographic expansion. Because the R_2 test is more suitable for the analysis of small samples than the F_s test (Ramos-Onsins & Rozas, 2002), we analysed *X. f. tenuirostris* ($N = 12$) using the R_2 test. The result was nonsignificant ($R^2 = 0.1800$, $P = 0.6960$), indicating that this taxa presented past demographic stability. Based on the estimations of τ, the demographic expansions of the northern and southern lineages of *X. f. fuscus* started approximately 57 000 and 19 000 years ago, respectively.

DISCUSSION

Phylogeographical structure of *X. fuscus* in the south-eastern Atlantic forest

The study of the control region of *X. fuscus* revealed a significant population genetic structure, which is in accordance with other Neotropical birds (Bates, 2000,

Table 2. Historical demographic analysis and expansion dates in years ago

Clade	*N*	F_s*	*P* (F_s)*	Θ_0	Θ_1	Expansion dates
Xiphorhynchus fuscus tenuirostris	12	–1.400	0.160	NA	NA	NA
Xiphorhynchus fuscus fuscus N	46	–7.080	0.001	0.0010	7.0460	10 130–57 370
Xiphorhynchus fuscus fuscus S	56	–3.789	0.015	0.0000	3415	4 690–19 460

N, sample size; *P* (F_s), probabilities of the F_s statistic value being lower than the observed one based on 1000 coalescent simulations; Θ_0 and Θ_1, parameters of the model of exponential growth of Rogers & Harpending (1992); NA, not applicable. *F_s was calculated *sensu* (Fu, 1997).

2002; Aleixo, 2004; Cheviron *et al*., 2005), and contrasts with the patterns observed with the same genetic marker in Paleartic passerine populations separated by thousands of kilometres (Merilä, Björklund & Baker, 1997; Kvist *et al*., 1998; Kvist *et al*., 1999a, b; Uimaniemi *et al*., 2003).

Three main mitochondrial lineages were revealed in the *X. fuscus* populations studied. One lineage was associated with the subspecies *X. f. tenuirostris* and the others with the subspecies *X. f. fuscus* (Figs 1, 2, 3). Haplotypes of *X. f. fuscus* northern lineage were found from the Doce river basin in Minas Gerais to north-eastern São Paulo. The other *X. f. fuscus* lineage was distributed from northern São Paulo to Santa Catarina and Misiones in Argentina. The two subspecies clades appear to have diverged in the late Pleistocene, around 130 000 years ago. The separation of the two *X. f. fuscus* clades was estimated to have occurred approximately 70 000 years ago. A contact zone between the two clades of *X. f. fuscus* was located to the south-west of VPSR (Fig. 1). We hypothesize that a contact zone between the *X. f. tenuirostris* clade and the northern *X. f. fuscus* lineage can occur along the Doce river in Espírito Santo and somewhere between the basins of the Jequitinhonha and the Doce rivers in the interior of Minas Gerais, as suggested by the distribution of these two subspecies (Fig. 1). Further studies are needed to test whether the Doce river can be a barrier to gene flow between the two subspecies. Another interesting result is that all southern localities (Misiones and Santa Catarina) present only three haplotypes (S1, S2, and S3). These haplotypes are grouped in the network and in the NJ tree and were only present in this region and in northern Paraná (Table 1). This suggests that there was a past range fragmentation somewhere between São Paulo and northern Paraná, and this needs to be tested in future studies with a more detailed sampling.

