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# Phenotypic evolution of an Atlantic Forest passerine (*Xiphorhynchus fuscus*): biogeographic and systematic implications

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We studied the phenotypic variation of the Atlantic Forest passerine *Xiphorhynchus fuscus* (Aves: Dendrocolaptidae) with the broad aim of addressing whether the history and type of forest affected the evolution of endemic taxa. We also tested whether the different subspecies and genetic lineages of *X. fuscus* could be considered full species. We collected plumage and body size measurements and, in combination with genetic data, used multivariate tests to evaluate the working hypotheses. Our results, combined with previous biogeographic analyses, indicate that vicariant events have been important determinants in the evolution of phenotypic characters of *X. fuscus*, once genetic isolation was complete. Our analysis also suggests that forest heterogeneity and ecotones are important factors in the early evolution of Atlantic Forest taxa, perhaps via divergent selection. Forest instability during the Pleistocene was critical in the evolution of phenotypic traits. We confirm that the subspecies *atlanticus* should be considered a full species. Other lineages or populations are also phenotypically differentiated but we do not suggest considering them as full species. They share high levels of gene flow and are part of a continuous latitudinal cline of phenotypic variation. Our study suggests that not all the historic events in the Atlantic Forest that affected the evolution of genetic lineages also influenced the evolution of phenotypic characters in the same direction and intensity. Undoubtedly, natural selection played a major role in the evolution of Atlantic Forest organisms. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **113**, 1047–1066.

ADDITIONAL KEYWORDS: Atlantic Forest – *Caatinga* – *Cerrado* – niche simulation – phenotypic evolution – phylogeography – woodcreepers.

## INTRODUCTION

Global climate cycles of the late Pleistocene are assumed to have contributed substantially to biological evolution, in particular in forested biomes (Haffer, 1969; Moritz *et al*., 2000; Behling & Negrelle, 2001; Bermingham, Dick & Moritz, 2005; Colinvaux, 2005; Ledru *et al*., 2005; Cheng *et al*., 2012; Ribas *et al*., 2012). For example, during the last glacial maximum the southern limit of the Atlantic Forest (Fig. 1A) was likely located 750 km northward of its current location; whereas the range of the rainforests expanded into some locations currently occupied by the *Cerrado* (Portuguese term for savanna) and the *Caatinga* (dry forests of northeastern Brazil) (Ledru,

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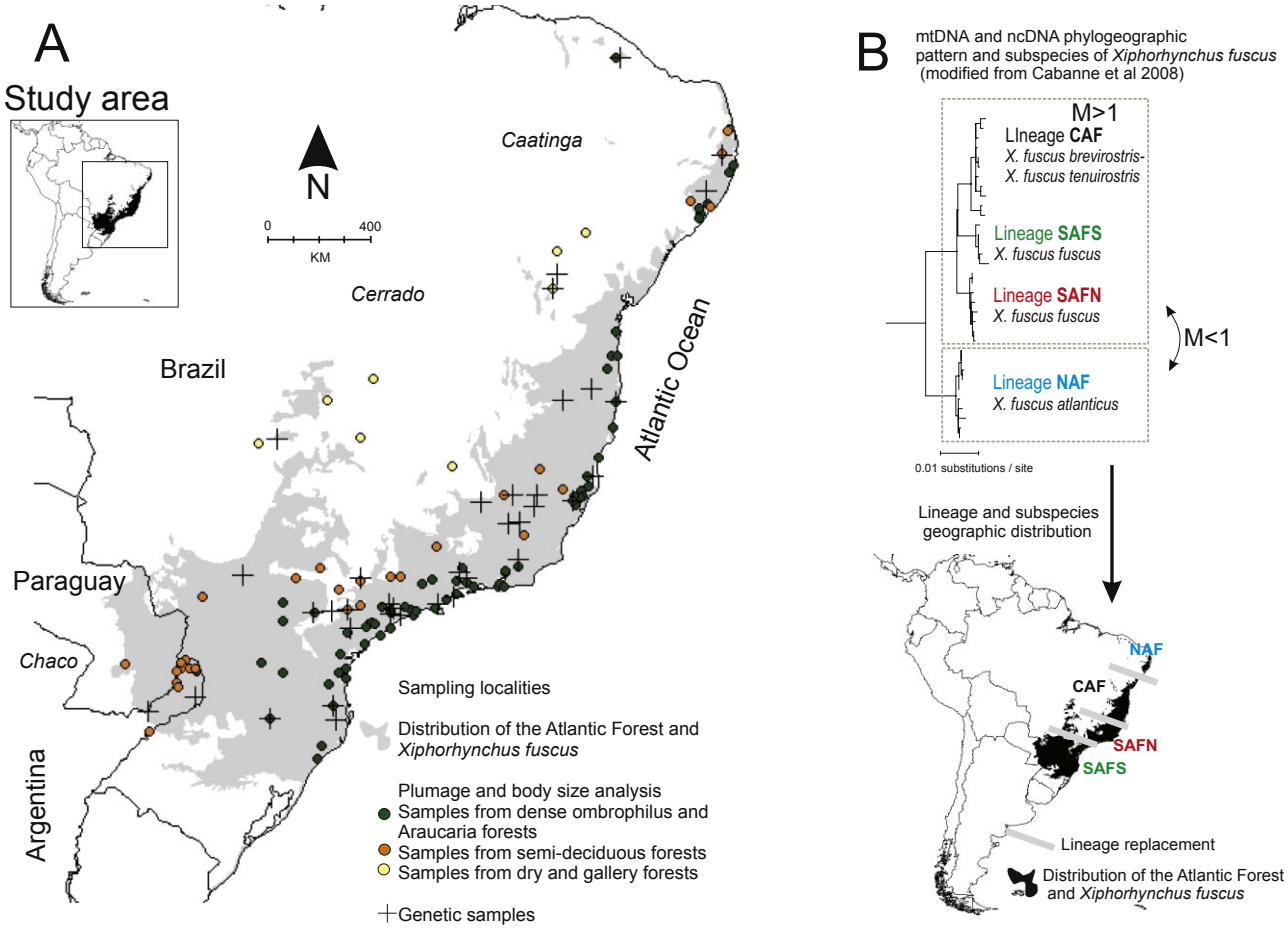



**Figure 1.** (A) Study area and distribution of samples of *Xiphorhynchus fuscus* for plumage, morphometric and genetic analysis. (B) Phylogeographic structure (based on mitochondrial and nuclear DNA) and gene flow pattern of the *Xiphorhynchus fuscus* (Cabanne *et al*., 2008). M > 1 denotes high gene flow among lineages and M < 1 denotes low gene flow. Subspecies *atlanticus* inhabits NE Brazil, northern the Rio São Francisco; subspecies *tenuirostris* occurs in the coastal Bahía and Espírito Santo states (E Brazil), from the Rio São Francisco to the Rio Doce; subspecies *brevirostris* inhabits the interior of Bahía state, and *fuscus* occurs from the Rio Doce to Argentina and Paraguay (Marantz *et al*., 2003). Lineages are: NAF, Northern Atlantic Forest; CAF, Central Atlantic Forest; SAFN, Southern Atlantic Forest North; SAFS, Southern Atlantic Forest South.

Salgado-Labouriau & Lorscheitter, 1998; Behling, 2002; Auler *et al*., 2004; Wang *et al*., 2004). This dynamic forest history modified, among other things, the degree of connectivity between populations of forest taxa, their population sizes, selection pressures, their competition regimes, etc. Altogether, these changes may have affected evolutionary processes at several hierarchical levels, from genes to phenotypes (Moritz *et al*., 2000). Recent genetic studies support this evolutionary scenario for the Atlantic Forest: many endemic taxa present strong phylogeographic structure highly congruent with the history of the Pleistocene forest (e.g. Cabanne *et al*., 2008; d'Horta *et al*., 2011; Batalha-Filho, Cabanne & Miyaki, 2012; Maldonado Coelho, 2012; Raposo do Amaral *et al*., 2013, but see Cabanne *et al*., 2013).

Even though the significance of forest range fluctuations on the evolution of forest dwelling organisms has been extensively studied and is of undeniable importance, in particular with respect to phylogeographic structure, their effects on the phenotype are not well understood (Schneider *et al*., 1999; Moritz *et al*., 2000; Cabanne *et al*., 2011). In this paper we address the hypothesis that the dynamic history of the forest has affected the evolution of complex characters such as those involved in the external phenotype of individuals; characters potentially more complex than those used to assess phylogeographic structure. Past forest fluctuations

significantly affected gene flow among populations (e.g. Carnaval *et al*., 2009) and, if these processes also affected the phenotype, its variation should follow historic patterns of gene flow. Historic gene flow patterns are represented in the phylogeographic structure of neutral characters [e.g. mitochondrial DNA (mtDNA) genetic variation]; therefore, variation in the phenotype should be correlated with phylogeographic lineages described by neutral markers. Violation of the aforementioned prediction would mean that the forest history, which affected neutral genetic structures, was not important for the evolution of phenotypic characters and that factors other than drift associated with population isolation, such as selection associated with current forest types, or phenotypic plasticity, should be considered.

