ORIGINAL ARTICLE

Phylogeny and comparative phylogeography of *Sclerurus* (Aves: Furnariidae) reveal constant and cryptic diversification in an old radiation of rain forest understorey specialists

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ABSTRACT

Aim To evaluate the role of historical processes in the evolution of *Sclerurus* leaftossers by integrating phylogenetic and phylogeographical approaches.

Location Humid forests of the Neotropical region.

Methods We reconstructed the evolutionary history of *Sclerurus* based on DNA sequences representing all species and 20 of the 26 recognized subspecies using one autosomal nuclear locus and three protein-coding mitochondrial gene sequences. Phylogenetic relationships were inferred using Bayesian and maximum-likelihood methods. We used Bayesian coalescent-based approaches to evaluate demographic changes through time, and to estimate the timing of diversification events. Based on these results, we examined the temporal accumulation of divergence events using lineage-through-time plots.

Results The monophyly of all *Sclerurus* species was strongly supported except for *Sclerurus mexicanus*, which was paraphyletic in relation to *Sclerurus rufigularis*, and for the sister pair *Sclerurus scansor*–*Sclerurus albigularis*, which were not reciprocally monophyletic in the nuclear tree. We found remarkably deep phylogeographical structure within all *Sclerurus* species, and overall this structure was congruent with currently recognized subspecies and Neotropical areas of endemism. Diversification within *Sclerurus* has occurred at a relatively constant rate since the Middle Miocene.

Main conclusions Our results strongly support the relevance of physiographical (e.g. Nicaragua Depression, Isthmus of Panama, Andean Cordillera, great rivers of Amazonia) and ecological barriers (open vegetation corridor) and ecological gradients (elevational zonation) to the diversification of Neotropical forest-dwelling organisms. Despite the high congruence among the spatial patterns identified, the variance in divergence times suggests multiple speciation events occurring independently across the same barrier, and a role for dispersal. The phylogenetic patterns and cryptic diversity uncovered in this study demonstrate that the current taxonomy of *Sclerurus* underestimates the number of species.

Keywords
Amazonia, Andes, areas of endemism, biogeographical barriers, cryptic diversity, elevational zonation, Great American Interchange, molecular clock, Neotropics, Sclerurinae.

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INTRODUCTION

The origin of the high biodiversity that characterizes the Neotropics has long been of interest to biogeographers (Wallace, 1853). Many historical and ecological mechanisms have been hypothesized to explain the origin of the biogeographical patterns within this region (e.g. Chapman, 1917; Haffer, 1969; Ayres & Clutton-Brock, 1992; Bush, 1994; Fjeldså *et al.*, 1999; Brumfield, 2012). In the Neotropical region different taxa often show similar distributions, which are used to define areas of endemism (Cracraft, 1985). Based on the conceptual framework of vicariance biogeography (Platnick & Nelson, 1978) some studies on the diversification of Neotropical birds used congruence among area relationships to infer the biogeographical processes (e.g. Cracraft & Prum, 1988; Marks *et al.*, 2002; Aleixo & Rossetti, 2007). However, ignoring temporal information obscures the connection between biogeographical patterns and their underlying causes (Donoghue & Moore, 2003) because a single pattern of area relationships can be achieved through different processes.

In the last decade temporal inferences have often been incorporated in studies of diversification of Neotropical bird lineages (e.g. Aleixo, 2004; Brumfield *et al.*, 2007; Derryberry *et al.*, 2011). Although many of these taxa have experienced the same history of landscape evolution, the results of these studies revealed differences among spatio-temporal patterns of diversification. The explanation of this may involve stochastic processes (e.g. dispersal and extinction) and ecological differences among taxa, such as dispersal ability and ecophysiological constraints (Burney & Brumfield, 2009).

Here we present a comprehensive study of the historical diversification of the *Sclerurus* leaftossers (Aves: Furnariidae). *Sclerurus* is an old, monophyletic group (Derryberry *et al.*, 2011) that comprises six species of strictly understorey, leaf-litter specialists, highly sensitive to habitat disturbance (Stotz *et al.*, 1996). The geographical ranges of *Sclerurus* species are dissected by the major biogeographical barriers of the Neotropics and encompass the main Neotropical forest biomes from southern Brazil to central Mexico.

Herein we analyse the evolutionary history, divergence time and diversification patterns of *Sclerurus* leaftossers across the Neotropical region. We maximized the geographical sampling of all taxa to minimize problems with potential cryptic diversity. Thus, we provide a comprehensive temporal and spatial analysis of the history of this group.