Even though studies on Atlantic forest organisms are scant, the biogeographical pattern observed in *X. fuscus* with three phylogeographical groups that appear to be limited by geographical landmarks or specific geographical regions, such as the VPSR and the transition between the basins of the Doce and the Jequitinhonha rivers, is compatible with patterns observed in other organisms. For example, small mammals present biogeographical divergence among north-eastern Minas Gerais, south-eastern Minas Gerais, and southwards regions (Mustrangi & Patton, 1997; Costa *et al*., 2000). In the gecko *G. darwinii* (sampled in the same geographical region studied here), Pellegrino *et al*. (2005) also found three main mitochondrial lineages. Geckos from southern Minas Gerais are divergent from those from São Paulo and southwards regions, and both clades are also separated from geckos from the Jequitinhonha river basin. Thus, *X. fuscus* and *G. darwinii* present similar phylogeographical patterns. Even considering that coalescence times present high variance and estimated divergence times may not be reliably compared, the difference between the splitting times among clades in the gecko (youngest divergence at least 0.9 Mya) and those among the bird clades (oldest divergence 130 000 years ago) does not support a common temporal origin of these patterns. Furthermore, Pellegrino *et al*. (2005) suggested that rivers were the barriers generating the phylogeographical pattern observed in the gecko. Some predictions for the hypothesis of rivers as primary gene flow barriers are that sister clades should occur across major rivers rather than along the same river bank and should not present signals of demographic expansion, which would favour secondary contact (Moritz *et al*., 2000). The samples that we had available did not permit these predictions to be tested in all the main river systems of the study area. For the area around the Paraíba do Sul river, the number of samples allowed us to start to explore this issue. Because the two *X. f. fuscus* lineages presented signals of demographic expansion (Table 2) and the northern lineage occurs on both sides and southwards of the VPSR (Fig. 1), this river does not appear to be a primary barrier for the gene flow of *X. fuscus*. Nevertheless, the limits of the distribution of the subspecies *X. f. tenuirostris* and *X. f. fuscus* appear to coincide with the Doce river (Fig. 1), especially in Espírito Santo, suggesting that this river could be a barrier. It is necessary to add more samples from key locations to test these hypotheses.

ON THE ORIGIN OF THE PHYLOGEOGRAHIC STRUCTURE OF *X. FUSCUS*

The partial regression analysis suggested that both isolation by geographical distance and the history of vicariance (geographical location) were important in shaping the population genetic structure of *X. fuscus*. The coalescence simulations supported the idea that *X. f. fuscus* lineages evolved in two populations instead of in a single one. At least two main vicariant phenomena were detected: one that separated the two subspecies lineages and the other which resulted in the divergence between the two *X. f. fuscus* phylogroups. The latter divergence is focussed on below.

Evolution in isolation and secondary contact provides a possible explanation for the phylogeographical structure of *X. f. fuscus*. Two vicariant events could have separated the ancestral population: the geological episodes that formed the VPSR or natural forest fragmentation.

The VPSR is a 173 km long and up to 2 km deep graben that separates the Serra da Mantiqueira and the northern Serra do Mar (Petri & Fulfaro, 1983) (Fig. 1). It is a contact area for birds (i.e. *Lepidocolaptes squamatus* and *Lepidocolaptes falcinellus*, Silva & Straube, 1996; *Heliobletus contaminatus contaminatus* and *Heliobletus contaminatus camargoi*; Silva & Stotz, 1992) and marsupials (i.e. *Marmosops paulensis* and *Marmosops incanus*; Mustrangi & Patton, 1997), and it separates mitochondrial lineages of the gecko *G. darwinii* (Pellegrino *et al*., 2005). Also, morphologically distinct populations of the passerine *Scytalopus speluncae* (Giovanni, 2005) come into contact in the same geographical sector where the two *X. f. fuscus* lineages meet. Silva & Straube (1996) postulated that the tectonic process that opened the valley and the consequent alteration of the forest cover could have been a significant vicariant event for forest species. Assuming that this proposition is correct, the two lineages of *X. f. fuscus* that meet near to the valley could have evolved in isolation after the formation of the valley and, when the conditions for dispersion improved, they came into contact. The predictions of this hypothesis are that populations at each side of the valley are differentiated, and that the separation time between the lineages is compatible with the valley's age. The first prediction is supported by morphological characters that diagnose each of the bird populations from Minas Gerais and from São Paulo (Albuquerque, 1996). The prediction regarding the age of the valley, however, is not accepted. According to Petri & Fulfaro (1983), the formation of the valley started in the Miocene-Pliocene (approximately 15 Mya) and lasted until the Early Pleistocene. The estimated divergence time of the two *X. f. fuscus* lineages was 50 000–100 000 years ago, which is too recent to match the formation of the valley. Thus, our data do not support the formation of the current VPSR as the vicariant event that produced the two *X. f. fuscus* lineages.

