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ORIGINAL ARTICLE

Matrilineal evidence for demographic expansion, low diversity and lack of phylogeographic structure in the Atlantic forest endemic Greenish Schiffornis *Schiffornis virescens* (Aves: Tityridae)

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Abstract Studies of Atlantic forest (AF) organisms suggest that the historical dynamics of the forest cover produced demographically stable populations in its central region and unstable populations in the southern regions. We studied the mitochondrial phylogeographic structure of an AF passerine, the Greenish Schiffornis *Schiffornis virescens* (Tityridae), and evaluated questions related to the history of the AF. We analyzed cytochrome *b* and control region sequences of the mitochondrial genome by traditional phylogenetic and population genetic methods based on summary statistics. In addition, we used coalescent simulations to evaluate specific models of evolution of the populations of *S. virescens*. The results did not support phylogeographic partitions of the genetic variability of *S. virescens*. The overall Φst was = 0.32 and gene flow between regions was moderate to high. The analyses suggested that the total population of *S. virescens* suffered a bottleneck followed by a demographic expansion in the late Pleistocene. The bottleneck might have contributed to the extinction of intraspecific lineages, and hence to the observed lack of a strong phylogeographic pattern and low genetic diversity. Our results suggest that some AF taxa have had all their populations similarly affected by the recent history of the biome, contrary to what has been revealed from most of the other phylogeographic studies in the region and as suggested by a model of AF refuges (the Carnaval–Moritz model). We suggest that the response of organisms to common histories may be idiosyncratic, and predictions about the history of the biome should take into account ecological characteristics and distribution of each specific taxa.

Keywords Atlantic forest · Phylogeography · mtDNA · *Schiffornis* · Historical demography

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Zusammenfassung

Matrilineare Hinweise auf demografische Expansion, geringe genetische Diversität und fehlende phylogeografische Struktur bei der Olivtrauerkotinga *Schiffornis virescens* (Aves: Tityridae), einer endemischen Vogelart der Mata Atlantica

Untersuchungen an Organismen der Mata Atlantica deuten darauf hin, dass die historische Dynamik der Waldbedeckung dort demografisch stabile Populationen in den inneren Bereichen und instabile Populationen in den südlichen Regionen hervorgebracht hat. Wir betrachteten

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die mitochondriale phylogeografische Struktur bei der Olivtrauerkotinga *S. virescens* (Tityridae), einem Sperlingsvogel der Mata Atlantica, und untersuchten Fragen im Zusammenhang mit der Geschichte dieses atlantischen Waldes. Wir analysierten Sequenzen von Cytochrom b und von Kontrollregionen des mitochondrialen Genoms unter Anwendung der gebräuchlichen phylogenetischen und populationsgenetischen Methoden auf der Grundlage statistischer Parameter. Zusätzlich verwendeten wir Coalescent Simulations, um spezifische Evolutionsmodelle für die Populationen von *S. virescens* zu testen. Die Ergebnisse konnten keine phylogeografischen Untereinheiten in der genetischen Variabilität von *S. virescens* belegen. Der Gesamtwert für Φ*st* betrug 0,32 und der Genfluss zwischen den Regionen war mäßig bis hoch. Die Analysen deuten an, dass die gesamte Population von *S. virescens* zunächst durch einen genetischen Flaschenhals ging, worauf anschließend im Oberen Pleistozän eine demografische Expansion erfolgte. Dieser Flaschenhals könnte zum Aussterben intraspezifischer Abstammungslinien und somit zum beobachteten Mangel an deutlichen phylogeografischen Mustern und zu der geringen genetischen Diversität beigetragen haben. Unsere Ergebnisse legen nahe, dass bei manchen Taxa der Mata Atlantica alle Populationen durch die jüngere Geschichte dieses Bioms ähnlich beeinflusst wurden. Dies steht im Gegensatz zu den Erkenntnissen der meisten anderen phylogeografischen Studien aus dieser Region und zu einem Modell atlantischer Waldrefugien (Carnaval–Moritz-Modell). Wir vermuten, dass verschiedene Organismen selbst auf eine gemeinsame Geschichte in ihrer ganz eigenen Weise reagieren. Prognosen über die Geschichte des Bioms sollten daher immer auch die ökologischen Eigenschaften und die Verbreitung jedes einzelnen Taxons berücksichtigen.

Introduction

The Atlantic forest (AF) of Brazil, northeastern Argentina and eastern Paraguay is amongst the richest and most endangered rainforests in the world (Galindo Leal and de Gusmão Câmara 2003) (Fig. 1). Congruence among palynological studies (i.e. Behling 2002; Behling and Negrelle 2001; Ledru et al. 2005), and models of paleo-distribution of forests and of some species of frogs (Carnaval and Moritz 2008; Carnaval et al. 2009), suggest that during the late Pleistocene the southern AF was strongly fragmented by the advance of grasslands and of other savanna-like vegetation. In contrast, the central and northern AF were relatively stable. More precisely, during the last glacial maximum (between 24,000 to 18,000 before present; Anderson et al. 2007), the southern grassland–forest ecotone seemed to have shifted 750 km north from its current location, which was possibly established in the late Holocene (Behling 2002). Under the former scenario of temporal and geographic stability–instability of the AF (Carnaval and Moritz (2008), hereafter the Carnaval–Moritz model, the majority of the forest-dependent taxa should have been similarly affected by the retraction and fragmentation of forests during the Pleistocene, and therefore the existence of shared phylogeographic patterns is expected.

The Carnaval–Moritz model has received particular support from some phylogeographic studies with forest birds (i.e. Maldonado-Coelho 2012; Cabanne et al. 2008; d'Horta et al. 2011), frogs (Carnaval et al. 2009) and bees (Batalha-Filho et al. 2010). But, on the other hand, the model has been recently challenged by a phylogeographic study of the *Rhinella crucifer* group of toads (Thomé et al. 2010) and by a study of planarians (Álvarez-Presas et al. 2011), which did not find support for a southern AF Holocene colonization and therefore suggest persistence of forested habitats in the south (a southern refuge). In addition, the existence of a southern refuge is further supported by a recent study with species distribution models of several endemic taxa (Porto et al. 2012).