MATERIALS AND METHODS

Sampling

We reconstructed the phylogeny of *Sclerurus* based on 119 ingroup samples representing all six species in the genus and 20 of the 26 subspecies (Remsen, 2003) (see Appendix S1 in Supporting Information). The outgroup taxa were defined based on previous phylogenies (Irestedt *et al.*, 2009; Derryberry *et al.*, 2011). We included two species of *Geositta* miners

(*Geositta poeciloptera* and *Geositta tenuirostris*), that is the sister-group of *Sclerurus*, and representatives of the closely related subfamilies, e.g. *Xenops minutus* (Furnariinae) and *Lepidocolaptes angustirostris* (Dendrocolaptinae). For most samples, we sequenced a total of 2962 bp from fragments of four loci: one autosomal nuclear locus, β-fibrinogen intron 7 (*Fib7*), and three protein-coding mitochondrial DNA (mtDNA) genes: cytochrome *b* (cyt *b*) and NADH dehydrogenase subunits 2 (*ND2*) and 3 (*ND3*). DNA extraction, amplification, sequencing and alignment procedures are described in Appendix S2.

Phylogenetic analyses

The phylogenetic analyses were performed separately for mtDNA (2408 bp) and for *Fib7* (914 bp). To estimate gene trees we used maximum-likelihood (ML) and Bayesian inference (BI) approaches. To select the simplest model of molecular evolution with the highest likelihood for the data, we applied a likelihood-ratio test (LRT) performed using MODELTEST 3.7 (Posada & Crandall, 1998). The ML searches were performed using RAxML 7.0.4 (Stamatakis, 2006), assuming a general time-reversible (GTR) model of evolution with distributed rate heterogeneity, four rate categories, and estimation of the proportion of invariable sites. The ML analyses of mtDNA were carried out considering three partitions (one partition per gene: cyt *b*, *ND2* and *ND3*). To determine if analyses had become trapped in local optima, we conducted 10 independent ML searches. The robustness of the nodes was determined by 1000 bootstrap replicates, using the 'fast bootstrap' algorithm of RAxML. The BI with Markov chain Monte Carlo (MCMC) sampling was implemented in MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). As in the ML analyses, a partitioned analysis was conducted with three locus-specific models, estimating parameters independently for each partition (nst = 6; rates = invgamma). We conducted two independent, parallel analyses with four simultaneous chains each for 10 million generations, sampling parameters and trees every 1000 generations. We evaluated the convergence between analyses by comparing the posterior probabilities of clades using AWTY (Nylander *et al.*, 2008). The first 2.5 million generations were discarded as burn-in and the posterior probabilities were estimated from the remaining trees.

Temporal patterns of lineage diversification

The timing of diversification events within *Sclerurus* was estimated using a subset of the mtDNA sequence matrix containing all species and one exemplar of each intra-specific clade that represented divergent populations (Appendix S1). Divergence times were estimated using the Bayesian approach implemented in BEAST 1.5.1 (Drummond & Rambaut, 2007) with the uncorrelated relaxed clock model (uncorrelated lognormal). The calibration used was based on the results of dating of the Furnariidae radiation obtained by Derryberry *et al.* (2011) in a very comprehensive study that included 97%

of furnariid species and used similar genetic markers (*ND2*, *ND3*, *CO2* and *Fib7*). We used the confidence intervals obtained by Derryberry *et al.* (2011) to set lower and upper limits of uniform prior distributions of ages for the most recent common ancestor for five clades within the *Sclerurus* phylogeny. Based on the resulting time-calibrated tree from beast, the temporal accumulation of divergence events was examined using lineage-through-time (LTT) plots to test for changes in lineage-splitting events along the evolutionary history of *Sclerurus*. Given the deep phylogeographical structure within *Sclerurus* species (see Results), we used the least inclusive terminal clades for this analysis. We used phylogenetic and likelihood-based statistical methods implemented in R 2.13.0 (R Development Core Team, 2011): packages ape 2.7 (Paradis *et al.*, 2004), laser 2.2 (Rabosky, 2006) and geiger (Harmon *et al.*, 2008).

We compared diversification events of *Sclerurus* among 10 major biogeographical areas to examine the spatial and temporal congruence of divergence in co-occurring lineages. The 10 biogeographical areas considered are: Central America – north (CAN; Mexico to Nicaragua), Central America – south (CAS; Costa Rica to Darién, north-western Colombia), Chocó (CHO; from Darién to western Ecuador), base of the Andes (BAN; eastern Andean foothill forests from southern Colombia to Bolivia), western Amazonia – south (WAS; lowland forests from the right bank of the Amazon/Ucayali rivers to the left bank of the Madeira River), western Amazonia – north (WAN; lowland forests from the left bank of the Amazon/Ucayali rivers to the right bank of the Negro River), Brazilian shields – west (BSW; the right bank of the Madeira River to the left bank of the Xingu River), Brazilian shields – eastern (BSE; the right bank of the Xingu River to the eastern limits of Amazonia), Guiana Shield (GUY; northern Amazonia from the left bank of the Negro River to the eastern limit of Amazonia) and Atlantic forest (ATF; southern Brazil, eastern Paraguay and north-eastern Argentina to north-eastern Brazil). These areas were designated based on their geological, geographical and palaeoecological similarities and distinctions (Bates, 2001; Campbell *et al.*, 2006; Aleixo & Rossetti, 2007) and based on patterns of distributions for terrestrial vertebrates (Haffer, 1974; Cracraft, 1985).