In this paper we also address the hypothesis that local demographic instability, a product of historical forest fluctuations, is important for evolution. The process of contraction and expansion of the forest (forest dynamics) has traditionally been viewed as a process that can drive evolution because it generates different levels of isolation among regions (vicariance) (Moritz *et al*., 2000). However, besides leading to vicariance, forest dynamics promote local demographic instability that could induce evolution at a local scale. Intense contraction and expansion of forests during glacial and inter-glacial periods, like the one which occurred in the southern Atlantic Forest (Behling, 1998; Behling, 2002), should have involved not only events of extinction and colonization, but also they may have impoverished forest habitats of many forest taxa. Accordingly, current populations of each forest taxon should present different degrees of historic demographic stability, ranging from highly stable in regions that maintained the forest during glaciation peaks (i.e. southern Bahía, Carnaval & Moritz, 2008), to fully unstable in regions where forests were replaced by grasslands during these same periods (e.g. southern Atlantic Forest, Behling, 2002). Demographic instability could drive evolution at the local scale because: (i) it can cause local population extinctions; (ii) it may fragment existing populations; and (iii) it reduces the effective size of populations. In turn, these processes may cause a reduction of genetic diversity and stochastic fixation of alleles in the affected population. Even though phenotypic traits are likely to be affected by selection, strong population reductions or instability increase the impact of drift and stochastic extinctions. Therefore, if demographic instability is important for the evolution of phenotypic characters at the local scale, we expect to find differences between regions of historical stability and instability, namely: (i) divergence in phenotypic traits between regions (e.g. different wing lengths); and (ii) lower phenotypic diversity in unstable populations (Roulin & Ducrest, 2013).

Last, we also evaluated the hypothesis that selective forces across different types of forests and ecotones can be important in shaping phenotypic characters (Smith *et al*., 1997, 2005b; Sulloway & Kleindorfer, 2013). Under selection, the phenotype may vary with factors other than neutral genetic markers, for example it may follow environmental gradients and habitat changes (Zink & Remsen, 1986; Meiri & Dayan, 2003). We believe the Atlantic Forest is a suitable system to study divergence across habitats because it presents different phytophysiognomies and climates, ranging from coastal ombrophilus and Araucaria forests without a dry season to semideciduous and deciduous forests with a strong dry season (Veloso, 1991; Galindo Leal & Câmara, 2003) (Fig. 1A). Another interesting feature of the biome is that towards the centre of the continent it borders, through ecotones, with a dry corridor formed by semiopen biomes, namely the *Caatinga*, *Cerrado* and *Chaco* (Veloso, 1991). Many Atlantic Forest taxa inhabit more than one forest type, or even occur in gallery forests and forest relicts within the dry corridor. All these forest types have different overall conditions (e.g. climate, luminosity, structure, etc.) and, thus, selective forces on phenotypic characters might vary across them. A prediction stemming from this hypothesis implies finding divergence in phenotypic traits among forest types that cannot be explained by genetic isolation alone (vicariance) (Smith *et al*., 1997, 2005a).

We studied here the phenotypic and genetic variation of the lesser woodcreeper *Xiphorhynchus fuscus* Vieillot (1818) (Aves, Dendrocolaptidae) and tested the aforementioned hypotheses on the effects of the recent history of forests on the evolution of endemic taxa. *X. fuscus* is a good model for forest biogeographic studies because it occurs in well preserved forests in most of the Atlantic Forest's range, from sea level up to 1200 m.a.s.l. (Fig. 1A). Additionally, in a previous study, we found that it can be divided into four main lineages that were not in full agreement with the four described subspecies (Marantz *et al*., 2003; Cabanne *et al*., 2008) (Fig. 1B). Only the subspecies *atlanticus* is monophyletic, isolated from the other populations, and currently considered a full species by some biologists (CBRO, 2014). The other subspecies (*brevirostris*, *tenuirostris* and *fuscus*) are not monophyletic, but if they diverged in phenotypic characters in a discrete way (not continuous clinal variation, Winker, 2010), they could be considered full species by some integrative species definitions, such as the General Lineage Species concept (de Queiroz, 1998). At the moment there is no available thorough description of the phenotypic variation of the lineages

and subspecies of *X. fuscus* that could be used to address these taxonomic questions.

The two main objectives in this paper are: (1) to evaluate the different hypotheses on the effects of recent forest dynamics on the evolution of endemic taxa; and (2) to address, from an evolutionary standpoint, the taxonomic status of lineages and subspecies of *X. fuscus*. To achieve our objectives we answered the following questions regarding our study model: (i) What is the effect of population isolation (evaluated through the phylogeographic lineages, Fig. 1B), of the different forest types and of demographic instability on plumage and body size traits?; (ii) Is the variation of phenotypic traits discrete or continuous?; (iii) Are the subspecies and lineages differentiated by phenotypic traits, and could they be considered species following the General Lineage Concept of species? To answer these questions we first collected plumage and body size measurements; used genetic data to get genetic diversity metrics; and simulated geographic distributions to describe demographically unstable populations. Then, we used multivariate statistics to study the effect on the phenotype of four factors (forest types, stability, genetic lineages and subspecies). Finally, we addressed taxonomic questions based on the General Lineage Concept of species, mainly because it is one of the most inclusive species concepts, and it is the one adopted by most ornithologists in the Neotropics (Aleixo, 2007).

## MATERIALS AND METHODS

### Plumage and morphometric data

To analyze plumage and body size variation we studied a total of 260 specimens from the following museums: Museu de Zoologia da Universidade de São Paulo, Brazil; Museu Nacional, Rio de Janeiro, Brazil; Coleção de Aves da Universidade Federal de Pernambuco, Recife, Brazil; and Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina.

The plumage colours in woodcreepers are mostly melanin-based, mainly because of the high prevalence of tones of brown, reddish-brown and black. Melanin-based plumages have an important genetic component (Mundy, 2006; Karell *et al*., 2013; Roulin & Ducrest, 2013), and thus are suitable for evolutionary studies because they are not expected to be subject to substantial phenotypic plasticity. We examined plumage variation in a subset of 197 adult specimens (101 males, 80 females and 16 undetermined) collected in 95 localities encompassing the entire range of the species (Fig. 1A and Table A3). We recorded ten plumage characters from each study skin. For each character, we defined a scoring system by subdividing the total range of colour or pattern variation and assigning a discrete state or score to each subdivision. See Table A1 for a description of characters. The number of states per character varied from two to five, depending on the degree of variation of each character. Specimens were scored by a single observer (GSC) by comparison with reference specimens. We described colours following the Munsell Soil Colour Charts (Munsell Color Company, 2000).

We examined variation in body size traits in a subset of 202 adult specimens (105 males, 85 females and 12 adults of undetermined sex) collected in 107 localities (Fig. 1A and Table A4). We recorded seven morphometric variables from each study skin: total length of the culmen, from base to tip (Bill I); length of culmen, from nostril to tip (Bill II); bill depth at nostril (Bill depth); width of bill at nostril (Width bill); tail length, from the base at the uropygial gland to the tip of the longer rectrix (Tail); tarsus length, from junction of tibiotarsus and tarsometatarsus to distal junction of hind toe and tarsometatarsus (Tarsus); and un-flattened wing length (Wing). The morphological traits that we studied have mid to high heritability in a number of passerine birds (e.g. Boag & van Noordwijk, 1987; Grant & Grant, 2008), therefore they are suitable for the present study. All measurements were performed by GSC using a caliper (accuracy of 0.1 mm).

Finally, to study body weight we used 37 museum specimens (22 males, 10 females and five unsexed), nine published records (Brooke, 1983; de Faria & de Paula, 2008; Mallet-Rodrigues, 2005; Reinert *et al*., 1996) and two weight measurements of live birds in the field (Cabanne, unpubl. data).

### Phylogroup assignments and genetic-phenotypic diversity study

We used the genealogy (mtDNA and the nuclear marker FIB5) obtained in Cabanne *et al*. (2008) (Fig. 1B) to test phenotypic differentiation of genetic lineages.

We also used genetic data to explore contact regions between genetic lineages and to obtain a metric of genetic diversity (nucleotide diversity), which we compared with phenotypic diversity. For this analysis we used a total of 138 mtDNA sequences (control region, 575 bp), with 113 sequences obtained from Cabanne, Santos & Miyaki (2007) and 25 sequences specifically obtained for this study following the methodology by the same author, and 68 nuclear DNA sequences (FIB5, 547 bp) available from Cabanne *et al*. (2008). GenBank accession numbers for the new sequences are KJ812122–KJ812145. See Table A2 for details of the samples used to obtain new sequences.

For exploring a model of phenotypic neutrality, we analyzed genetic diversity by dividing the study

region in latitudinal bands of a width of one degree. We pooled genetic data from specimens coming from each band, and compared uncorrected nucleotide diversities for both mtDNA and ncDNA markers with phenotypic diversity (see Multivariate statistical analysis).