Brown & Ab'Saber (1979) suggested that the forest cover along the VPSR was not discontinued in the last glacial period. Based on this hypothesis, Silva & Straube (1996) suggested that the putative forest fragmentation that resulted in the distribution pattern of *L. falcinellus* and *L. squamatus* could not be related to this historical climatic oscillation. Clapperton (1993), however, suggested that, in the last glacial maximum, there were two main forest refuges in this area: one in the northern Serra do Mar and the other in the Serra da Mantiqueira and part of the Serra do Espinhaço in Minas Gerais; and these refuges were separated by a grassland area coincident with the VPSR. Under the scenario of Clapperton (1993), the palaeorefuge hypothesis could explain the diversification of clades in *X. f. fuscus*, and thus the current contact area of the *X. f. fuscus* lineages should be secondary (Moritz *et al*., 2000). A prediction of this hypothesis is that clades involved in the secondary contact should exhibit evidence of range expansion (Hewit, 2000; Moritz *et al*., 2000; Cheviron *et al*., 2005) and concomitant demographic expansion. The demographic analysis supports this prediction for the two *X. f. fuscus* lineages. Furthermore, the contact area between *X. f. fuscus* lineages has a high proportion of derived haplotypes, especially from the northern lineage (i.e. haplotypes N1, N4, and N5; Table 1, Fig. 2), which is also compatible with population expansion and secondary contact (Templeton, Routman & Phillips, 1995).

According to Behling (1998, 2002), grassland dominated the southern and south-eastern Brazilian landscape during the Late Pleistocene, where diverse forest ecosystems exist today. The current southern limit of the Atlantic forest biome contacts the grasslands biome of southern Brazil at latitude 28–27°S in the north of Rio Grande do Sul, and Behling (2002) concluded that, during the Late Pleistocene, this limit extended 750 km northwards, at least to latitude 20°S (southern Minas Gerais and northern São Paulo). Behling (2002) also proposed that the modern forest cover of south and south-east Brazil was established only in the Late Holocene. Under this historic scenario of dynamic forest cover, it is expected that forest dependent organisms presented strong demographic expansion in response to the forest advance in the Late Holocene, especially in regions southwards to the Late Pleistocene northern limit of grassland. Thus, we suggest that the demographic expansion of *X. f. fuscus* lineages is related to their geographical expansion that followed the forest advance in the Holocene, as this is compatible with the estimated expansion dates. The southern lineage possibly expanded from the Serra do Mar mountain range or from forest refuges along the Paraná river (Ledru

et al., 1998). On the other hand, *X. f. tenuirostris* presented no demographic expansion, possibly because their forest habitat was less affected by Pleistocene climatic alterations (Behling, 1998, 2002).

The hypothesis of refuges, the influence of geography, and river barriers are among the most discussed models in the study of Neotropical diversification. In conclusion, our data did not support the primary influence of geographical barriers (VPSR) or rivers in the divergence between the two main mitochondrial lineages of *X. f. fuscus* of the south-eastern Atlantic forest. Instead, the data supported the influence of isolation by geographical distance, recent vicariance events, and demographic expansions in shaping the phylogeographical structure of *X. fuscus*. These vicariance and expansions events appear to be related to recent natural forest landscape dynamics. There are more than 1000 bird species in the Atlantic forest, many of them distributed along the same geographical range of *X. fuscus* and with comparable ecological requirements. It is plausible that the same forest dynamics that appear to have modelled the phylogeographical structure of *X. fuscus* could have also affected other forest-dependent birds. More studies are needed to understand the importance of those evolutionary and demographic factors in shaping the Atlantic forest biota.