Comparative demography

We examined the molecular signature of past demographic events in Amazonian populations of each species using the mtDNA and *Fib7* data. We used a Bayesian approach implemented in phase 2.0 (Stephens *et al.*, 2001; Stephens & Donnelly, 2003) to identify heterozygous haplotypes of *Fib7*. Heterozygous indel positions were not found. To evaluate the existence of significant evidence of recombination in *Fib7*, the pairwise homoplasy index (PHI) test (Bruen *et al.*, 2006) was performed for each lineage using SplitsTree 4.10 (Huson & Bryant, 2006). We calculated population genetic summary statistics and estimated parameters such as genetic diversity (Θ) and population growth rate (g) using the lamarc package (Kuhner, 2006). lamarc implements a MCMC method for

sampling genealogies and calculates a likelihood curve used to determine the values of Θ and g that maximize the probability of originating the empirical data. lamarc analyses were performed with five replicates of 10 short chains each (500 genealogies sampled each 50 interactions and a burn-in of 1000 genealogies), and two long chains (20,000 genealogies sampled each 50 interactions and a burn-in of 1000 genealogies). For these analyses we did not consider the migration effect among populations. Because the PHI test indicated that the null hypothesis of no recombination could not be rejected, the parameter r (recombination) was not considered in the lamarc analyses. In addition, we used Tajima's D (Tajima, 1989), Fu's F_S test (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) to test the scenarios of population expansion. The significance of the tests was determined based on 10,000 coalescent simulations, which assumed neutrality and equilibrium conditions. These analyses were performed using DnaSP 4.10.9 (Rozas *et al.*, 2003).

RESULTS

Data characteristics

An alignment of 2048 bp of mtDNA (119 individuals) contained cyt *b* (1022 bp; 117 individuals), *ND2* (1041 bp; 118 individuals) and *ND3* (345 bp; 119 individuals). We obtained 914 bp of the nuclear intron *Fib7* from 107 individuals. No indels were present in the mitochondrial alignment, but some were identified in the *Fib7* dataset. All sequences were deposited in GenBank under the accession numbers JQ903619–JQ904023.

The mtDNA dataset was characterized by 826 (40.3%) variables and 785 (38.3%) parsimony-informative sites, and the *Fib7* dataset by 93 (10.2%) variables and 73 (8.0%) parsimony-informative sites. The GTR + I + Γ evolutionary model (pinv = 0.5353 and α = 1.3229) was selected for all partitions of mtDNA, while the HKY + Γ model (Γ = 0.2708) was selected for *Fib7*.

The uncorrected mtDNA distance between *Sclerurus* and its sister genus *Geositta* (Irestedt *et al.*, 2009; Derryberry *et al.*, 2011) was 18.4%. Within *Sclerurus*, mitochondrial genetic distances ranged from 16.1% (South American *Sclerurus mexicanus* versus *Sclerurus scansor*) to 2.7% (*S. scansor* versus *Sclerurus albigularis*). Although pronounced genetic structure was found within all species, the mean genetic distances among populations within species were quite variable, ranging from 12.1% between the two major clades of the broadly defined *S. mexicanus* (Central versus South American populations) to 0.16% among *S. scansor* populations (Appendix S3).