### Mapping areas of historical instability

To identify areas with *X. fuscus* populations that were most likely to have remained stable during the late Pleistocene, a period of intraspecific diversification of the species and of important geographic changes in the Atlantic Forest distribution (Cabanne *et al*., 2008), we constructed current and past distribution maps using ecological niche modelling, and interpreted overlapping areas among maps as regions with putative stable populations (e.g. Carnaval & Moritz, 2008; Thomé *et al*., 2010). We used the maximum entropy algorithm implemented in MAXENT (Phillips, Anderson & Schapire, 2006), with geographic records obtained from the phenotypic and genetic study and also from the ORNIS database (http://www.ornisnet.org/). See MAXENT input file in Supporting Information, Table S1. We obtained climatic variables from WorldClim (Global Climate Data, http://www.worldclim.org) (Hijmans *et al*., 2005) and used them with a resolution of 2.5 arcminutes. Variables used in our final analyses, selected following a rationale of permutation importance > 5%, were: mean diurnal range of temperature (BIO2), isothermality (BIO3), temperature seasonality (BIO4), mean temperature of wettest quarter (BIO8), mean temperature of coldest quarter (BIO11), annual precipitation (BIO12), precipitation of warmest quarter (BIO18) and precipitation of coldest quarter (BIO19). We also selected variables according to the criterion of elimination of correlated variables (Peterson *et al*., 2011). However, because results obtained with both methods of variable selection did not differ, we only present those based on the first criterion. The model obtained with current conditions was projected in the following past periods: last glacial maximum (LGM), 21 000 years BP (models MIROC3.2 and CCSM, data downloaded from WorldClim) and last inter-glacial (LIG; ~120 000–140 000 years BP) (from WorldClim; Otto-Bliesner *et al*., 2006). General conditions for all analyses were: random test points: 25; replicates: 10; replicate type: subsample; maximum iterations: 5000. We produced binary maps by adopting the threshold definition of equal training sensitivity and specificity criterion. The models were evaluated with the underlying area (AUC) of Receiver Operating Characteristics Curve (ROC) for the modelling algorithm. Models were transformed to binary and overlapped to obtain a final stability map in DIVA-GIS 7.5 (http://www.diva-gis.org).

### Multivariate statistical analysis

We first extracted principal components from both plumage and morphometric phenotypic variables and then applied multivariate analyses of variance (MANOVA) (Hair *et al*., 2010) to evaluate how each factor affected the phenotype. For plumage data we extracted principal components with a non-linear principal component analysis (non-linear PCA, also known as categorical PCA, Gifi, 1990). Non-linear PCA has the same objectives as a traditional PCA (Quinn & Keough, 2002), but it is suitable for variables of mixed measurement levels (e.g. nominal and continuous variables), such as the plumage variables studied here (nominal variation). For this analysis variables were considered unordered. For continuous body size variables we extracted principal components with a linear PCA by using a correlation matrix and Varimax rotation. For both body size and plumage data we selected the first principal components that accounted for more than 80% of the original variation.

We studied principal components by using MANOVA to evaluate the role of the following factors on phenotypic variation. (1) Phylogeographic lineages (Lineages), as a proxy for historic isolation: we assigned samples of plumage and morphometric analyses to a specific lineage according to its geographic distributions (Fig. 1B and results of the genetic analysis). Samples from locations where two or more genetic lineages overlapped geographically were not considered. (2) Population instability: we classified samples based on the collection site and its stability/instability condition defined by the niche simulations. (3) Forest type: we classified samples according to the dominant forest at the collection locality in: (i) dense ombrophilus forests from the coastal range (lowlands and Serra do Mar ranges) and Araucaria forests, (ii) semi-deciduous forests, and iii) dry (xeromorphic) and gallery forests within *Cerrado* and *Caatinga* (hereafter dry/gallery forests). We used forests classification of Veloso (1991) (Fig. 1A). (4) Subspecies: we assigned specimens to subspecies based on the geographic distribution of the intraspecific taxa (Marantz *et al*., 2003). For the MANOVA we considered collection year and sex as covariate and co-factor (no fixed effects), respectively. We considered collection year as covariate because the colour of museum skins may have faded with age since collection. Plumage studies should control for specimen age, otherwise old specimens may look different from newer specimens. Before running our analyses we checked prerequisites for MANOVA (Hair *et al*., 2010).

After each MANOVA, results were confirmed by ANOVAs and post hoc Scheffé tests on each single principal component (Hair *et al.*, 2010). When data violated the prerequisites for ANOVA we applied Kruskal–Wallis tests (Quinn & Keough, 2002). We used SPSS 15.0 (Windows, SPSS Inc., Chicago, IL) and Rundom Pro 3.14 (Jadwiszczak, 2009) for statistical analyses.

### Clinal analysis of traits

For the taxonomic discussion we studied whether specific traits varied according to a continuous or step cline across latitude, by adjusting both a linear and a polynomial function to the relationship between most of the trait variation (i.e.: first principal component) and latitude. We only studied a latitudinal cline because subspecies and clades are distributed across a latitudinal axis. If variation follows a step cline we expect the polynomial function to have a better goodness of fit to the data than the linear function. We evaluated absolute goodness of fit using the formula $D = \sum\left(\sqrt[2]{(E-O)^2} \div \sqrt[2]{O^2}\right)$, where $D$ is the sum over all cases of absolute deviations between the predicted value according to the adjusted equation (E) and the observed value (O).

## RESULTS

### Genetic data analysis

Results of the phylogenetic evaluation of the mtDNA complete data set were in full agreement with the phylogeny obtained in our previous study (Fig. 1A, results not shown). We used the mitochondrial data set and the nuclear sequences to obtain nucleotide diversity values to be compared with phenotypic diversity (Supporting Information, Table S2).

### Species distribution models

We collected a total of 268 occurrence records, which after eliminating records from the same locality (duplicates) totalled 112 records to be used in MAXENT. Figure 2 presents the results of the niche models for the present, LGM, LIG and the final model of stability across all periods. The support of the MAXENT model was good (mean AUC = 0.944). We used the stability model to define regions of population stability/instability for the multivariate analyses. This stability model suggested that most regions outside the coastal ranges were unstable, because they did not present the species during the four periods considered. Interestingly, the three regions of instability that reached the coast, or that were proximate to it (Fig. 2D), coincided with regions of lineage transitions in the species (Fig. 1B).

### Plumage, data reduction

A Mann–Whitney test on each single plumage character indicated that *X. fuscus* did not present plumage sexual dimorphism ($P > 0.05$ for all tests, with Bonferroni correction for number of characters). Therefore, we lumped data for both sexes for the following plumage analyses.

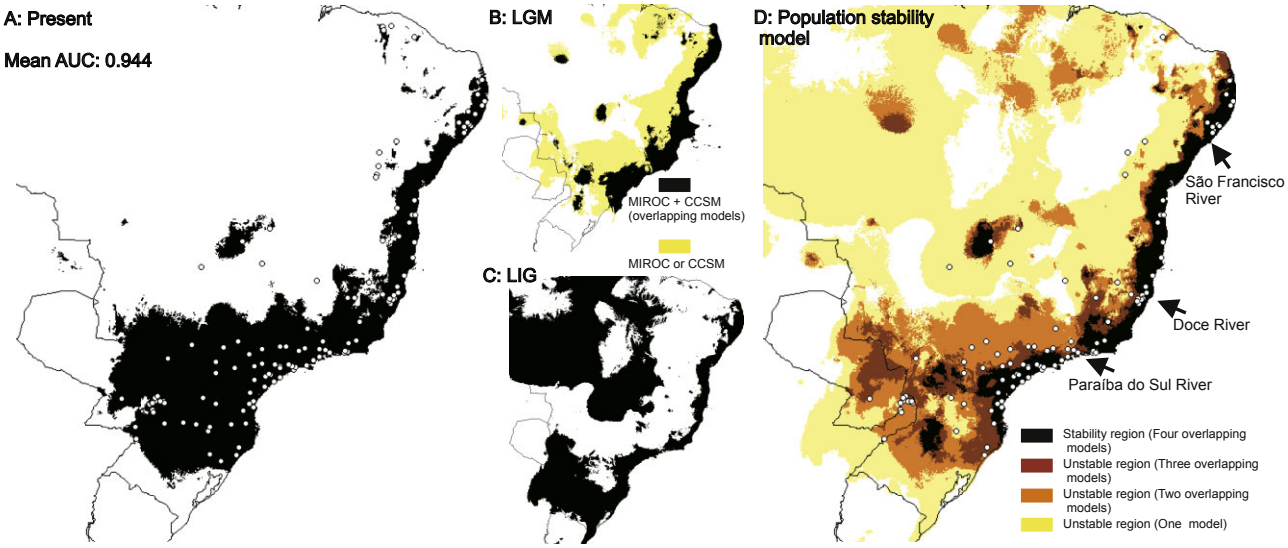



**Figure 2.** Simulated models of the distribution of *Xiphorhynchus fuscus* obtained in MAXENT in the (A) present, (B) last glacial maximum (LGM), (C) last inter-glacial period (LIG), and (D) a model of population stability across all the periods. Regions of lineage transitions that match unstable regions are also depicted (arrows in D). White dots in A, B and C represent records used for MAXENT simulations. White dots in D represent samples for the plumage and morphometric study.