In the present study, we used sequence data derived from mitochondrial DNA (mtDNA) to explore the population genetic structure of the Greenish Schiffornis *Schiffornis virescens* (Tityridae; Remsen et al. 2009) (Fig. 1a) and to address questions of the AF history. *S. virescens* is a good model to address issues related to the AF history because it is endemic to the biome and occurs with low to medium density in well-preserved forests (Stotz et al. 1996). The species does not present geographic variation in plumage, song or morphometry (Ridgely and Tudor 1996). Since it inhabits the understory of preserved forests and is sensitive to forest fragmentation (Anjos et al. 2011; Stotz et al. 1996), chances to have been affected by Pleistocene fragmentation of habitat, and therefore to present a strong population genetic structure, are high (Burney and Brumfield 2009). Assuming that most AF organisms were affected similarly by the Pleistocene forest dynamics, we expected to find in *S. virescens* results similar to those obtained by other studies of AF organisms, which apparently revealed a shared phylogeographic pattern (Maldonado-Coelho 2012; Batalha-Filho et al. 2010; Grazziotin et al. 2006; Cabanne et al. 2007, 2008; Mustrangi and Patton 1997; Carnaval et al. 2009; d'Horta et al. 2011; Pessoa 2007). This shared pattern for each species consists of the existence of two main mtDNA clades south of the Doce river that come into contact in south-central São Paulo where no barrier to gene flow is evident (Fig. 1a, b). In addition, some species present a third lineage north of the Doce river (Cabanne et al. 2008; d'Horta et al. 2011; Maldonado-Coelho 2012). Another characteristic of the shared phylogeographic pattern is that the corresponding southern clades present

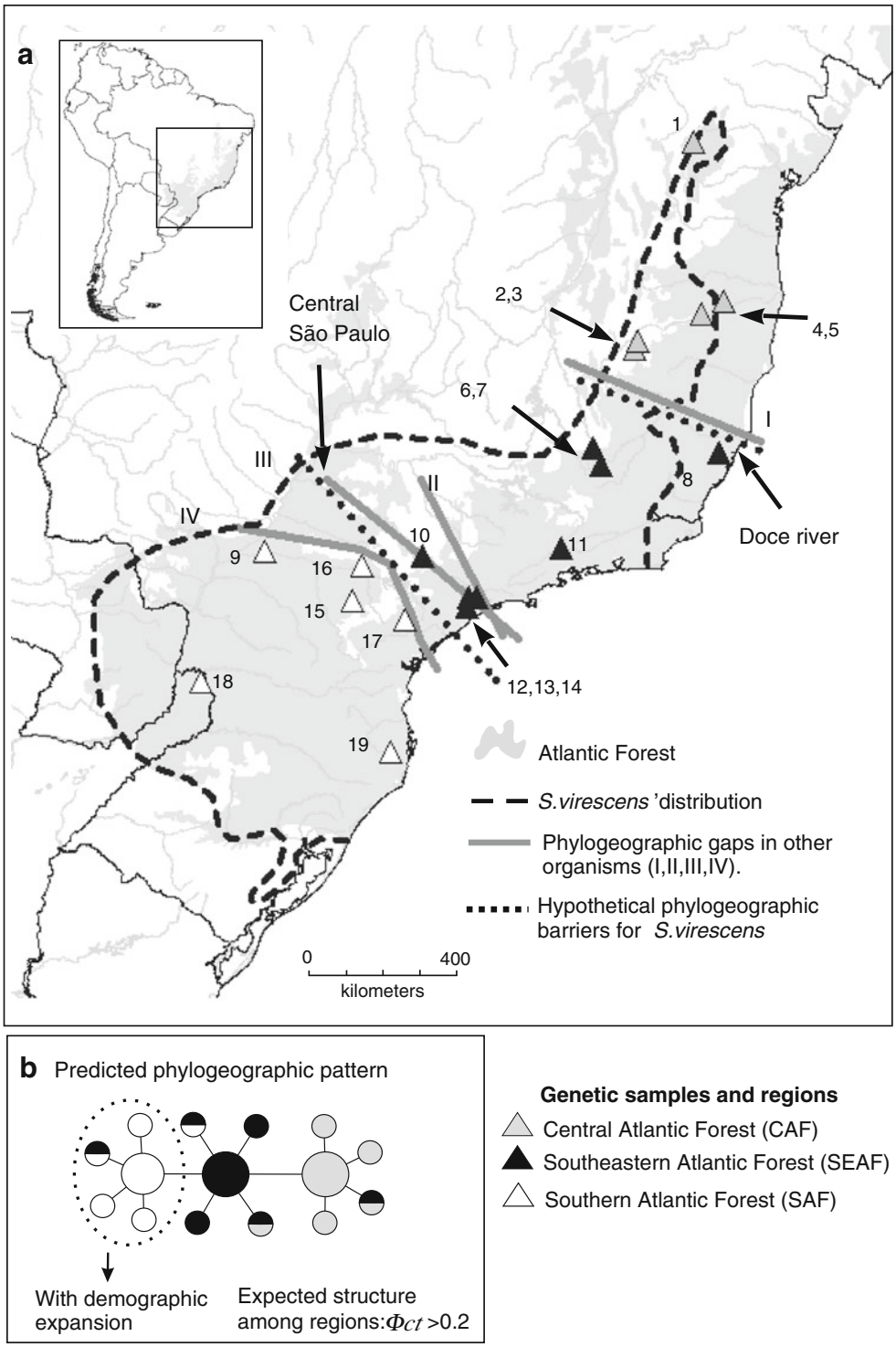

Fig. 1 **a** Distribution of the Atlantic forest (AF), of *Schiffornis virescens* and of the sampling localities for the genetic study. See "Appendix 1" for details of sampling localities. Phylogeographic barriers observed in other AF organisms are: barrier *I*, the passerine *Sclerurus scansor* (d'Horta et al. 2011), the passerine *Xiphorhynchus fuscus* (Cabanne et al. 2008), the passerine *Pyriglena leucoptera* (Maldonado-Coelho 2012), frogs of the genus *Hypsiboas* (Carnaval et al. 2009), and the toad *Rhinella crucifer* (Thomé et al. 2010); barrier *II*, opossum *Marmosops incanus* (Mustrangi and Patton 1997), the passerines *Sclerurus scansor* (d′Horta 2009) and *Conopophaga lineata* (Pessoa et al. 2006; Pessoa 2007); barrier *III*, the passerines *Xiphorhynchus fuscus* (Cabanne et al. 2007, 2008) and *Sclerurus scansor* (d'Horta et al. 2011), frogs of the genus *Hypsiboas* (Carnaval et al. 2009), and monkeys of genus *Alouatta* (Martins et al. 2011); barrier *IV*, the passerine *Dendrocolaptes platyrostris* (Cabanne et al. 2011), the bee *Melipona quadrifasciata* (Batalha-Filho et al. 2010), the viper *Bothrops jararaca* (Grazziotin et al. 2006), the frogs *Hypsiboas faber* (Carnaval et al. 2009), *Proceratophrys boiei* (Amaro et al. 2012) and another frog of the genus *Thoropa* (Fitzpatrick et al. 2009). **b** Predicted phylogeographic pattern in *S. virescens* based on the genetic constitution of other AF species (see **a**). The predicted genetic structure among regions ($\Phi ct > 0.2$) is related to a level of gene flow that is not sufficient to avoid allele fixation and strong divergence (Wang 2004)

evidence of population expansion, differently to clades north of the Doce river. These shared phylogeographic features could be the product of alterations in the forest cover that occurred during the late Pleistocene, as would be expected from the Carnaval–Moritz model.