Conservation implications

The present study has revealed a strong phylogeographical structure in *X. f. fuscus* that defined two divergent populations in different geographical areas with dissimilar histories in the southern Atlantic forest: (1) south-eastern Minas Gerais plus southern Rio de Janeiro and (2) São Paulo plus southwards regions. As indicated by AMOVA, genetic differences between the two regions explained approximately 31% of the mitochondrial variation of *X. f. fuscus.* Unfortunately, current taxonomy does not recognize this diversity within *X. f. fuscus*, and even though the taxon is not threatened, this data is important for local conservation. It is possible that other forest birds present a similar condition. Thus, the importance of the characterization of the distribution of the genetic diversity of threatened species is further reinforced by the present data, as divergences not yet detected may be ignored in their conservation plans.

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REFERENCES

Albuquerque JLB. 1996. Padrões de variação geográfica em algumas espécies de aves da floresta atlântica brasileira: o papel de barreiras e eventos vicariantes. Unpublished DPhil Thesis, Universidade Federal do Rio Grande do Sul.

Aleixo A. 2004. Historical diversification of a *terra-firme* forest bird superspecies: a phylogeographic perspective on the role of different hypothesis of amazonian diversification. *Evolution* **58:** 1303–1317.

Baker AJ, Marshall HD. 1997. Mitochondrial control region sequences as tools for understanding evolution. In: Mindell DP, ed. *Avian molecular evolution and systematics*. New York, NY: Academic Press, 51–82.

Bates JM. 2000. Allozyme genetic structure and natural habitat fragmentation: data for five species of Amazonian forest birds. *Condor* **102:** 770–783.

Bates JM. 2002. The genetic effects of forest fragmentation on five species of Amazonian birds. *Journal of Avian Biology* **33:** 276–294.

Behling H. 1998. Late Quaternary vegetational and climatic changes in Brazil. *Review of Palaeobotany and Palynology* **99:** 143–156.

Behling H. 2002. South and southeast Brazilian grasslands during Late Quaternay times: a synthesis. *Palaeogeography, Palaeclimatology, Palaeoecology* **177:** 19–27.

Behling H, Negrelle RRB. 2001. Tropical rain forest and climate dynamics of the atlantic lowland, southern Brazil, during the Late Quaternary. *Quaternary Research* **56:** 383–389.

Brown KS, Ab'Saber AN. 1979. Ice-age forest refuges and evolution in Neotropics: correlation of paleoclimatoligical, geomorphological and pedological data with biological endemism. *Paleoclimas* **5:** 1–30.

Bruford MW, Hanotte O, Brookfield JFY, Burke T. 1992. Single-locus and multilocus DNA fingerprinting. In: Hoelzel AR, ed. *Molecular genetic analysis of populations – a practical approach*. New York, NY: IRL Press, 287–336.

Cheviron ZA, Hackett SJ, Caparella AP. 2005. Complex evolutionary history of a Neotropical lowland fores bird (*Lepidothrix coronata*) and its implications for historical

hypothesis of the origin of Neotropical diversity. *Molecular Phylogenetics and Evolution* **36:** 338–357.

Clapperton C. 1993. *Quaternary geology and geomorphology of South America*. New York, NY: Elsevier, 143–162.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1659.

Costa LP, Leite YL, Fonseca GAB, Fonseca MT. 2000. Biogeography of South American forest mammals: endemism and diversity in the atlantic forest. *Biotropica* **32:** 872–881.

Crandall KA, Templeton RR. 1996. Applications of intraspecific phylogenetics. In: Harvey PH, Leigh AJ, Brown X, Smith JM, Nee S, eds. *New uses for new phylogenies*. New York, NY: Oxford University Press, 81–99.

Excoffier L, Smouse P, Quattro J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human DNA restriction data. *Genetics* **131:** 479–491.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* **39:** 783–791.

Fu YX. 1997. Statistical test of neutrality against population growth, hitchhiking and background selection. *Genetics* **147:** 915–925.

Geise L, Smith MF, Patton JL. 2001. Diversification in the Genus *Akodon* (Rodentia: Sigmodontinae) in southeastern South America: mitochondrial DNA sequence analysis. *Journal of Mammalogy* **82:** 92–101.