Phylogeny of *Sclerurus* species

The phylogenetic ML and BI analyses of the mitochondrial and nuclear data sets strongly support the monophyly of the genus *Sclerurus*. Two main clades were identified within *Sclerurus* (Fig. 1). The first includes four light-throated species segre-
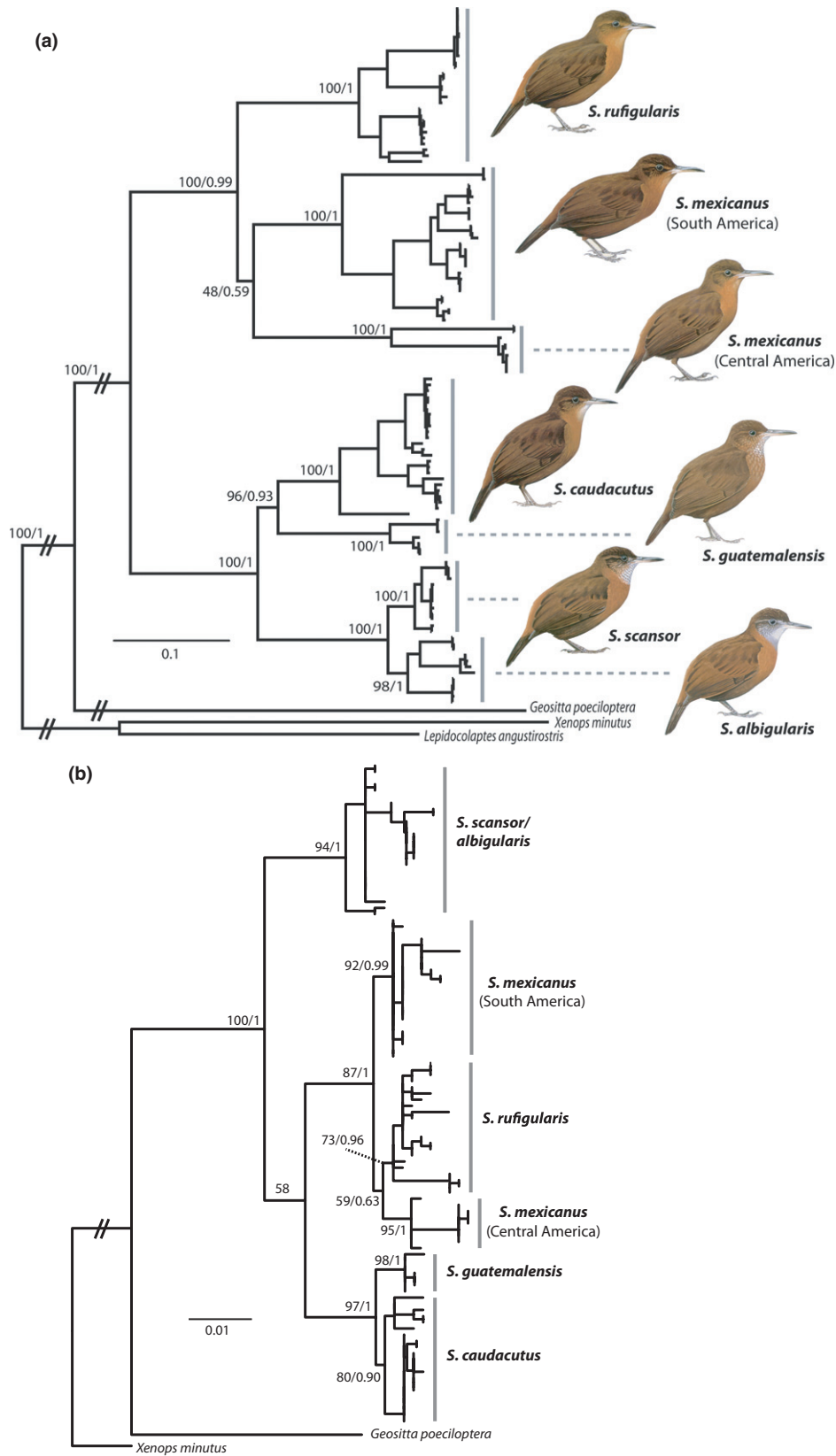
Figure 1 Phylogenetic relationships of *Sclerurus* leaftossers. Maximum likelihood tree inferred from (a) 2408 bp of mitochondrial DNA (mtDNA; cyt *b*, *ND2* and *ND3*) and (b) from 914 bp of *Fib7*. Maximum likelihood bootstrap (1000 replicates) and Bayesian inference posterior probability values are shown at the nodes. Illustrations are from nominate subspecies, except for *Sclerurus mexicanus* from South America, which is represented by subspecies *S. m. obscurior* (courtesy of Lynx Edicions; *Handbook of the birds of the world*, Vol. 8, 2003).

gated into two clades: (1) a sister pair of cross-Andes lowland species, *Sclerurus caudacutus* and *Sclerurus guatemalensis*; and (2) a sister pair of a circum-Amazonian species of foothill montane forests, *S. albigularis* and an Atlantic forest species, *S. scansor* (Fig. 1). The second main clade in *Sclerurus* includes the rufous-throated species: the Amazonian lowland *Sclerurus rufigularis* that forms a polytomy with two deeply divergent *S. mexicanus* clades, one from Central America (Mexico to Darién) and the other from South America (Fig. 1). Except for *S. mexicanus*, all currently recognized species of *Sclerurus* were found to be monophyletic according to the mtDNA gene tree with high statistical support (Fig. 1a). Although the *Fib7* gene tree largely reflects the same phylogenetic structure of mtDNA, the reciprocal monophyly between *S. scansor* and *S. albigularis* was not recovered. However, because the geographical sampling for *S. albigularis* was the least comprehensive of all, our current results do not allow us to draw strong conclusions about the phylogeography of that species (Fig. 1b).