A categorical principal component analysis reduced variation in plumage data. We selected the first two principal components, which accounted for 81% of the variability of the original data (Table 1). See Supporting Information, Figures S1 and S2 for a PC1 – PC2 plot.

### Plumage analysis: subspecies

A MANOVA model indicated that subspecies (Pillai's trace = 1.283, $F_{8, 380} = 84.907$, $P < 0.001$, Partial squared Eta = 0.642 and Power = 1) and collection year (Pillai's trace = 0.087, $F_{2, 189} = 8.974$, $P < 0.001$, Partial squared Eta = 0.087 and Power = 0.972) are significant factors for plumage variation. Subspecies presented the largest effect size according to partial squared Eta, while the contribution of year of collection was very small. This result was further corroborated with Kruskal–Wallis tests and ANOVAs on each principal component (Fig. 3). Post hoc tests on PC1 separated *atlanticus* from *tenuirostris* and both from *brevirostris* and *fuscus*. For variables correlated to PC1 (Table 1) the largest divergence occurred between *atlanticus* and the other subspecies, where more than 87% of the plumage characters presented different median scores (Fig. 3). PC2 only separated *tenuirostris* from the other samples. Subspecies *brevirostris* strongly diverged from the other subspecies but post hoc tests were not significant, perhaps due to the low sample size ($n = 4$, Table A3).

**Table 1.** Loadings of categorical principal components of plumage of *Xiphorhynchus fuscus*. Percentage of explained variance between parentheses

| Plumage character | PC1 (68.32%) | PC2 (12.73%) |
|---|---|---|
| CChead | 0.988 | –0.139 |
| CEXhead | 0.620 | 0.475 |
| Throat | 0.988 | –0.138 |
| PCchest | 0.988 | –0.138 |
| PEXchest | 0.988 | –0.138 |
| MCmantle | 0.988 | –0.138 |
| MEXmantle | 0.276 | 0.753 |
| Tail | 0.621 | 0.177 |
| Pchpattern | 0.353 | 0.579 |
| Under-tail | 0.988 | –0.138 |

An analysis for further testing the phenotypic identity of the subspecies detected diagnostic characters only for *atlanticus* (Table A3). The specific six character states for *atlanticus* were: CChead state 1 (very dark brown), Throat state 1 (brownish yellow), PCchest state 1 (brownish yellow), PEXchest state 1 (dark yellowish brown), MCmantle state 1 (dark brown) and Under-tail state 1 (plain). In summary, *X. fuscus atlanticus* was the darkest and most brownish population and the only one presenting plain under-tail coverts, while all the other populations presented striated under-tail coverts.

We studied the relationship between plumage variation (plumage PC1, 68.32% of original variation) and latitude to evaluate whether plumage variation was continuous across latitude. We adjusted both polynomial and linear equations to the data and observed that the goodness of fit D of the polynomial function was 1.73 times larger than D of the linear equation, which indicated that plumage of *X. fuscus* did not vary following a latitudinal continuous cline (Fig. 4A).

### Plumage analysis: biogeography

A MANOVA model for testing lineages, forest type, forest stability and year of collection found that

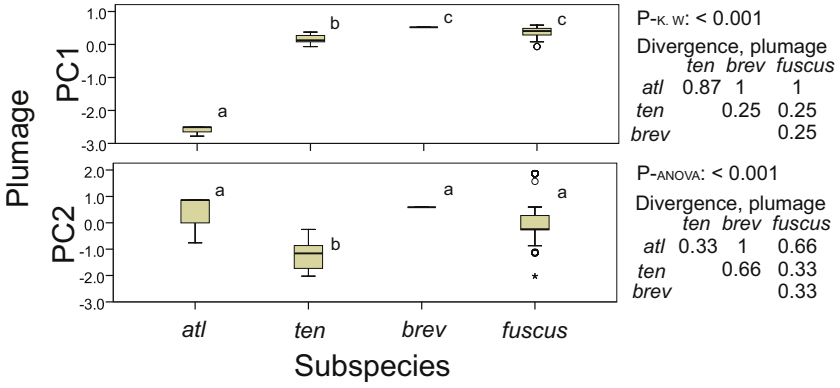



**Figure 3.** Analysis of subspecies' plumage based on categorical principal components. Lowercase letters within graphs indicate grouping after post hoc tests (Scheffé tests). $P_{-K.W.}$ indicates significance of Kruskal Wallis test. $P_{-ANOVA}$ indicates significance of ANOVA test. A matrix of divergence between subspecies is also shown. Divergences represent the proportion of characters associated to the specific PC with different median scores. Subspecies are: atl (*atlanticus*), ten (*tenuirostris*), brev (*brevirostris*) and *fuscus*.

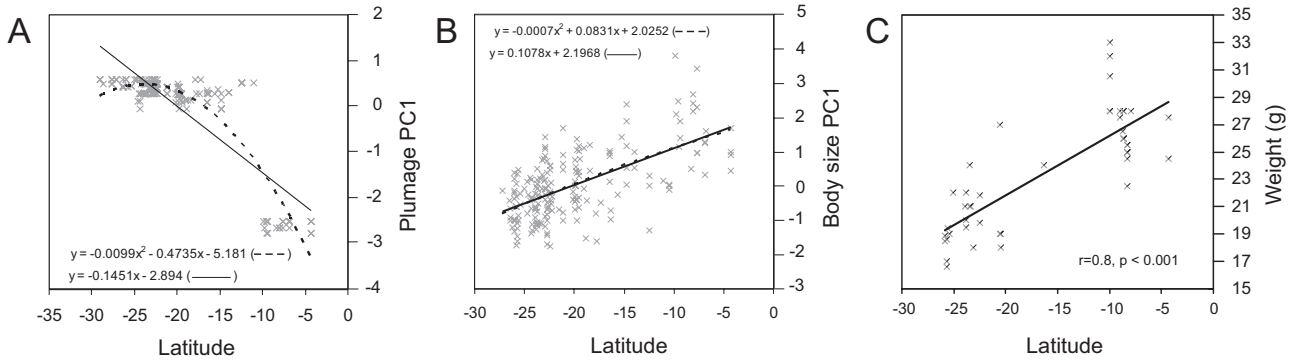



**Figure 4.** (A) *Xiphorhynchus fuscus*' plumage, (B) body size traits and (C) body weight variation across latitude.

**Table 2.** Multiple analysis of variance based on plumage (a) and body size (b) traits of *Xiphorhynchus fuscus*

| Factor | Pillai's trace | F | d.f. | *P*-value | Partial squared Eta | Power |
|---|---|---|---|---|---|---|
| a-plumage | | | | | | |
| Lineage | 1.221 | 89.295 | 6, 342 | < 0.001 | 0.610 | 1.000 |
| Forest type | 0.021 | 0.904 | 4, 342 | 0.462 | 0.01 | 0.287 |
| Forest stability | 0.009 | 0.785 | 2, 170 | 0.458 | 0.009 | 0.182 |
| Collection year (covariate) | 0.063 | 5.748 | 2, 170 | 0.004 | 0.063 | 0.862 |
| b-body size | | | | | | |
| Lineage | 0.812 | 21.162 | 9, 513 | < 0.001 | 0.271 | 1.000 |
| Forest stability | 0.122 | 7.863 | 3, 169 | < 0.001 | 0.122 | 0.989 |
| Forest | 0.199 | 3.592 | 6, 340 | 0.002 | 0.06 | 0.952 |
| Sex | 0.213 | 15.276 | 3, 169 | < 0.001 | 0.213 | 1.000 |

lineages and year of collection affected plumage, with lineage being the strongest factor according to partial squared Eta (Table 2). A low power may have compromised the test of forest type and stability. This result was confirmed by individual ANOVA and Kruskal–Wallis tests on each principal component (Fig. 5). A post hoc test on PC1 separated the lineage Northern Atlantic Forest (NAF) from the lineage Central Atlantic Forest (CAF), and both of them from the more southern lineages Southern Atlantic Forest North (SAFN) and Southern Atlantic Forest South (SAFS) (Fig. 1B). For variables associated with PC1 the largest divergence was between lineage NAF and the others, where 62% to 87% of the plumage characters presented different median states. PC2 only separated lineage CAF from the other samples with divergence at the associated characters of 33 to 66%.

A Levene test did not detect differences in variances for plumage PC1 and PC2 between regions of stable and unstable populations of Figure 2D ($P > 0.05$). Also, most of the plumage diversity (variance of plumage PC1) was not correlated with mitochondrial or nuclear genetic diversity ($P > 0.05$).

### Body measurements: sexual dimorphism and data reduction

*X. fuscus* did present sexual dimorphism in three body measurements (*t*-test, $P < 0.01$ for all tests), specifically in beak width (3.4% narrower beak in males), wing length (3.1% shorter wings in females) and tail length (2.2% shorter tails in females). Thus, we used sex as a co-factor in the subsequent analyses of variance.