Phylogeographic studies of AF organisms are still scant and a robust synthetic hypothesis about the evolution of AF organisms is not yet possible (but see Carnaval et al. 2009; Martins et al. 2011). Up to now, the selection of AF species for phylogeographic studies was not only driven by biogeographic questions but also by systematic issues in polymorphic taxa (i.e. Cabanne et al. 2008). This aspect may have biased the possibility of finding complex phylogeographic patterns because variation in external

characters (i.e. plumage and voice) is frequently correlated with genetic structure. *S. virescens* has no described geographic variation in external characters, therefore an alternative working hypothesis might be to find no phylogeographic structure.

Nyari (2007) showed that *S. turdina*, which is the sister species of *S. virescens,* presents a substantial intra-specific genetic structure. Because both species are very similar in song and plumage, the evolutionary relationship between them is not yet clear. The study of Nyari (2007) included samples of *S. virescens*, but without sampling all the variation. Thus, before addressing our biogeographic questions, we evaluated whether the species is monophyletic.

The objective of this study was to describe the mtDNA genetic variation of *S. virescens* to address the following questions: (1) Is the species monophyletic? (2) Does it present the phylogeographic structure described for other AF organisms? (3) How did the species' phylogeographic pattern originate? and (4) What are the implications of results for understanding the evolution of AF organisms?

Methods

Genetic datasets

In the phylogenetic analysis, we included 28 *S. virescens*, 14 *S. turdina* representing the variation of that species (Nyari 2007), as well as 1 *S. major* and 2 *Laniocera hypopyrra* as the outgroup. We used an alignment consisting of 840 bp of the cytochrome *b* (cyt*b*) and 476 bp of the first domain of the mtDNA control region (CR). Sequences of cyt*b* from *S. turdina*, two *S. virescens* and the two outgroups were obtained from the study of Nyari (2007) (see GenBank accession numbers in Fig. 2). We were unable to collect CR sequences for some *S. turdina* and for the outgroups, so we coded these as missing data. Amplification and sequencing of cyt*b* was done following Cabanne et al. (2008). See "Appendix 1" for details of the samples used in this study.

For the population genetic study, we used CR sequences (476 pb) from 57 *S. virescens* collected in 19 localities (Fig. 1a). Amplification and sequencing of the CR was performed by specifically designed primers: forward LSvCB GATCTATCCCCAATAAACTTGGAGG and reverse HSvCR GATTACGAACTCGAAGTTGGACGG. PCR annealing temperature for CR was 60 °C for *S. virescens* and 53 °C for *S. turdina*. In order to avoid working with possible copies of the CR translocated to the nucleus, we only used sequences obtained from clean amplification bands, presenting the expected length, single peaks, and a base composition compatible with mtDNA (Baker and Marshall 1997).

Since both cyt*b* and CR are physically linked in a single molecule (mitochondrial genome), results obtained with both markers are totally compatible because they reflect a single genealogy and history (Avise 2000). Genbank accession numbers for cyt*b* and for CR are JX477687–JX477770. For evaluating whether the used sequences were representative of the genetic constitution of *S. virescens*, 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.

Phylogenetic analyses and molecular clock

Molecular evolution parameters were selected in MODELTEST 3.7 (Posada and Crandall 1998) or directly estimated during phylogenetic reconstruction. To obtain phylogenetic trees, we used bayesian and maximum likelihood approaches as implemented in MrBAYES 3.1.2 (Ronquist and Huelsenbeck 2003) and PHYML 2.4.4 (Guindon and Gascuel 2003), respectively. MrBAYES analysis used a MCMC of 5×10^6 generations with sampling every 1,000 generations. We discarded the first 1,750 genealogies (burn-in), and parameters of molecular evolution between genes (cyt*b* and CR) were unlinked. CR sequences of some taxa were coded as missing data. As missing data might potentially mislead phylogenetic inference (Lemmon et al. 2009), we analyzed the dataset with and without CR sites. As topologies did not change, the final phylogenetic analyses used the dataset with missing data (cyt*b*+CR).

We used BEAST 1.6.1 (Drummond and Rambaut 2007) to estimate node ages by using a relaxed uncorrelated lognormal molecular clock analysis based on the cyt*b* partition, a molecular clock calibration of 2.1 % divergence per million years (Weir and Schluter 2008), and a tree prior of constant size. From this analysis in BEAST, we estimated a substitution rate for the CR in relation to the cyt*b* rate. Node ages obtained with the cyt*b*-CR dataset (with missing data) and with only the cyt*b* (no missing data) were similar, and therefore we based our dating estimation in the cyt*b*-CR dataset.

To study the constancy of the molecular clock we used two procedures. First, we evaluated the parameter *ucld.stdev* (standard deviation of the molecular rate of cyt*b*), estimated in BEAST. If the parameter estimate is close to zero then the data is clock-like. If the *ucld.stdev* has an estimated value much greater than one, then the data exhibits substantial rate heterogeneity among lineages (Drummond and Rambaut 2007). Secondly, we used a likelihood-ratio test, as implemented in MODELTEST, by