Giovanni NM. 2005. Taxonomy of southern populations in the *Scytalopus speluncae* group, with description of a new species and remarks on the systematics and biogeography of the complez (Passeriformes: Rhinocryptidae). *Ararajuba* **13:** 5–26.

Goudet J. 2002. *Fstat*, Version 2.9.3.2 Lausanne: Institute of Ecology, UNIL.

Guindon S, Gascuel. O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.

Guindon S, Lethiec F, Duroux P, Gascuel O. 2005. PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* **33:** W557–W559.

Gusmão Câmara I. 2003. Brief history of conservation in the Atlantic forest. In: Galindo-Leal C, Gusmão Câmara I, eds. *The state of the hotspots: the Atlantic forest*. Washington, DC: Island Press, 31–42.

Haffer J. 1969. Speciation in Amazonian birds. *Science* **165:** 131–131.

Haffer J, Prance GT. 2001. Climatic forcing in Amazonia during the Cenozoic: on the refuge theory of biotic differentiation. *Amazoniana* **16:** 579–607.

Hasegawa M, Kishino H, Yano T-A. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22:** 160–174.

Hewit G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907–913.

Klicka J, Zink RM. 1999. Pleistocene effects on North American songbird evolution. *Proceedings of the Royal Society of London Series B, Biological Sciences* **266:** 695–700.

Knowles LL. 2001. Did the Pleistocene glaciations promote divergence? Test of explicit refugial models in montane grasshopprers. *Molecular Ecology* **10:** 691–701.

Kumar S, Tamura K, Nei M. 2004. MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5:** 150–163.

Kvist L, Ruokonem M, Lumme J, Orell M. 1999a. Different population structure in northern and southern populations of the European blue tit (*Parus caeruleus*). *Journal of Evolutionary Biology* **12:** 798–805.

Kvist L, Ruokonem M, Lumme J, Orell M. 1999b. The colonization history and present-day population structure of the Europeian great tit (*Parus major major*). *Heredity* **82:** 495–502.

Kvist L, Ruokonen M, Thessing A, Lumme J, Orell M. 1998. Mitochondrial control region polymorphism reveal high amount of gene flow in Fennoscandian willow tits (*Parus montanus borealis*). *Hereditas* **128:** 133–143.

Ledru MP, Salgado-Labouriau ML, Lorscheitter ML. 1998. Vegetational dynamics in southern and central Brazil during the last 10 000 yr B. P. *Review of Palaeobotany and Palynology* **99:** 131–142.

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research* **25:** 955–964.

Maddison WP, Maddison DR. 2004. *Mesquite, a modular system for evolutionary analysis*, Version 1.02. Available at http://mesquiteproject.org.

Marantz CA, Aleixo A, Bevier LR, Patten MA. 2003. Family Dendrocolaptidae (Woodcreepes). In: del Hoyo J, Elliot A, Christie D, eds. *Handbook of the birds of the world*, Vol. 8. Broadbills to tapaculos. Barcelona: Lynx Edicions, 358–447.

Merilä J, Björklund M, Baker AJ. 1997. Historical demography and present day population structure of the greenfinch (*Carduelis chloris*): an analysis of mtDNA control region sequences. *Evolution* **51:** 946–956.

Milot E, Gibbs HL, Hobson KA. 2000. Phylogeography and genetic structure of northern populations of the yellow warbler (*Dendroica petechia*). *Molecular Ecology* **9:** 667–681.

Mindell DP, Sorenson MD, Dimcheff DE. 1998. Multiple independent origins of mitochondrial gene order in birds. *Proceedings of the National Academy of Science of the United States of America* **95:** 10693–10697.

Moritz C. 2002. Strategies to protect biological diversity and the evolutionary process that sustain it. *Systematic Biology* **51:** 238–254.

Moritz C, Patton JL, Schneider JC, Smith JB. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annual Review in Ecology and Systematics* **21:** 533–563.

Mustrangi MA, Patton JL. 1997. *Phylogeography and systematics of the slender mouse opossum Marmosops (Marsupialia: Didelphidae). Zoology*, Vol. 130. Berkeley, CA: University of California Press.