A principal component analysis was used to reduce variation in body size measurements. We selected the first three principal components, which together accounted for 81.4% of the total variability (Table 3). See Supporting Information, Figures S1 and S2 for bivariate plots of principal components.

### Body measurements: subspecies

A MANOVA model found that subspecies (Pillai's trace = 0.710, $F_{9, 546} = 18.825$, $P < 0.001$, Partial squatted Eta = 0.237 and Power = 1) and sex (Pillai's trace = 0.179, $F_{3, 180} = 13.040$, $P < 0.001$, Partial squatted Eta = 0.179 and Power = 1) were significant for body measurements variation, subspecies having the

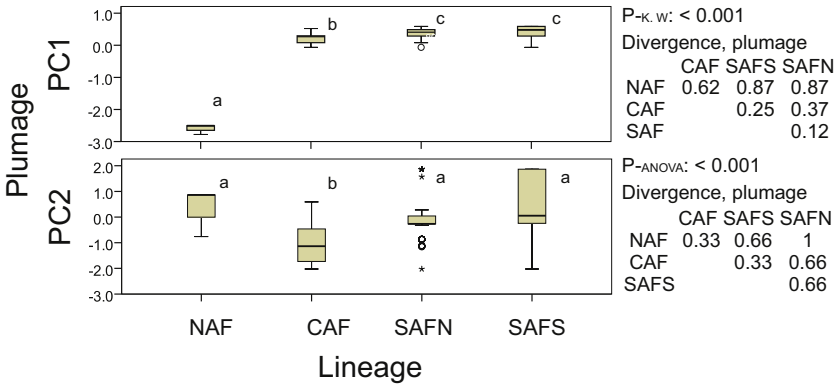



**Figure 5.** Analysis of lineages and plumage based on principal components. See Figure 1B for phylogenetic and geographic relationships of lineages. Lowercase letters within graphs indicate grouping after post hoc tests (Scheffé tests). $P_{K.W.}$ indicates significance of Kruskal Wallis test. $P_{ANOVA}$ indicates significance of ANOVA test. A matrix of divergence between lineages is also shown. Divergences represent the proportion of characters associated to the specific PC with different median scores.

**Table 3.** Loadings of principal component of body size traits of *Xiphorhynchus fuscus*. Percentage of explained variance in parentheses

| Body size traits | PC1 (53.73%) | PC2 (15.61) | PC3 (12.1%) |
|---|---|---|---|
| BILL I | 0.893 | 0.243 | 0.134 |
| BILL II | 0.853 | 0.195 | 0.203 |
| BILL WIDTH | 0.798 | 0.062 | 0.019 |
| BILL DEPTH | 0.738 | 0.426 | –0.016 |
| WING | 0.504 | 0.730 | –0.085 |
| TAIL | 0.100 | 0.929 | 0.075 |
| TARSUS | 0.125 | 0.012 | 0.980 |

largest effect size. This result was further corroborated by individual ANOVA tests on each principal component (Fig. 6). A post hoc test on body traits PC1 separated *atlanticus* from *tenuirostris* and both from *brevirostris* plus *fuscus*. Subspecies *atlanticus* was the largest one and divergence with the other subspecies at the variables associated with PC1 was on average 7.7% to 13.7%, while maximum average divergence between the other subspecies was 6.5%. PC2 presented the same variation tendency observed in PC1, but post hoc tests did not have enough power to differentiate groups. PC3 did not vary in a clear way among subspecies. See Table A4 of Appendix for mean values of body measurements for each subspecies.

### Body measurements: latitude and weight

We studied whether body size measurements varied along a latitudinal cline by studying the relationship between PC1 and latitude. We adjusted polynomial and linear equations to the data and observed that both functions presented similar goodness of fit (ratio of D between functions = 1.0031), thus we concluded that body measurements did vary following a continuous latitudinal cline (Fig. 4B). A similar result was obtained with PC2 (results not shown).

Most of the studied specimens did not have available weight data; therefore we did not include weight in body size multivariate analyses. Figure 4C indicates that body weight decreased from northern to southern populations. That is, average weight in the northernmost population (subspecies *atlanticus*, mean weight = 26.98 g, $n = 23$) and the southernmost population (subspecies *fuscus*, mean weight = 20.05 g, $n = 22$) was statistically different ($t$-test $P < 0.001$). The fact that body weight varied across latitude in a similar way to the variation found for PC1 and PC2 (Fig. 4B), corroborated that the principal components are good indicators of body size.

### Body measurements: biogeography

A MANOVA model for testing lineages, forest type, population stability and sex found all factors significant (Table 2b), with lineages and sex having the largest effect size, followed by population stability and forest type.

This result was further corroborated by individual ANOVAs with sex as co-factor (Fig. 7). A post hoc test on body size PC1 separated all lineages, where the largest divergence in variables correlated to PC1 (bill) occurred between lineage NAF (subspecies *atlanticus*) and the others (divergence range 8.5–15.24%). A post hoc test on PC2 separated lineage NAF from the others, with divergence in the range of 6.4–12%. PC3 did not vary among lineages in a meaningful way.

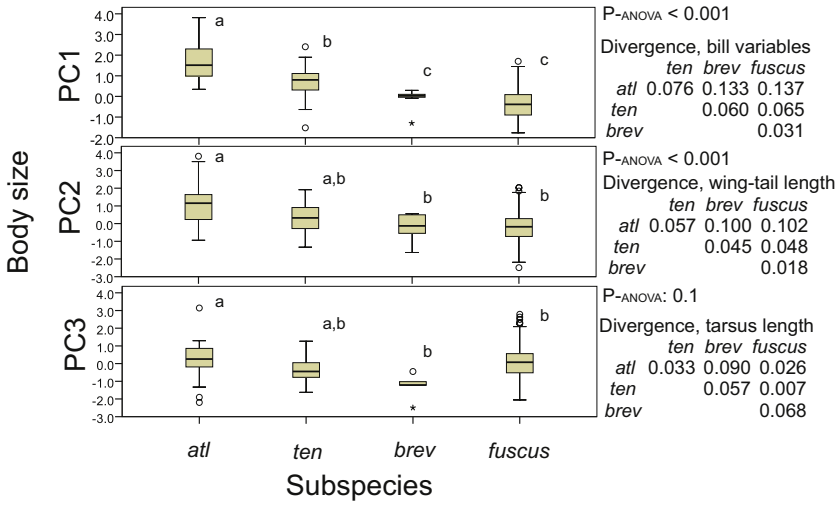



**Figure 6.** Analysis of subspecies based on principal components of body size traits. Lowercase letters within graphs indicate grouping after post hoc tests (Scheffé tests). P-ANOVA indicates significance of ANOVA test. A matrix of divergence between subspecies is also shown. Divergences represent the average absolute proportional difference, in relation to the higher value, in measurements of traits associated to each principal component. For example, for PC1, divergence between *atlanticus* and *fuscus* is 0.137, which means that in average *fuscus* is 13.7% smaller than *atlanticus* in variables associated to PC1.

Post hoc tests on body measurements PC1 did not separate forest types in a clear way, but PC2 (wing and tail) and PC3 (tarsus) did separate dry/gallery forest from the other continuous forests (coastal, Araucaria and semi-deciduous). Birds from continuous forests presented shorter wings and tails (body PC2, in average 1.4–2.6% shorter), while birds from dry/gallery forest had shorter tarsi (body PC3, 4.6–5.9% shorter).

Regarding forest stability, body size PC1 did show difference between stable and unstable populations, with unstable populations having, on average, shorter bills (average divergence 5.5%). PC2 also separated both types of populations, with stable ones presenting longer wings and tails on average (average difference 4.1%). In addition, a Levene test rejected equal variances between stable and unstable populations in body PC1 ($P = 0.006$, 53% higher variance in stable populations), in body PC2 ($P = 0.003$, 58% higher variance in sable populations) and in body size PC3 ($P < 0.001$, 41% higher variance in unstable populations). Finally, most of the body size diversity (variance of body size PC1) was not correlated with mitochondrial or nuclear genetic diversity ($P > 0.05$).

## DISCUSSION

### Effect of isolation: did forest dynamics affect phenotypic evolution?

The results of this study suggest that not all the events that affected the evolution of the genetic lineages of a forest bird also affected the evolution of its phenotypic traits in the same direction and with the same intensity. Factors other than historical isolation should be considered in order to understand phenotypic evolution. Most genetic lineages of *X. fuscus* (Fig. 1B), which evolved under different levels of isolation during the Pleistocene (see Cabanne *et al.*, 2007, 2008), were differentiated by plumage or body size traits. However, only the most divergent and completely isolated lineage of *X. fuscus atlanticus* (gene flow $M < 1$ individual/generation, Cabanne *et al.*, 2008) was associated with a clear step transition in phenotypic traits (Figs 3, 5 and 7). The other lineages, which shared high gene flow levels among each other (Cabanne *et al.*, 2008), were located along a latitudinal continuous cline of colour and body size and, thus, did not represent clear biological entities (Fig. 4 and Supporting Information, Figs S1 and S2).