Intra-specific differentiation in *Sclerurus* leaftossers

A striking pattern recovered by the wide geographical sampling is the pervasive population genetic structure within all *Sclerurus* species (Fig. 2). We found congruence between *c.* 80% of the subspecies sampled and the intra-specific lineages in the mtDNA tree, suggesting an overall good correspondence between phenotypic and phylogeographical breaks in *Sclerurus*. Exceptions were the subspecies of *S. mexicanus* from north-western South America and Darién (see below). However, the observed phylogeographical patterns are even more complicated than those suggested by the currently recognized subspecies. These patterns are largely consistent with the known Neotropical areas of endemism (Figs 2 & 3) and are bounded by either known physiographic barriers such as the Nicaragua Depression, the Andes, the Madeira, Negro and Amazon/Solimões rivers, open diagonal (Caatinga, Cerrado and Chaco), or by ecological gradients such as different elevational zones along the Andean foothills (Figs 2 & 3).

Temporal patterns of divergence

Divergence time estimates were obtained for 27 nodes distributed throughout the *Sclerurus* phylogeny (Fig. 3). Diversification within the genus was estimated to have occurred over the last 12 million years and at a relatively constant rate. A pure-birth model of diversification provided the best fit to the data [diversification rate = 0.28, Akaike information criterion (AIC) = −11.41, ΔAIC = 0, log-likelihood = 6.71], although the LTT curve tended to border the lower end of the expected diversification rate under that model (Fig. 4). Likelihood ratio tests did not reject the pure-birth model as the best fit to the data in comparison with birth–death and two-rate models. Intra-specific lineages of different species separated by a common geographical feature were compared to test for spatial and temporal congruence. Few

patterns were common in both spatial and temporal aspects within *Sclerurus* (Fig. 3, Table 1). The divergence between lineages from Andean foothills and Amazonian lowlands (red line) occurred at similar times both in *S. albigularis* (Fig. 3, node 19) and in South American *S. mexicanus* (Fig. 3, node 6). Also, the split associated with the Amazon River (orange line) occurred at similar times in both *S. rufigularis* (Fig. 3, node 10) and *S. caudacutus* (Fig. 3, node 23).

Historical demography in Amazonia

Tests based on summary statistics suggest distinct demographic scenarios for different populations of *Sclerurus* leaftossers (Table 2). The F_S results for both mtDNA and *Fib7* datasets, as well as the R_2 result for the mtDNA dataset, indicate recent population expansion within the *S. rufigularis* population from WAN. Significant values were also obtained for the *S. caudacutus* and *S. rufigularis* populations from BSW. For *S. caudacutus* the F_S test was significant for *Fib7*, and the R_2 test was significant for *Fib7* and mtDNA. The F_S and R_2 tests within *S. rufigularis* were only significant for the mtDNA dataset. Tajima's *D*-test results were not significant for any of the studied populations (Table 2). A similar scenario emerged from LAMARC analyses (Fig. 5). Populations of *S. rufigularis* and *S. peruvianus* from GUY and BAN, respectively, exhibited 95% confidence intervals (CI) of the population growth rate (*g*) that overlapped with zero (Fig. 5); hence, the null hypothesis of constant effective population size was not rejected. On the other hand, positive *g* values were obtained for populations associated with WAN, WAS and BSW, suggesting scenarios of population expansion (Fig. 5).

DISCUSSION

Origin, diversification and systematics

Sclerurus is one of the oldest genus-level lineages in the Furnariidae radiation (mean estimated age of 22 Ma; *c.* 26 Ma in Derryberry *et al.*, 2011), yet the genus is relatively homogeneous in morphology and coloration, and has comparatively few species. Our phylogeographical results demonstrated deep genetic divergence within *Sclerurus* species, suggesting that the low phenotypic diversity in *Sclerurus* may obscure cryptic species-level diversity. Our results provide an opportunity for a better taxonomic interpretation of *Sclerurus* diversity, particularly in *S. mexicanus*.