Nei M, Kumar S. 2000. *Molecular evolution and phylogentics*. New York, NY: Oxford University Press.

Pellegrino KCM, Rodrigues MT, Waite AN, Morando M, Yassuda YY, Sites JW. 2005. Phylogeography and species

limits in the *Gymnodactylus darwinii* complex (Gekkonidae, Squamata): genetic structure coincides with river system in the Brazilian Atlantic Forest. *Biological Journal of the Linnean Society* **85:** 13–26.

Pereira SL, Baker AJ. 2004. Low number of mitochondrial pseudogenes in the chicken (*Gallus gallus*) nuclear genome: implications for molecular inference of population history and phylogenetics. *BMC Evolutionary Biology* **4:** 1–8.

Petri S, Fulfaro VJ. 1983. *Geologia do Brasil – Fanerozoico*. São Paulo: Editora da Universidade de São Paulo.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Quinn PQ, Keough MJ. 2002. *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press.

Ramos-Onsins S, Rozas J. 2002. Statistical properties of new neutrality test against population growth. *Molecular Biology and Evolution* **19:** 2092–2100.

Riccomini C, Peloggia AUG, Salón JCL, Kohner MW, Figueira RM. 1989. Neotectonic activity in the Serra do Mar rift system (southeastern Brazil). *Journal of South American Earth Science* **2:** 191–197.

Rogers AR, Harpending H. 1992. Population growths makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9:** 552–569.

Rozas J, Sánchez-Del Barrio JC. 2003. DnaSP, DNA polymorphism analysis by the coalescent and other methods. *Bioinformatics* **19:** 2496–2497.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4:** 406–425.

Schneider S, Roessli D, Excoffier L. 2000. *Arlequin vers 2000: a software for population genetics data analysis*. Geneva: University of Geneva, Genetics and Biometrics Laboratory.

Silva JMC, Stotz DF. 1992. Geographic variation in the Sharp-billed Treehunter *Heliobletus contaminatus*. *Bulletin of the British Ornithologists Club* **112:** 98–101.

Silva JMC, Straube FC. 1996. Systematics and biogeography of Scaled Woodcreepers (Aves: Dendrocolpatidae). *Studies in Neotropical Fauna and Environments* **31:** 3–10.

Slatkin M, Maddison WP. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics* **123:** 603–613.

Smouse PE, Long JC, Sokal RR. 1986. Multiple regression and correlation extension of the Mantel test of matrix correspondence. *Systematic Zoology* **35:** 627–632.

Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP. 1999. Primers for a PCR-base approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution* **12:** 105–114.

Swofford D. 2001. *PAUP*: phylogenetic analysis using parsimony (** and *other methods)*, Beta Version 4.0.B10. Sunderland, MA: Sinauer Associates Inc.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in human and chimpanzees. *Molecular Biology and Evolution* **10:** 512–526.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic association with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132:** 619–633.

Templeton AR, Routman E, Phillips C. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mtDNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* **140:** 767–782.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal–windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24:** 4876–4882.

Uimaniemi L, Orell M, Kvist L, Jokimäki J, Lumme J. 2003. Genetic variation of the Siberian tit *Parus cinctus* population at the regional level: a mitochondrial sequence analysis. *Ecogeography* **26:** 98–106.

Vanzolini PE, Williams EE. 1970. South american anoles: the geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria: Iguanidae). *Arquivos de Zoologia* **19:** 1–298.

Veloso HP. 1991. *Classificação da vegetação Brasileira, adaptada a um sistema universal.* Rio de Janeiro: Fundação Instituto Brasileiro de Geografia e Estatística.

Watterson GA. 1975. On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology* **7:** 256–276.

Wilson AC, Cann RL, Carr SM, George M, Gylensten UB, Helm-Bychowski KM, Higuchi RG, Palumbi SR, Prager EM, Stoneking M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* **26:** 375–400.

Zimmer JT. 1947. New birds from Pernambuco, Brazil. *Proceedings of the Biology Society of Washington* **60:** 99–106.