Our findings corroborate that partial isolation within the same environment does not always produce phenotypic divergence, indicating that factors other than drift associated with vicariance are important for phenotype evolution (e.g. selection) (Schneider *et al.*, 1999; Milá *et al.*, 2009). The action of selection is further suggested by the lack of correlation between genetic and phenotypic diversity. The fate of phenotypic traits with genetic basis during forest fluctuations may depend on the levels of selection affecting them (Saether *et al.*, 2007; Whitlock, 2008). Even though neutrality of phenotype seems to be unlikely (Zink & Remsen, 1986), we explored this

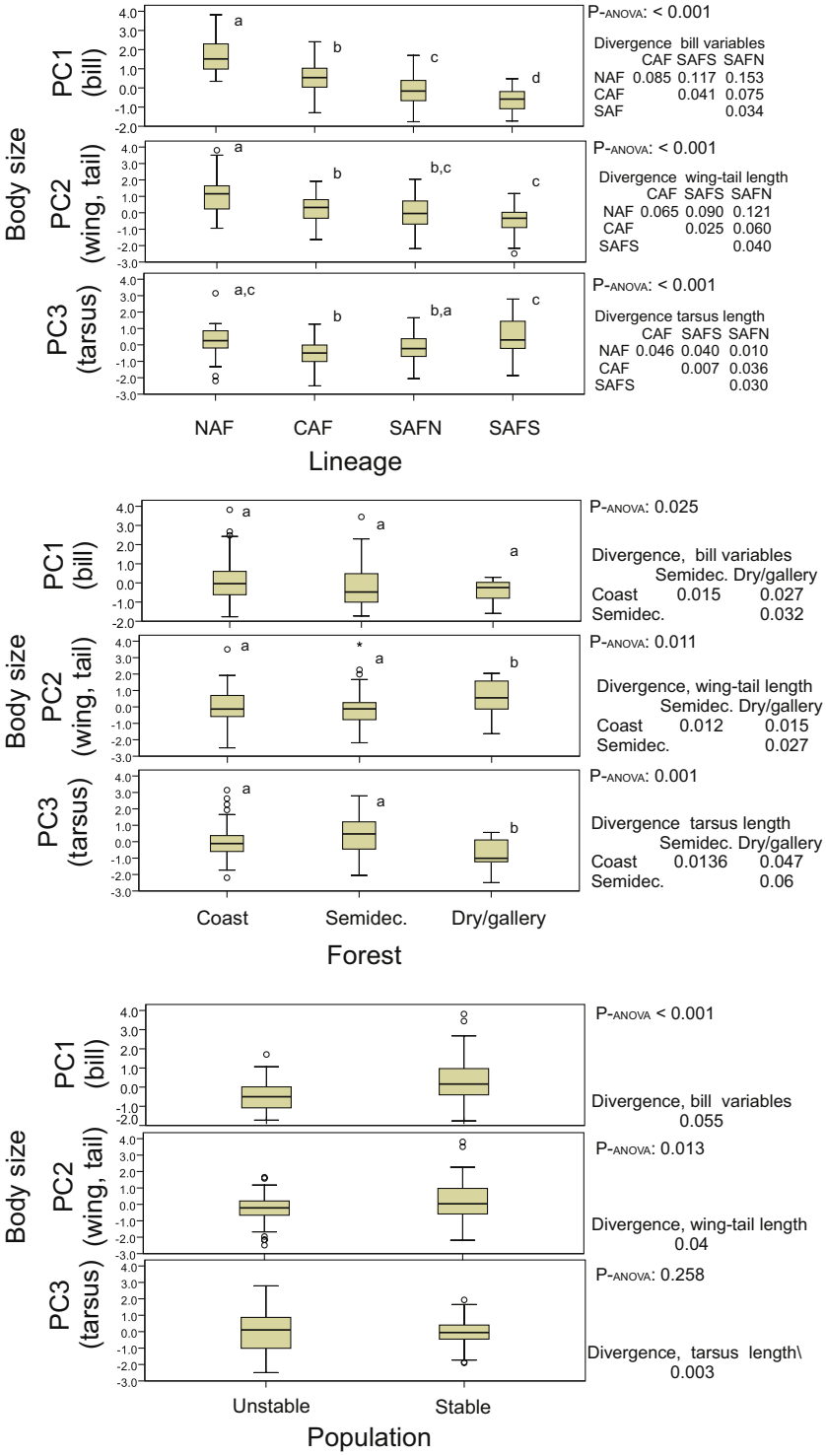



**Figure 7.** Analysis of the effects of lineages, forest type and population stability–instability based on body size traits principal components. Lowercase letters within graphs indicate grouping after post hoc tests (Scheffé tests). P-ANOVA indicates significance of ANOVA test. A matrix of divergence between subspecies is also shown. The divergences represent the average absolute proportional difference, in relation to the higher value, in measurements of traits associated to each principal component. For example, for PC1, divergence between NAF and SAFS is 0.153, which means that in average SAFS is 15.3% smaller than NAF in variables associated to PC1.

hypothesis as a null model (Storz, 2002). An expectation for this scenario of neutrality would be to find a positive correlation between diversity of neutral genetic markers and of phenotypic characters, in addition to correspondence between the neutral genetic structure and the phenotypic trait variation. This is because according to population genetic theory, alleles governing the putative neutral phenotypic traits and the neutral genetic markers should have been affected by similar evolutionary forces [e.g. direction and intensity of gene flow, drift, population demography, etc., but not selection, (Hedrick, 2005)]. However, because neutral genetic diversity and the diversity of the phenotypic traits were not correlated, the idea of a neutral phenotype evolving only by drift is not supported.

Our results are in accordance with studies of other organisms describing how morphological divergence might be related to habitat differences and not strictly to isolation (Schneider *et al*., 1999; Smith, Kelt & Patton, 2001; Smith *et al*., 2005a, 2011; Milá *et al*., 2009; Cabanne *et al*., 2011; Sulloway & Kleindorfer, 2013). For example, Cabanne *et al*. (2011) studied the phylogeographic structure and plumage variation of the Planalto woodcreeper (*Dendrocolaptes platyrostris* Spix, 1824), a bird of the Atlantic Forest that also inhabits gallery and dry forests of the *Cerrado*, *Chaco* and *Caatinga*. They found that most of the variation in plumage colour in this species followed changes in habitat type (continuous rainforest versus dry/gallery forest) instead of population historical divergence (phylogeographic gaps). This suggests that selection associated with habitat type may be directly responsible for the evolution of the plumage of this species.

### Did population instability affect phenotype?

Population instability also seems to be important for the evolution of body size traits. Specifically, we found bill, wings and tail to be affected by forest instability; and, even though effect size was small (e.g. average divergence 5.5% in bill traits), it was comparable to the effect described for forest type. In addition to the observed changes in mean values, we also found that variability of most body size traits (variances in body size PC1 and PC2) was smaller in unstable forests (Variability approximately 100% higher in stable populations), as expected according to our working hypothesis. We believe that this difference may be a consequence of higher extinction of alleles affecting the studied phenotypic traits during periods of forest fragmentation.

Population instability is also important for gene flow and the resulting phylogeographic patterns in *X. fuscus*, as shown in other birds and forests (e.g. Arbeláez-Cortés, Milá & Navarro-Siguenza, 2014). This is supported by the observed correspondence between the geographic distribution of the phylogeographic lineages of *X. fuscus* and the areas of stability (Figs 1B, 2D). The four main phylogeographic lineages are associated with regions of population stability. Moreover, these lineages replace each other in regions where instability approaches the coastal range; specifically at the mouth of the São Francisco River, at the Doce River and at the Valley of the Paraíba do Sul River (Fig. 2D).

Our results support the fact that the distribution of stable areas of the Atlantic Forest (forest refuges) does not match stable areas of specific endemic taxa (taxa refuges), in particular at the southern portion of the biome. In a previous study, Carnaval & Moritz (2008) simulated the distribution of forest refuges and found no important regions of forest stability in the south-eastern Atlantic Forest (south to the Doce River, Fig. 2D). However, our simulations with *X. fuscus*, and the occurrence of an endemic lineage of the species in that region (Fig. 1B, lineage SAFS of Cabanne *et al*., 2008), suggest that such stable forests existed. Similar results were obtained by other studies, like Thomé *et al*. (2010), Grazziotin *et al*. (2006), Porto, Carnaval & Rocha (2012), Fitzpatrick *et al*. (2009) and Raposo do Amaral *et al*. (2013). Therefore, it seems necessary to consider multiple evolutionary models for understanding the history of complex biomes such as the Atlantic Forest as a whole, perhaps one for each endemic taxon, because a single general model seems not plausible.