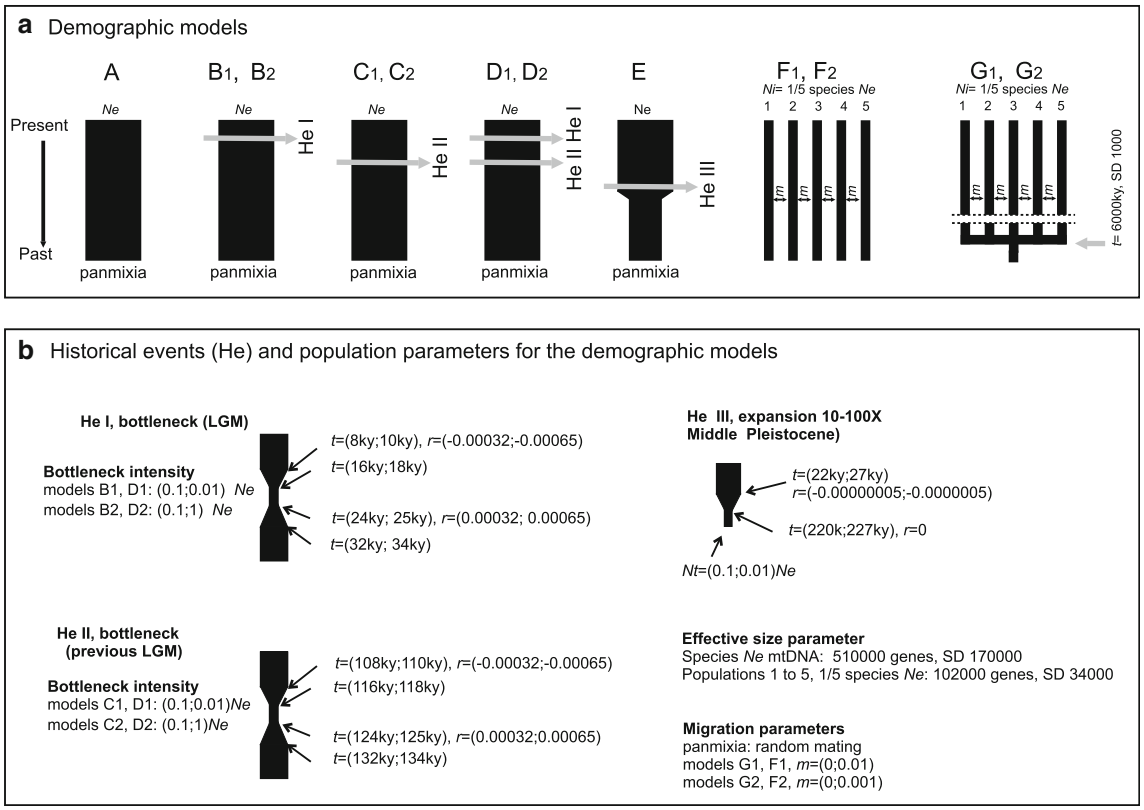

Fig. 2 **a** Demographic models of *Schiffornis virescens* evaluated by coalescent simulations in BAYESSC. We simulated populations (shown in *black*) that suffered different historical events (He): stability (*A*), bottlenecks (*B–D*), expansion (*E*), and the evolution in an island system (*F*, *G*). **b** Details of historical events and population parameters used in simulations. The prior distribution of time of events (t), migration (m) and of the demographic growth rate (r) are specified. Continuous priors are denoted between parentheses. Normally distributed priors are denoted by their mean and standard distribution (SD). Intensities of bottlenecks are indicated by the proportion of the current effective size (*Ne*) that persisted during the bottleneck. Time is expressed in kilo-years (ky). *LGM* last glacial maximum

comparing the log-likelihood values of maximum likelihood trees with and without enforcing a molecular clock.

Lastly, visualization of relationships among CR haplotypes was done by a median-joining network constructed in NETWORK 4.1.0.8 (http://www.fluxus-engineering.com).

Population genetic analysis

All the population genetic analyses used only the CR dataset because this marker was more variable than the cyt*b*. To test the existence of phylogeographic barriers (gene flow barriers between populations), we used analyses of molecular variance (AMOVA) in ARLEQUIN 3.1 (Excoffier et al. 2006) based on uncorrected distances. Specifically, we evaluated the Φ*ct* statistic, which represents the proportion of the total molecular diversity variance that is explained by genetic differences among geographic regions. These regions were delimited by the hypothetical phylogeographic barriers (Fig. 1a). $\Phi_{ct} = Va/Vt$, where *Va* is the molecular variance among groups and *Vt* is the total molecular variance (Excoffier et al. 1992). We also evaluated $\Phi_{st} = (Va + Vb)/Vt$, where *Vb* is the molecular variance among populations within groups (Excoffier et al. 1992). Significance and confidence intervals of fixation indices were calculated based on 20,000 permutations.

We estimated the parameter Θ by a maximum likelihood coalescent method as implemented in LAMARC 2.1.2b (Kuhner 2006). We also used LAMARC to evaluate the demographic signature by estimating the exponential population growth rate *g* of the model $\Theta_t = \Theta_{\text{now}} e^{-gt}$, where Θ_{now} is the current Θ and Θ_t is the value of the parameter *t* time ago. Positive *g* values indicate population growth and negative values indicate population decline. Runs used the F84 model of sequence evolution with empirical base frequencies and transition/transversion ratios, and default characteristics of the Markov Chain Montecarlo.

We also evaluated the historical demography using the neutrality tests of Tajima (1989) and of Fu (1997) in DNAsp 5.1 (Librado and Rozas 2009). If the marker is selectively neutral (not positively selected), significant negative values of the test statistics suggest demographic expansion. In addition, to evaluate the hypothesis of

positive selection, we used the compound test HEW and DHEW (Zeng et al. 2007), performed with *S. turdina* as outroup in the NH software (kindly provided by K. Zeng). The tests HEW and DHEW evaluate the null hypothesis of neutrality and are significant only when positive selection has acted in the target sequence or linked regions (hitchhiking) (Zeng et al. 2007). The skyride analysis (Minin et al. 2008) for studying the change of population effective size through time was performed in BEAST 1.6.1 with 10 million generations, sampling every 1,000 generations and a burn-in of 1,000 genealogies. We used an uncorrelated log-normal clock model, time aware smoothing and a generation time of 1 year (Klicka and Zink 1999).

Estimation of gene flow assuming an island model in equilibrium was obtained using the formula $M = (1 - \Phi st)/2\Phi st$ (Hedrick 2000). Also, we used the isolation–migration (IM) model (Nielsen and Wakeley 2001; Hey and Nielsen 2004), implemented in IM (v. April 21, 2008), to estimate non-equilibrium gene flow rates and divergence times between regions. We applied the HKY model of evolution, similar population parameter theta values ($\Theta_1 = \Theta_2 = \Theta_a$) and $m_1 = m_2$. A total of 500,000 iterations were used for burn-in and analyses were stopped when the smallest effective sample size (ESS) was higher than 50 and parameter trend lines stabilized, usually after 20 million iterations. We assumed a generation time of 1 year.

Coalescent simulations

In order to evaluate different demographic histories, we simulated demographic scenarios in the program BAYESSC, a modification of software SERIAL SIMCOAL (Anderson et al. 2005; Chan et al. 2006), and evaluated the goodness of fit of the observed data (CR sequences) to the simulations; see Richards et al. (2007) and Knowles (2009) for reviews on this approach. Because genetic signals of expansion may be found in a demographically stable island system (Nielsen and Beaumont 2009; Peter et al. 2010), the main objective of this section was to investigate if the phylogeographic pattern could have been generated in an island system with demographic stability or in a non-structured system that expanded demographically. The demographic models assessed, detailed in Fig. 2, differed in the timing, intensity, and numbers of bottlenecks, and also in the number of populations. The populations in model G were originated before the Pleistocene, as suggested by the divergence of *S. virescens* with its sister species (see "Results"). In order to obtain a metric of population genetic structure (Φst) for the models of single populations and to simulate the island models, we organized the sampling localities by geographic proximity into five populations (pop). This procedure is a conventional practice in this kind of analysis (i.e. Fabre et al. 2009; Belle et al. 2006). In preliminary analyses, we tested several other population configurations and results did not change. The final organization of sampling localities was: pop 1: localities 1–5; pop 2: localities 6–8, 11; pop 3: localities 12–14; pop 4: localities 9, 10, 15–17; pop 5: localities 18 and 19. See localities in "Appendix 1".