In some cases, intra-specific divergence times, as observed in *S. mexicanus* (3.09–5.49 Ma) and *S. caudacutus* (2.43–3.97 Ma), surpassed the divergence times between distinct species, as in *S. scansor* and *S. albigularis* (1.74–2.81 Ma), and between many sister species pairs in the Furnariidae (Derryberry *et al.*, 2011) and other passerine birds (Roy *et al.*, 1997; García-Moreno *et al.*, 1999; Chesser, 2004). The high genetic diversity within *Sclerurus* species, the long time of isolation of differentiated populations and the potential geographical overlap in some of these forms highlight the need for an
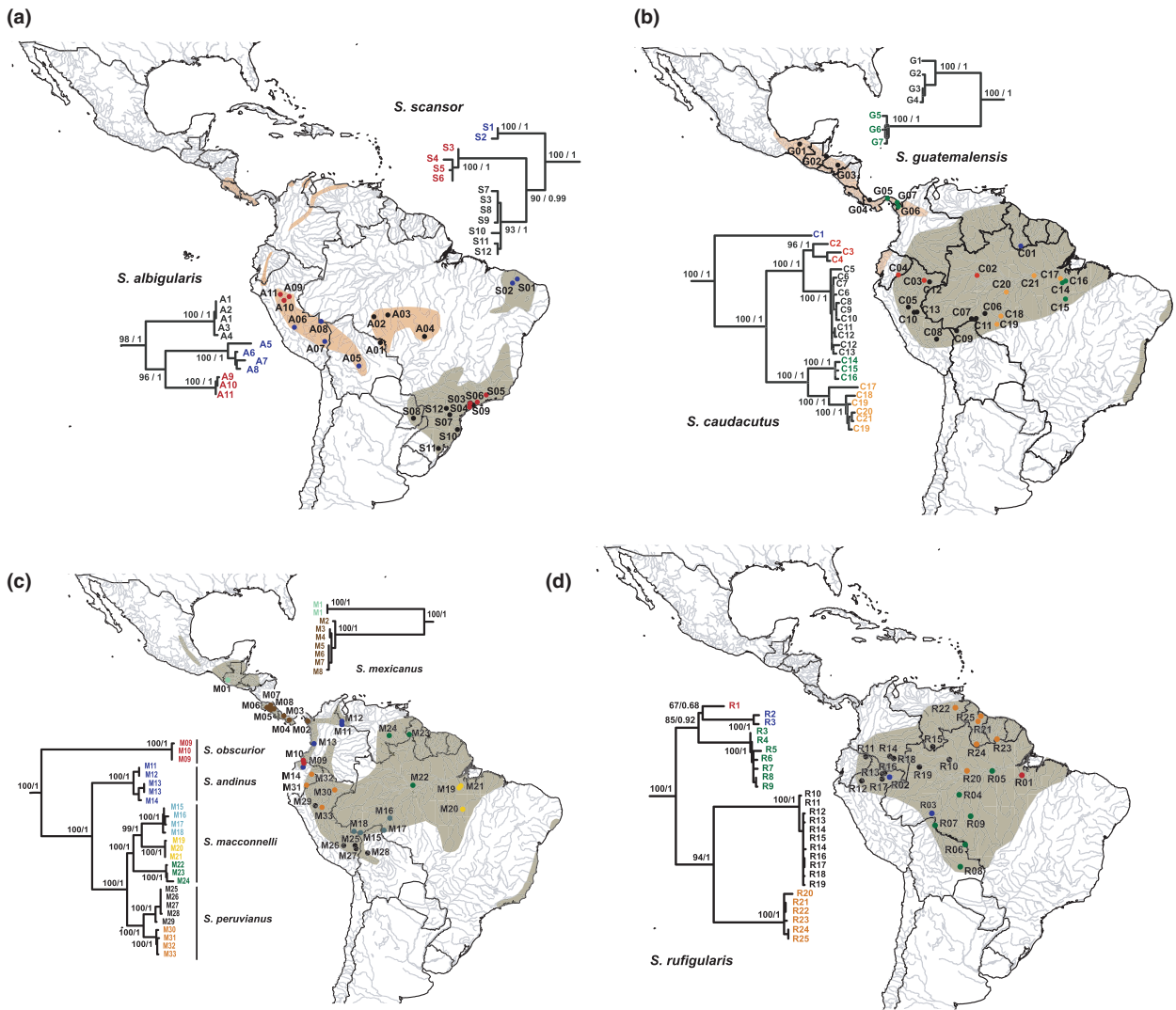
Figure 2 Geographical distribution of genetic variation in *Sclerurus* species in the Neotropics. Phylogeographies are depicted as maximum likelihood trees based on a mitochondrial DNA concatenated dataset with bootstrap and posterior probability values at the node. Individuals are labelled as in Appendix S1 and coloured according to geography and lineage. Each map depicts the overall geographical range of each species and the location of sampled localities coloured and labelled as in each tree and Appendix S1: (a) *Sclerurus albigularis* and *S. scansor*; (b) *S. caudacutus* and *S. guatemalensis*; (c) *S. mexicanus* of Central America (upper right), and *S. obscurior, S. andinus, S. peruvianus* and *S. macconnelli* of South America (lower left); and (d) *S. rufigularis*.

integrative systematic revision of this group to properly reflect this diversity in its taxonomic classification.

The non-monophyly of *S. mexicanus* given the phylogenetic uncertainty of its two major clades in relation to *S. rufigularis*, as well as the relatively ancient time of isolation among major clades, and the elevational parapatry of divergent lineages along the Andes slopes (see below) support a redefinition of species boundaries in this complex. First, the Central American lineages that include samples referable to the subspecies *Sclerurus mexicanus mexicanus* and *Sclerurus mexicanus pullus* (M1 to M8, Fig. 2c), should be regarded minimally as one full species. Second, divergent South American populations of *S. mexicanus* exhibit elevational parapatry on both sides of the Andes, which is a strong indication of evolutionary isolation and supports the elevation of these populations to species rank.