### Effect of forest types: is the phenotypic variation a product of different forest types?

Our analysis suggests that habitat differences between the continuous forest and the dry/gallery forests of the *Caatinga* and *Cerrado* are important for incipient evolution of forest taxa. Only body size traits were affected in *X. fuscus* by forest type transitions. Specifically, we found that in dry/gallery forest tarsi were shorter (up to 6%), and wing and tail were longer than in continuous forests (i.e. up to 2.7% shorter in semi-deciduous forest). This result supports the prediction that phenotypic change may be driven by divergent selection across habitats. We found no gene flow barriers among forests (we controlled for lineage in MANOVAs) while there was an incipient divergence in phenotypic traits. A similar result was obtained by Milá *et al*. (2009) in another member of the Family Dendrocolaptidae, the wedge-billed woodcreeper *Glyphorynchus spirurus* Vieillot (1819). Milá and colleagues studied ecological divergence along a gradient of altitude and forest types in the Andes, finding that tarsus length was associated

with altitude and abundance of moss over tree trunks and branches. They also found that traits related to flight (wing and tail length) were negatively correlated to tree density. The authors also studied the genetic population structure of this species finding that divergence occurred in the presence of gene flow and that historic isolation was not a good indicator of divergence in phenotypic traits. They hypothesized that the phenotypic changes observed in *G. spirurus* could have been driven by selection because the tarsi of birds that hitch trunks (e.g. woodcreepers and woodpeckers) are expected to be shorter when moss is less abundant, and also because traits related to flight should be affected by forest structure. That is, longer flights are expected in forests with low tree density than in forests with high tree density (Winkler & Leisler, 1992), and therefore selection for longer flight feathers is expected in forests with lower tree density.

In our study, we found that changes in body size observed in *X. fuscus* may have been driven by selection. This suggestion is supported by studies describing differences in body size of *G. spirurus* and of other birds across forests types (Smith *et al*., 1997, 2008; Milá *et al*., 2009). These studies propose that these changes were driven by selection across habitats. The phenotypic changes in *G. spirurus* and in the other birds are similar to those found in our focal species. Longer tails and wings might facilitate longer flights of *X. fuscus* through the network of dry/gallery forests, and longer tarsi might help in climbing trunks and branches with high abundance of moss (Zeffer & Norberg, 2003; Milá *et al*., 2009). Dry and gallery forests in *Cerrado* and *Caatinga* represent, for continuous-forest taxa such as *X. fuscus*, an intrinsically fragmented and more open system than semi-deciduous and ombrophilus forests. Forest fragmentation reduces resource availability and quality (e.g. in relation to food, shelter, and nesting places) and some forest birds respond to it by enlarging their home ranges, including several habitat fragments and gallery forests (Hansbauer *et al*., 2008). Accordingly, *X. fuscus* may have larger home ranges in these forests than in continuous forest, a situation that would require longer flights and might select for longer flight feathers (Winkler & Leisler, 1992; Smith *et al*., 1997, 2008). Similarly, abundance of moss over trunks and branches, which is highly dependent on overall humidity conditions, is lower in gallery and dry forest than in humid coastal and continuous forests (Cabanne, unpubl. data; Veloso, 1991). Lower abundance of moss can, in turn, select for shorter tarsi in gallery and dry forests. Thus, phenotypic changes observed in *X. fuscus* might have been induced by the mentioned differences between forests, and a comparison of home ranges and flight behaviour between regions might contribute to test this hypothesis.

The process of divergence across the continuous forests, and the dry and gallery forests of *Cerrado* and *Caatinga* might also have been important for diversification at biological levels deeper than the differentiation found within species. The open vegetation corridor and its network of dry/gallery forests are contiguous with the Atlantic and Amazon forests, and also, in some locations, with the Andean humid forests. There are several taxa that occur in both continuous and gallery forest habitats that might have diverged across the ecotone in the presence of gene flow. For example, the pair of sister species *Thamnophilus ruficapillus* Vieillot (1816) and *T. torquatus* Swainson (1825) (Brumfield & Edwards, 2007), which are mainly differentiated by plumage, may have diverged by selection in different habitats. *Thamnophilus ruficapillus* is the darkest and occurs in lower growth, borders, and secondary growth forest in the Atlantic Forest and tropical forests of the Andes; whereas *T. torquatus* occurs in scrubs and lower growth forest in the *Cerrado* and *Caatinga*. Another example of interspecific differentiation might be the pair of sister taxa *Syndactyla rufosuperciliata* Lafresnaye (1832) and *S. dimidiatus* Pelzeln (1859) (Derryberry *et al*., 2011), the former being found in the Atlantic and Andes forests and the latter from gallery forests of the *Cerrado*. Finally, another similar situation might be the pair *Basileuterus culicivorus* Deppe (1830) and *B. hypoleucus* Bonaparte (1850). The former is the darkest species and occurs in most of the forested regions of the Neotropics, whereas the latter occurs in forest borders and scrubs of the *Cerrado*. However, these species are not reciprocally monophyletic (Vilaça & Santos, 2010), plumage differentiation is subtle, and they hybridize (Robbins, Faucett & Rice, 1999).

We did not find evidence for forest type as a factor related to variation in plumage traits, opposite to what was expected according to our working hypothesis and a study with another Atlantic Forest woodcreeper, *Dendrocolaptes platyrostris* (Cabanne *et al*., 2011). One explanation could be that both *D. platyrostris* and *X. fuscus* have different geographic distributions and microhabitats in dry/gallery forest, and therefore selection pressures over plumage might be stronger in *D. platyrostris*. *Xiphorhynchus fuscus* only marginally penetrates the gallery and dry forests of the open vegetation corridor (Fig. 1A), while *D. platyrostris* inhabits these forests in most of the open vegetation corridor (Silva, 1996). Moreover, *D. platyrostris* also inhabits forested savannas with low tree density and palm savannas of the *Chaco* region (Cabanne, unpubl. data). Therefore, it is expected that the *X. fuscus* population from the dry/

gallery forests will be smaller than the population from the continuous forest, and thus absolute gene flow from continuous forest to dry/gallery forest might be high enough to preclude local adaptations in the later forest. However, we cannot rule out that this lack of evidence for forest type to be related to plumage traits may be due to low statistical power of the analysis (Table 2).

### Violation of Bergmann's rule?

Even though the evaluation of ecogeographical rules was out of the scope of this study, it is worth noting that our focal species violated Bergmann's rule, because larger individuals occurred in northern warmer regions instead of southern colder regions (Zink & Remsen, 1986) (Fig. 4B, C). A possible explanation for this result might be that northern populations inhabit the region with the most seasonal and dry climate of the *X. fuscus* range. Specifically, northern birds (subspecies *atlanticus*) occur in the coastal forests and in small relicts of humid forests located in highlands surrounded by dry seasonal forests (*Caatinga*). The overall climate of the *Caatinga* is dry, with rainy seasons shorter than 3 months and with 300–600 mm average rainfall each year [except for highland areas and close to the sea, where up to 2000 mm could be registered in a year (Prado, 2003)]. If we abide by an alternative definition of Bergmann's rule, body size may follow not only temperature but also seasonality of climate, or of other important resources such as water and food availability (Boyce, 1979; Lindstedt & Boyce, 1985; Murphy, 1985). *Xiphorhynchus fuscus* is a humid forest bird, but *atlanticus* inhabits a region where rain seasonality is very strong, while central and southern Atlantic Forest populations occur in regions with higher rainfall, with shorter or no dry season. Even though *atlanticus* is restricted to the most humid forests within the *Caatinga*, it might be affected in some way by the overall seasonality of the regional climate. Then, the larger body sizes found in northern regions might be a result of adaptations to a strong dry season, as occurs in some other organisms (Murphy, 1985). Also, and regarding our initial questions about which factors affected phenotypic traits (if stochastic or deterministic), the fact that body size traits seem to follow seasonal environmental conditions supports the idea that such traits evolved by deterministic factors.

### *Xiphorhynchus fuscus atlanticus* should be considered a full species

Subspecies *atlanticus* should be considered an independent evolutionary lineage and therefore a full species according to the General Lineage Species concept, which is in accordance with CBRO (2014). *X. fuscus atlanticus* can be differentiated by plumage (Figs 3, 5) and body size (Figs 6, 7), and a previous study showed that it is monophyletic and completely isolated from the other con-specific populations (Cabanne *et al*., 2008). Subspecies *atlanticus* is the darkest and most brownish population and the only one presenting plain under-tail coverts (Table A3). In relation to body size (Table A4), *X. fuscus atlanticus* represents the population with the largest birds. The other populations have a bill 7.5–14% smaller than in *atlanticus*, as well as wing and tail length 5–10% smaller and tarsi 2.5–9% smaller than the ones found in *atlanticus* (Fig. 6).

Other species concepts such as the phylogenetic and the biological species concepts can also be used to recognize *atlanticus* as a species, because it is monophyletic and completely genetically isolated (Cracraft, 1983; Cabanne *et al*., 2008) (Fig. 1B). The other subspecies can be differentiated by plumage or body size measurements (e.g. subspecies *tenuirostris*, Figs 3, 6). However, they do not represent independent evolutionary lineages because they are not monophyletic, they share high levels of gene flow among each other (Cabanne *et al*., 2008), and are part of a continuous latitudinal cline of colour and body size (Fig. 4 and Supporting Information, Figures S1 and S2). A similar conclusion can be drawn for the three phylogenetic lineages aside from *atlanticus* (Fig. 1B). Therefore, we do not suggest considering them as full species under any species concept.