For each model, we performed 1,000 simulations and used ARLEQUIN to estimate five summary statistics for the complete dataset: number of segregating sites, nucleotide diversity, Tajima's *D* (Tajima, 1989), Fu's *Fs* (Fu 1997), and Φst amongst populations (Excoffier et al. 1992). To evaluate the plausibility of each model, we first estimated the proportion *pi* of simulated values equal and higher than the observed summary statistics, and then we reported a global *p* value for each model by combining individual *pi* values by a nonparametric procedure described in Voight et al. (2005) and Fabre et al. (2009). See electronic supplementary material for further methodological details.

Results

Genetic data, neutrality and mtDNA diversity

The cyt*b* and CR concatenated alignment and the CR alignment presented probabilities to represent the complete gene genealogy equal to 93 and 96.5 %, respectively, which indicated that the samples are suitable to the study. The phylogenetic analysis showed that *S. virescens* is a well-supported monophyletic lineage (Fig. 3a) that diverged from its sister species, *S. turdina,* approximately 6.2 Mya (million years ago) (HPD95 % 4.3–8.14 Mya). Contrary to what we hypothesized, no intraspecific matrilines genetic structure was present in *S. virescens*. The CR dataset presented 17 haplotypes and the network confirmed the lack of phylogeographic structure (Fig. 3b). The parameters *cytb.ucld.stdev* = 0.293 (HPD95 % 0.0000015–0.76) and *CR.ucld.stdev* = 0.3757 (HPD95 % 0.0000119–1.05) estimated in BEAST indicated a slight deviation from the constant molecular clock in cyt*b* and CR sequences, respectively. This result was further confirmed by the likelihood ratio test for the dataset *S. virescens*/*S. turdina* and for the datasets of each species ($p < 0.00001$). The divergence rate of CR estimated in BEAST was 2.34 % divergence per million years, slightly higher than the cyt*b* rate (2.1 % divergence per million years). We used this rate in the following analyses with CR.

We used cyt*b* to compare the genetic diversity of *S. virescens* with other AF birds. We reviewed the available studies on AF birds and found that the nucleotide diversity of a sample of 12 species (including *S. virescens*) ranged from 0.01574 to 0.00187 (average = 0.0086, see

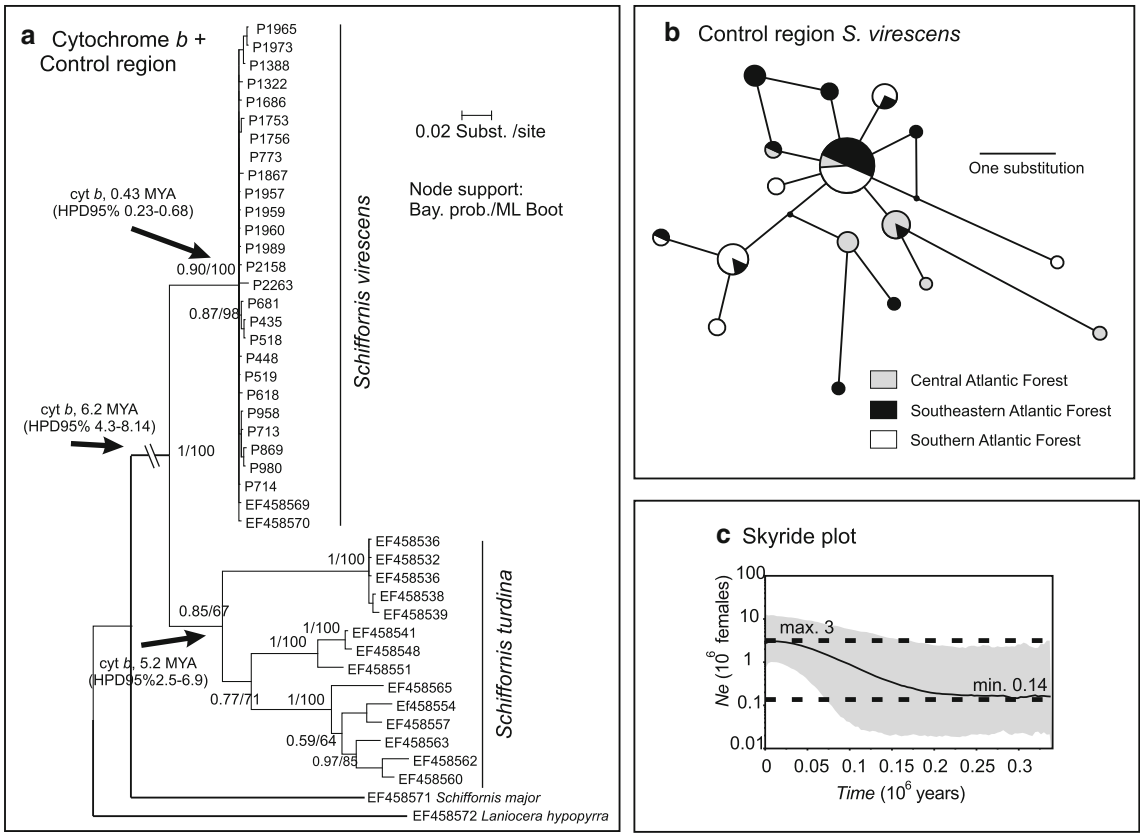

Fig. 3 **a** Bayesian phylogenetic tree based on 1,533 bp of the cyt*b* and control region of the mtDNA of *Shiffornis virescens* and closely related species. Each gene partition used the model of molecular evolution GTR+I+G with parametes specifically estimated. Posterior probability obtained in MrBAYES (*Bay. prob*) and maximum likelihood bootstrap (*ML Boot*, 500 replicates) are presented at nodes. Divergence dates were obtained in BEAST based in the cyt*b* molecular clock (Weir and Schluter 2008). **b** Median joining network based on 476 bp of the mtDNA control region of *S. virescens*. **c** Skyride plot showing the variation in population effective (*Ne*) size through time

"Appendix 2" for details). *S. virescens* presented the smallest diversity (cyt*b* nucleotide diversity = 0.00187) among the 12 species considered.