In particular, along the elevational gradient of the western Andes two deeply divergent clades were identified: one including the birds from the lowlands of Esmeraldas, Ecuador, near the type locality of the taxon *Sclerurus mexicanus obscurior* (M9 and M10, Fig. 2c); and other from the highlands (*c.* 1100–2000 m) of Ecuador, Colombia and Venezuela (M11–M14, Fig. 2c) corresponding to the range of *Sclerurus andinus*. In the eastern Andean slope we also identified two parapatric lineages along the elevational gradient: one referable to the taxon *Sclerurus macconnelli* is widespread throughout the Amazonian lowlands (M15–M24, Fig. 2c), while *Sclerurus peruvianus* includes populations from the Andean foothills and outlying ridges (M25–M33, Fig. 2c).

Therefore, we suggest the recognition of five species within the current *S. mexicanus*: (1) *Sclerurus mexicanus* Sclater, 1856
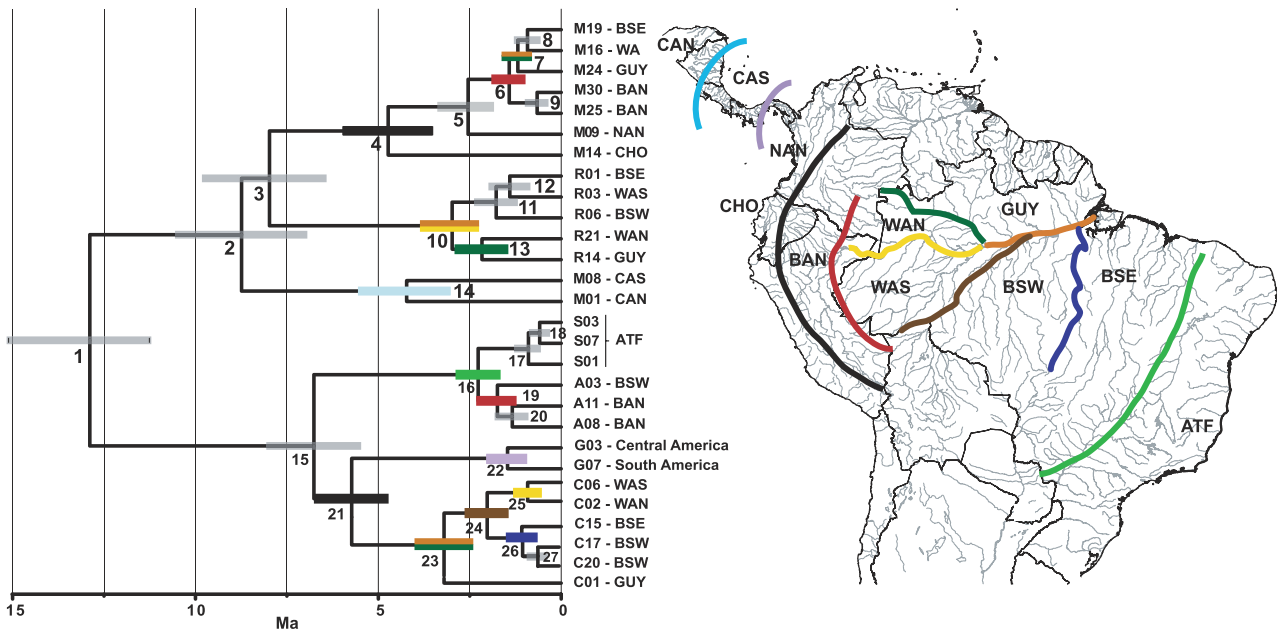
Figure 3 Chronogram of *Sclerurus* diversification in the Neotropics from BEAST analysis based on the concatenated mitochondrial DNA dataset. The bars on nodes represent the 95% confidence intervals around the mean divergence times, and are coloured according to the biogeographical break separating sister clades. Individuals analysed are identified as in Appendix S1. Biogeographical areas are: CAN (Central America – north), CAS (Central America – south), CHO (Chocó), NAN (northern Andes), BAN (base of the Andes), WAS (western Amazonia – south), WAN (western Amazonia – north), BSW (Brazilian shields – west), BSE (Brazilian shields – eastern), GUY (Guiana Shield), and ATF (Atlantic forest).
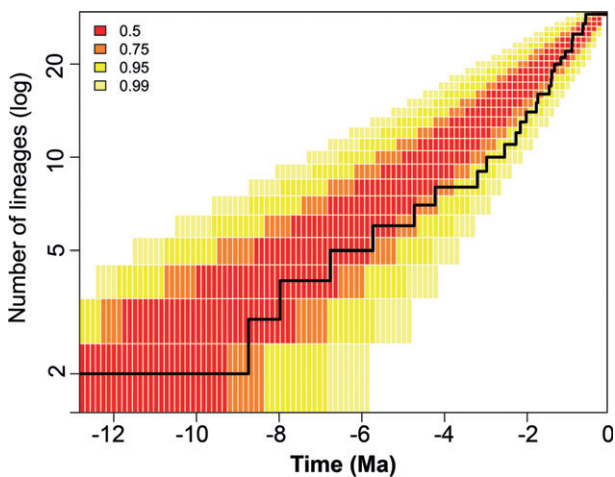