Our conclusions regarding the phenotypic variation of subspecies *brevirostris* should be considered with care because the size of the studied sample is small. However, because our previous genetic study indicated that *brevirostris* is not monophyletic (Cabanne *et al*., 2008), we do not believe that studying a larger sample would change its taxonomic status.

## CONCLUSIONS

Our current results, together with our previous analyses, indicate that vicariant events were significant factors for the evolution of phenotypic characters of a forest bird such as *X. fuscus*, but only when isolation was complete. The analyses also suggest that forest heterogeneity can promote incipient evolution in the Atlantic Forest, perhaps by means of divergent selection across regions. Also, forest instability during the Pleistocene may have led to the evolution of phenotypic traits.

Our study suggests that not all historic events of the Atlantic Forest which affected the evolution of phylogeographic patterns, affected in turn the evolution of phenotypic traits in the same direction and

with the same intensity. Natural selection must also have played a major role in the evolution of Atlantic Forest organisms.

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## APPENDIX

**Table A1.** Studied plumage characters of *Xiphorhynchus fuscus*. Colour definition is according to Munsell Color Company (2000). Hue, value/chroma and colour name are presented

| Characters | Description of characters and states* |
|---|---|
| CChead | Characteristics of contour feathers of the crown, central feather region. *State 1*, 10YR 2/2 (very dark brown); *State 2*, 7.5YR 3/3 (dark brown) to 3/1 (very dark grey); *State 3*, 10YR 2/1 (black); *State 4*, 5YR 6/8 (reddish yellow) |
| CEXhead | Characteristics of contour feathers of the crown, external feather region. *State 1*, 10YR 6/6 (brownish yellow); *State 2*, 10YR 7/3 (very pale yellow); *State 3*, 10YR 7/4 (very pale brown) |
| Throat | Throat contour feathers. *State 1*, 10YR 6/6 (brownish yellow) to 7/4 (very pale brown); *State 2*, 10YR 8/3 (very pale brown); *State 3*, 10YR 7/6 (yellow) |
| PCchest | Characteristics of contour feathers of the chest, central feather region. *State 1*, 10YR 6/6 (brownish yellow); *State 2*, 10YR 8/3 (very pale brown); *State 3*, 10YR 7/6 (yellow), *State 4*, 10YR 7/4 (very pale brown), *State 5*, 10YR 7/3 (very pale yellow) |
| PEXchest | Characteristics of contour feathers of the chest, external feather region. *State 1*, 10YR 4/4 (dark yellowish brown); *State 2*, 2.5Y 3/3 (dark yellow brown); *State 3*, 2.5Y 4/3 (olive brown), *State 4*, 2.5Y 5/4 (light olive brown) |
| MCmantle | Characteristics of contour feathers of the mantle, central feather region. *State 1*, 7.5YR 3/3 (dark brown) to 4/4 (brown); *State 2*, 7.5YR 3/4 (dark brown); *State 3*, 7.5YR 4/6 (strong brown) |
| MEXmantle | Characteristics of contour feathers of the mantle, external feather region. *State 1*, 10YR 6/6 (brownish yellow) to 6/4 (light yellowish brown); *State 2*, 10YR 8/3 (very pale brown); *State 3*, 10YR 7/6 (yellow) |
| Tail | Tail colour. *State 1*, 10R 3/4 (dark red); *State 2*, 10R 3/6 (dark red) |
| Pchpattern | Overall chest patterns. *State 1*, scaled; *State 2*, scaled/striated. *State 3*, striated. *State 4*, strongly striated |
| Under-tail | Overall pattern of under-tail coverts. *State 1*, plain pattern; *State 2*, striated or scaled |

*The following specimens of the museums Museu de Zoologia da Universidade de São Paulo (MZUSP), Brazil; Museu Nacional (MN), Rio de Janeiro, Brazil, were taken as reference for character description: MN24702, MN28084, MN35556, MN14017, MN26146, MN35980, MN14028, MN20436, MN14035, MZUSP37330, MZUSP37325, MZUSP41659, MZUSP41161, MZUSP26023, MZUSP34523, MZUSP76121, MZUSP62541, MZUSP75031, MZUSP75046, MZUSP75565, MZUSP28218.

**Table A2.** Tissue samples used in this study to obtain sequences of the control region of the mtDNA

| Sample* | Locality |
|---|---|
| B1401, B1821, B1822, B1823 | Mata do Paraíso, Viçosa, Minas Gerais, Brazil (Br). Lat. −20.773922°, long. −42.874248° |
| LGEMAP1433 | Botuverá, Santa Catarina (SC), Br. Lat. −27.256987°, long. −49.147655° |
| LGEMAP1435, LGEMAP1436, LGEMAP1437 | Rio Pequeno das Areias, Serra da Armação, Celso Ramos, SC. Lat. −27.633511°, Long. −51.337165° |
| LGEMAP1443, LGEMAP1444 | Água Azul, Vicência, Pernambuco, Br. Lat. −7.668423°, long. −35.327456°. |
| LGEMAP1644 | Reserva Biológica Augusto Ruschi, Santa Teresa, Espírito Santo, Br. Lat. −19.947357°, long. −40.598141°. |
| LGEMAP1754, LGEMAP1769, LGEMAP1771, LGEMAP1788 | Rancho Queimado, SC. Lat. −27.693350°, long. −48.997675°. |
| LGEMAP1807 | Fazenda Santa Adelia, Jataí, Goiás, Br. Lat. −17.892841°, long. −51.715134°. |
| LGEMAP1809, LGEMAP1810, LGEMAP1811 | Estação Veracruz, Porto Seguro, Bahía, Br. Lat. −16.12°, long. −39.6°. |
| FMNH399195, FMNH399196, FMNH399197, FMNH399199, FMNH399199 | Ibateguara, Alagoas, Br. Lat. −8.354°, long. −35.312° |

*Tissue collections: LGEMA – Laboratório de Genética e Evolução Molecular de Aves, Universidade de São Paulo, São Paulo. FMNH – Field Museum of Natural History, Chicago. B – Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte.

**Table A3.** Median scores and range of variation (between parentheses) of plumage characters of the *Xiphorhynchus fuscus*. Median scores in bold are fixed and diagnostic for a specific taxon

| Subspecies | Sample size | CChead | CEXhead | Throat | PCchest | PEXchest | MCmantle | MEXmantle | Tail | Pchpattern | Under-tail |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *atlanticus* | 25 | **1** | 1 | **1** | **1** | **1** | **1** | 1 | 1 | 3 (1–3) | **1** |
| *tenuirostris* | 24 | 2 (2–3) | 1 (1–3) | 2 | 2 | 2 | 2 | 1 (1–2) | 2 (1–2) | 2 (2–4) | 2 |
| *brevirostris* | 4 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 (3–4) | 2 |
| *fuscus* | 142 | 2 (2–4) | 3 (1–3) | 2 (2–3) | 2 (2–5) | 3 (2–4) | 2 (2–3) | 1 (1–3) | 2 (1–2) | 3 (2–4) | 2 |

**Table A4.** Mean values (95% CI) of body trait measurements (mm) of subspecies of *Xiphorhynchus fuscus*

| Subspecies | Sample size | BILLl I | BILL II | BILL WIDTH | BILL DEPTH | WING | TAIL | TARSUS |
|---|---|---|---|---|---|---|---|---|
| *atlanticus* | 21 | 31.230 (30.618, 31.841) | 21.919 (21.410, 22.427) | 4.304 (4.180, 4.428) | 5.617 (5.436, 5.797) | 86.939 (85.182, 88.696) | 81.882 (79.401, 84.364) | 17.738 (17.326, 18.150) |
| *tenuirostris* | 25 | 28.965 (28.302, 29.628) | 20.041 (19.555, 20.526) | 4.026 (3.915, 4.137) | 5.145 (5.025, 5.025) | 82.694 (81.288, 84.100) | 76.504 (75.023, 77.984) | 17.136 (16.894, 17.379) |
| *brevirostris* | 7 | 27.922 (27.285, 28.560) | 19.454 (18.811, 20.097) | 3.582 (3.311, 3.854) | 4.794 (4.536, 5.051) | 78.297 (75.502, 81.091) | 73.765 (70.855, 76.676) | 16.148 (15.743, 16.553) |
| *fuscus* | 144 | 27.322 (27.116, 27.529) | 19.104 (18.941, 19.266) | 3.776 (3.733, 3.818) | 4.642 (4.595, 4.690) | 76.543 (75.897, 77.189) | 74.855 (74.204, 75.506) | 17.255 17.116, (17.394) |

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Plumage data of the *Xiphorhynchus fuscus*. Principal components (PC1 and PC2) plots for subspecies (A) and lineages (B). Full range ellipses are presented.
**Figure S2.** Body size traits data of the *Xiphorhynchus fuscus*. Principal components (PC1–PC2–PC3) plots for subspecies (A) and lineages (B). Full range ellipses are presented.
**Table S1.** Input file for MAXENT analysis.
**Table S2.** Diversity of plumage, body size traits and genetic diversity of the *Xiphorhynchus fuscus*.