The AMOVA results did not support any of the hypothesized genetic barriers (Table 1). All Φ*ct* values were small and not significant, which indicates that most of the variation in the mtDNA of *S. virescens* was organized among and within populations, and not among regions separated by the tested barriers. In addition, the global Φ*st* value (Φ*st* = 0.3184) indicated a moderate population genetic structure among localities, which corresponded to moderate equilibrium gene flow of ~1.1 females per generation among populations. The analysis in IM indicated moderate to high gene flow between regions separated by the putative phylogeographic barriers (Fig. 1a): $M_{CAF–SAF}$: 0.8 females (HPD95 % 0–6.5), $M_{CAF–SEAF}$: 5.5 females (HPD95 % 1.6–42.7); and $M_{SAF–SEAF}$: 10.4 females (HPD95 % 1.7–?). All divergence times correspond to the late Pleistocene: $T_{CAF–SAF}$: 0.16 MYA (HPD95 % 0.04–0.7), $T_{CAF–SEAF}$: 0.1 MYA (HPD95 % 0.0014–0.5); and $T_{SAF–SEAF}$: 0.06 MYA (>0.012). However, as all posterior distributions of divergence estimations are very wide (results not shown), populations cannot be considered as independent, which confirms the absence of population structure in *S. virescens*.

The summary statistics tests collectively suggested a demographic expansion in *S. virescens* (Table 2). The neutrality–demographic tests suggested a demographic expansion because Fu's tests were significant. Results of the test of Tajima were borderline but compatible with the Fu's tests because the *D* statistics were negative. Results of the positive selection tests did not support the action of positive selection; however, some *p* values are borderline significant. The demographic analysis in LAMARC further corroborated that *S. virescens* expanded recently (positive $g = 932$, HPD95 % CI: 367–1966). The Skyride plot analysis showed the existence of a moderate expansion during the late Pleistocene, near 21 times the initial *Ne* (Fig. 3c). The region of origin of the demographic expansion should present the highest level of genetic diversity. However, because the three regions into which we divided the study area (Fig. 1a; CAF, SEAF and SAF) presented

Table 1 Analyses of molecular variance of *Schiffornis virescens* based on the mtDNA control region and the testing of phylogeographic barriers

Tested barrier[a]	Population structure[b]	Φ*st* (95 % c.i.)	Φ*st p* value	Φ*ct* (95 % c.i.)	Φ*ct p* value
No barrier	[(2–3), (4–5), (6–7), 10, 11, (12–13), 14, 9, (15–15), 17, 18, 19]	0.3185 (0.0710–0.4717)	<0.00001	–	–
CSP	[(2–3), (4–5), (6–7), 10, 11, (12–13), 14] [9, (15–15), 17, 18, 19]	0.3348 (0.0911–0.4825)	<0.00001	0.0528 (0.0148–0.0896)	0.0733
DR	[(2–3), (4–5)] [(6–7), 10, 11, (12–13), 14, 9, (15–15), 17, 18, 19]	0.3367 (0.0513–0.5044)	<0.00001	0.0405 (0.1253–0.2871)	0.2308
CSP and DR	[(2–3), (4–5)] [(6–7), 10, 11, (12–13), 14] [9, (15–15), 17, 18, 19]	0.3227 (0.0766–0.4720)	<0.00001	0.0220 (0.0627 –0.1598)	0.1899

[a] See Fig. 1a for localities and tested phylogeographic barriers (*CSP*, Central-São Paulo, and *DR* Doce river

[b] Numbers represent localities from Table 1. Specific structures indicated by samples within square brackets. Localities within parenthesis were pooled to form a new sample. Localities 1 and 8 were excluded due to small sample sizes

Table 2 Diversity indices and test of significance of neutrality–demography in *Shiffornis virescens*

Alignment[a]	*n*	Number of segregating sites	Nucleotide diversity	Neutrality–demographic test *p* values		Positive selection test *p* values	
				Tajima's test (statistic)	Fu's test (statistic)	HEW	DHEW
cyt*b* and CR (1533 pb)	28	13	0.00272 (SE 0.00059)	0.04 ($D = -1.578$)	0.001** ($Fs = -8.484$)	0.0706	0.0425
CR (476pb)	57	13	0.0041 (SE 0.0013)	0.038 ($D = -1.47$)	0.002** ($Fs = -7.5$)	0.027	0.0178

[a] *cytb* cytochrome *b, CR* control region

** Significant test at 1 % level after Bonferroni correction for four tests

similar levels of genetic diversity (results not shown), the origin of the expansion was not detected.

Simulations results

Results of the evaluation of demographic models by coalescent simulations are presented in Table 3. The model A of panmixia and demographic stability, the model E of panmixia and expansion, and the models of islands and demographic stability F and G obtained low global probabilities, indicating they all have low plausibility. All the models with high global probability (*p* value >0.1: models B, C, and D) suggest that *S. virescens* suffered bottlenecks during the glacial maxima followed by posterior recovering of the population effective size. In particular, the model with highest *p* value (model C1, $p = 0.84$) suggests that *S. virescens* is conformed by a single population that suffered an intense bottleneck before the last glacial maxima.

The results of these simulations are compatible with the analysis performed in LAMARC, as well as with the demographic summary statistics and the skyride plot, collectively suggesting that the species suffered an expansion during the late Pleistocene. A genetic pattern compatible with demographic expansion could also result from an island system with demographic stability, but our simulations did not support this situation (models F and G).

Discussion

Schiffornis virescens was found to be monophyletic, and presented low mtDNA genetic diversity and a weak population genetic structure. In addition, the genetic partitions across genetic barriers important for other organisms were not found to be influential for *S. virescens*. These results are in contrast to what was found in its sister species, *S. turdina*, and in other AF taxa dependent on preserved forests. *S. turdina* has high genetic diversity and substantial phylogeographic structure (Nyari 2007). Within the lineage *S. virescens*/*S. turdina*, the most ancient split separated birds from the AF (*S. virescens*) from birds associated primarily to the Amazon and Central America forests (*S. turdina*). Interestingly, and as observed in some other taxa (i.e. Cabanne et al. 2008; Ribas et al. 2005), the AF lineage showed shorter branches and lower genetic distances among terminals (Fig. 3a), a pattern that suggests different evolutionary dynamics in both *Schiffornis* species (Ricklefs 2007). Also, contrary to what was expected according to other Neotropical rainforest organisms from

Table 3 Results of evaluation of demographic scenarios of *Schiffornis virescens* using coalescence simulations using BAYESSC

Model description	Global *p* value	Model description	Global *p* value
A-Panmixia, stability	0.0620	D2-Panmixia, two bottlenecks, *Nt*/*No* = 10–100 %	0.0542
B1-Panmixia, bottleneck LGM, *Nt*/*No* = 1–10 %	0.3270	E-Expansion, 10–100X	0.0640
B2-Panmixia, bottleneck LGM, *Nt*/*No* = 10–100 %	0.0482	F1-Stable islands, *m*:0–0.01	0.0242
C1-Panmixia, bottleneck pre LGM, *Nt*/*No* = 1–10 %	0.8440	F2-Stable islands, *m*:0–0.001	0.0780
C2-Panmixia, bottleneck pre LGM, *Nt*/*No* = 10–100 %	0.1530	G1-Stable islands, *m*:0–0.01, origin 6 MYr ago	0.0980
D1-Panmixia, two bottlenecks, *Nt*/*No* = 1–10 %	0.3430	G2-Stable islands, *m*:0–0.001, origin 6 MYr ago	0.0540

LGM last glacial maximum; *Nt* and *No* effective population size at the end and at the beginning of the bottleneck, respectively; *m* migration rate parameter

the AF or from outside the AF (i.e., Cabanne et al. 2011; d'Horta et al. 2011; Carnaval et al. 2009; Bates 2002; Aleixo 2004; Cheviron et al. 2005; Nyari 2007), *S. virescens* did not present a strong phylogeographic structure.

The differences in matrilineal population structures among birds could not be explained by ecological divergence between *S. virescens* and the other AF birds. Ecology predicts levels of intraspecific genetic divergence in birds from Amazonia and likely from other regions (Burney and Brumfield 2009). For example, canopy birds tend to be less genetically differentiated than understory birds. In addition, very specialized bird taxa are more diversified than generalist birds (Salisbury et al. 2012). Even though comparative ecological studies of AF birds are scarce (i.e., Anjos 2006; Ribon et al. 2003), there are not remarkable ecological differences between *S. virescens* and the AF birds that present strong phylogeographic structure (Cabanne et al. 2008, 2011; d'Horta et al. 2011; Maldonado-Coelho 2012; Pessoa et al. 2006; Lacerda et al. 2007). *S. virescens,* as well as most of the other studied birds, inhabits the understory and is a specialist of well-preserved forests. A pattern of low genetic structure, such as the one found in *S. virescens*, is expected for species with high gene flow and low sensitivity to forest fragmentation. An example of such a species is *Basileuterus leucoblepharus* (Anjos 2006), which is a bird co-distributed with *S. virescens* whose mtDNA and nuclear DNA are not geographically structured and that presented demographic stability during the late Pleistocene (Batalha-Filho et al. 2012).

The mtDNA genealogical pattern of low diversity and no genetic structure of *S. virescens* could have been generated by a selective sweep (positive selection) (Nielsen 2005). However, we utilized specific tests for positive selection, HEW and DHEW, and the results did not support the hypothesis of selection (Table 2). Therefore, the genetic constitution of *S. virescens* could have been modeled by factors other than positive selection, such as demographic processes.

S. virescens is a relatively young species, as shown by its time to the most recent common ancestor (*TMRCA*) (0.43 Mya; Fig. 3a), and thus not enough time may have passed for the evolution of intraspecific structure. However, *S. virescens* is not younger than other AF species that do present a strong phylogeographic structure, such as *Sclerurus scansor* and *Dendrocolaptes platyrostris* (d'Horta et al. 2011; Cabanne et al. 2011), therefore species age alone cannot account for the lack of phylogeographic structure.

The difference between the splitting time between *S. virescens* and *S. turdina* (6.2 Mya) and the *TMRCA* of *S. virescens* (0.43 Mya) seems to be high when compared to other pairs of sister taxa of the region. For example, in other AF species such as *S. scansor* (d'Horta et al. 2011) and *Xiphorhynchus fuscus* (Cabanne et al. 2008), the divergence with their sister clades are ~1.1 and ~3 Mya ago, respectively, while the maximum intraspecific divergence are about 0.6 and 0.8 Mya, respectively. Also, in *D. platyrostris,* another passerine from the AF and the gallery forests of central South America savannas, the divergence with its sister taxa is about 1.6 Mya while the intraspecific divergence is 0.22 Mya (Cabanne et al. 2011). We expected a higher intraspecific differentiation in *S. virescens*, and thus an older *TMRCA*, because of the old splitting time with *S. turdina* and because of the high intraspecific diversity documented within the latter species. The matrilineal constitution of *S. virescens* suggests an evolutionary history that resulted in low intraspecific diversification. Perhaps, lower diversification rates or higher extinctions rates in *S. virescens* could be responsible for this pattern (Ricklefs 2007).

The phylogeographic pattern of *S. virescens* could have been originated through the combined effects of gene flow and historical demography. Our results indicated that gene flow rates among populations are moderate to high, enough to avoid allele fixation and strong divergence (Wang 2004). Also, different analyses collectively revealed a recent population expansion. Finally, *S. virescens* presented lower levels of mtDNA genetic diversity than other AF birds ("Appendix 2"), suggesting that the demographic expansion may have occurred after a population bottleneck that

reduced genetic diversity. This bottleneck might have contributed to the extinction of intraspecific mtDNA lineages, leading to the observed lack of a strong phylogeographic pattern and low genetic diversity in *S. virescens.*

Different effective population sizes, demography, or the lack of recognition of different species in *S. turdina* might be responsible for the differences in mtDNA genetic diversity and structure between *S. virescens* and *S. turdina*. *S. turdina* inhabits all the Amazonian basin forests, part of Central America forests, and part of northeastern AF. Thus, its geographic range is nearly five times larger than that of *S. virescens*, which may imply a larger population and therefore higher genetic diversity for *S. turdina*. In addition, *S. turdina* has several highly divergent phylogroups that, with further study, might be considered as good species (Nyari 2007).

The phylogeographic pattern of a species can be shaped by the history of the biome in which it is found (Hewitt 2000, 2004). Our results, specifically the lack of a strong phylogeographic structure, the low genetic diversity, a most recent common ancestor in the mid-Pleistocene, and the evidence of demographic expansion for the whole species, suggest that the *Ne* of *S. virescens* was reduced greatly during the late Pleistocene, likely to a population restricted to a single continuous forest (refuge) of uncertain location. Interestingly, the magnitude of the demographic expansion of *S. virescens* ($\sim 21 \times$ initial *Ne*; Fig. 3c) is similar to that described for some taxa from the northern hemisphere that were highly affected by the cycles of forest shrinkage—expansion that occurred during global glaciations (e.g., *Catharus ustulatus*; Ruegg et al. 2006).

Most populations of *S. virescens* inhabit a region whose rainforest coverage fluctuated during the Pleistocene, which could be the reason why they presented a shared demographic history. Specifically, the majority of the range of the bird is located in the southern portion of the AF, which was the most affected by natural fragmentation and expansion of savanna-like vegetation during the global glaciations (Carnaval et al. 2009; Behling 2002; Behling et al. 2002). This is different from those taxa that persisted in the same period in more than one population, and whose geographic ranges also span historically stable forests in central and northern AF (e.g., d'Horta et al. 2011; Cabanne et al. 2008, 2011; Thomé et al. 2010).

Our results were not fully compatible with the predictions derived from the Carnaval–Moritz model (Fig. 1b), but they were still in accordance with some aspects of the hypotheses of the AF history. Most published studies on AF organisms suggest that the Pleistocene dynamics produced stable populations in the central region and unstable populations in the southern portion. We did not recover this pattern in *S. virescens*, but rather documented that its association to the southern AF and relatively short age was crucial for this resulting pattern. The Carnaval–Moritz model is not powerful enough to predict the distribution of Pleistocene refugia of organisms restricted to the southeast–south portion of the AF (Porto et al. 2012), and our results suggested that its power to predict phylogeographic patterns of those organisms is also low. Independent of the origin of the phylogeographic structure within *S. virescens*, our results indicated that some AF taxa have had all their extant populations similarly affected by the recent history of the biome, contrary to what has been revealed from most of the other phylogeographic studies of species from the region. Therefore, the response of organisms to common histories may be idiosyncratic, and predictions about the history of the biome should take into account ecological characteristics and distribution of each specific taxa.

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Appendix 1

See Table 4.

Table 4 *Schiffornis virescens* and *S. turdina* sampling localities and sample information used in the present study

Taxa	Locality	Sample size	Tissue ID[TISSUE]
S. virescens	1-Bonito, Bahia. 11°56'S, 41°15′W (pop1)	1	LGEMA P2263[b]
	2-Ecological station Acauã, Leme do Prado, Minas Gerais (MG). 17°4′S, 42°40′W	1	B2284[b]
	3-Turmalina, MG. 17°16′S, 42°43′W	7	B1990[b], B1989[b], B2279[b], B2280[b], B2281[b], B2282[b], B2283[b]
	4-Jequitinhonha (Mata Escura), MG. 16°20′S, 41°00′W	1	B1091[b]
	5-José Gonçalves de Minas, MG. 15°00′S, 40°25′W	1	B2285[b]
	6-Nova Lima, MG, 19°50′S, 43°49′W	1	B1570[b]
	7-São Bartolomeu (Grande Mata), MG, 20°17′S, 43°35′W	1	B1022[b]
	8-Santa Teresa, Espírito Santo (ES). 19°56′S, 40°34′W	1	B1711[b]
	9-Morro do Diabo State Park, São Paulo (SP). 22°30′S, 52°18′W	10	All from LGEMA: P1965[b], P1867[b], P1957[b], P1960[b], P1959[b], P1973[b], P2158[b], P1494[b], P1989[b], P1388[b]
	10-Barreiro Rico, SP. 22°38′S, 48°13′W	2	LGEMA P1703[M], LGEMA P1686[M]
	11-Itatiaia Natl. Park, Rio de Janeiro. 22°25′S, 44°36′W.	5	Al from LGEMA: P1322[b], P1346[b], P1339[b], P1332[b], P1333[b]
	12-Piedade, SP.	1	LGEMA P681[M]
	13-Morro Grande State Park, SP. 23°42′S, 46°59′W	10	All from LGEMA: P519[b], P493[b], P542[b,] P463[b], P435[b], P448[b], P461[b], P544[b], P618[b], P518[b]
	14-Juquitiba, SP, 23°53′S, 47°00′W	3	LGEMA P714[M], LGEMA P773[M], LGEMA P713[M]
	15-Pinhalão, Paraná (PR). 23°46′S, 50°3′W	1	LGEMA P869[M]
	16-Wenceslau Braz, PR. 22°5′S, 48°47′W	2	LGEMA P958[M], LGEMA P980[M]
	17-PETAR, Caboclos station, SP, 24°28′S, 48°35′W	3	LGEMA P1211[b], LGEMA P1220[b], LGEMA P1221[b]
	18-State Park Urugua-í, Misiones, Argentina, 25°51′S, 53°55′W	5	All from LGEMA: 10369[M], 10341[M], 10348[M], 10359[M], 10370[M]
	19-Rancho Queimado, Santa Catarina. 27°40′S, 49°1′W	2	LGEMA P1753[b], LGEMA P1756[b]
S. turdina	Novo Airão, Amazonas. 1°57′S 62°21′W		LGEMA 9705[M]

Samples have been deposited at the Laboratório de Genética e Evolução Molecular de Aves (LGEMA), Instituto de Biociências, Univ. de São Paulo, Brazil, and at the Departamento de Biologia Geral, Instituto de Ciências Biológicas, Univ. Federal de Minas Gerais, Brazil (B). All samples are from Brazil, except when otherwise indicated

TISSUE tissue type, [b] blood, [M] muscle

Appendix 2

See Table 5.

Table 5 Nucleotide diversity of cytochrome b (mtDNA) from studies of Atlantic forest passerines

Species	Π—nucleotide diversity	Sample size	Length alignment (bp)	Reference
Xiphorhynchus fuscus	0.01574 ($SD = 0.0022$)	35	1,110	Cabanne et al. (2008); Aleixo (2002); Irestedt et al. (2004)
Dendrocolaptes platyrostris	0.004454 ($SD = 0.0012$)	34	1,006	Cabanne et al. (2011); Irestedt et al. (2004)
Automolus leucophtalmus	0.00242 ($SD = ?$)	22	900	D′Horta (2009)
Sclerurus scansor	0.01044 ($SD = 0.0020$)	47	1,046	d'Horta et al. (2011)
Thamnophilus ambiguus	0.005 ($SD = 0.002$)	22	837	Lacerda et al. (2007)
Thamnophilus pelzelni	0.003 ($SD = 0.002$)	21	837	Lacerda et al. (2007)
Scytalopus speluncae	0.0395 ($SD = 0.006$)	31	357	Mata et al. (2009)
Scytalopus pachecoi	0.0056 ($SD = 0.0023$)	14	344	Mata et al. (2009)
Scytalopus petrophilus	0.0039 ($SD = 0.0019$)	12	344	Mata et al. (2009), Whitney et al. (2010)
Schiffornis virescens	0.00187 ($SD = 0.00062$)	28	840	This study
Tachyphonus coronatus	0.00512 ($SD = 0.00042$)	50	990	Cabanne and Miyaki, unpublished
Basileuterus leucoblepharus	0.0058 ($SD = 0.0010$)	55	914	Batalha-Filho et al. (2012)

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