Figure 4 Lineage-through-time plot (logarithmic scale) and 95% confidence intervals (according to the inset legend) under the pure-birth model of *Sclerurus* lineage diversification in the Neotropics.

(type locality: Veracruz, Mexico) of Central America, comprising the ranges of *S. m. mexicanus* Sclater, 1856 and *S. m. pullus* Bangs, 1902; (2) *Sclerurus obscurior* Hartert, 1901 (type locality: Lita, Esmeraldas, Ecuador) of the Chocó lowlands of Ecuador and Colombia; (3) *Sclerurus andinus* Chapman, 1914 (type locality: Buenavista, above Villavicencio, Colombia) of the humid Andean slopes of western Ecuador, Colombia and western Venezuela; (4) *Sclerurus macconnelli* Chubb, 1919

(type locality: Ituribisci River, Guyana), comprising the Amazonian lowlands and, probably, the central Atlantic forest, corresponding to the ranges of subspecies *Sclerurus mexicanus macconnelli* and *Sclerurus mexicanus bahiae,* respectively; (5) *Sclerurus peruvianus* Chubb, 1919 (type locality: Yurimaguas, Loreto, Peru), comprising the populations of eastern Andean foothills from Bolivia to eastern Colombia, also reaching the lowlands and outlying ridges in north-western Amazonia. Hereafter we will follow this classification and refer to *mexicanus* taxa of Central American as *S. mexicanus*, and South American taxa as *S. obscurior*, *S. andinus*, *S. macconnelli* and *S. peruvianus*.

Biogeography

Central–South America disjunction

Despite the uncertainty of evolutionary relationships in the rufous-throated group, our estimates of divergence times indicate that the split between the Central (i.e. *S. mexicanus*) and all the South American lineages (i.e. *S. obscurior*, *S. andinus*, *S. macconnelli*, *S. peruvianus* and *S. rufigularis*) occurred during the Middle Miocene, between 6.5 and 10.5 Ma (nodes 2 and 3; Fig. 3, Table 1), and divergence between *S. guatemalensis* clades occurred during Late Pliocene–Early Pleistocene (from 1.0 to 1.9 Ma; node 22; Fig. 3, Table 1). Two hypotheses invoke specific timings of the origin of the Panamanian land bridge between Central and South America. It is generally held that the final closure of the

Isthmus of Panama occurred around 3.5 Ma (Coates *et al.*, 1992). An emerging alternative hypothesis based on recent fossil dating suggests that the final closure occurred between 12 and 20 Ma (Farris *et al.*, 2011; Montes *et al.*, 2012). Considering the ecological characteristics of *Sclerurus* (e.g. presumed low dispersal ability), this new temporal hypothesis is attractive because the dispersion between Central and South America, followed by the differentiation (node 2; Fig. 3, Table 1), would have occurred after the final closure of the Isthmus of Panama, and not before, as under the traditional geological dating. Differences in divergence times across the isthmus have also been observed in other terrestrial organisms (Koepfi *et al.*, 2007; Cody *et al.*, 2010; Smith & Klicka, 2010; Johnson & Weckstein, 2011).

Table 1 Ages and 95% confidence intervals (CI) for nodes of the time-calibrated phylogeny of *Sclerurus* leaftossers (Fig. 3). Minimum and maximum ages were determined for five nodes following Derryberry *et al.* (2011); see text for details.

Node	Age (Ma)	CI (Ma)	Calibration
1	12.89	11.26–15.11	11.26–16.55
2	8.74	6.99–10.51	
3	7.98	6.47–9.77	6.56–11.87
4	4.73	3.61–5.95	
5	2.55	1.92–3.31	
6	1.42	1.06–1.83	
7	1.19	0.85–1.54	
8	0.92	0.62–1.24	
9	0.66	0.40–0.94	
10	2.98	2.28–3.80	
11	1.78	1.24–2.33	
12	1.41	0.89–1.94	
13	2.17	1.53–2.84	
14	4.23	3.09–5.49	
15	6.76	5.52–8.01	5.40–8.60
16	2.27	1.74–2.81	1.72–3.43
17	0.90	0.61–1.23	
18	0.59	0.36–0.83	
19	1.74	1.30–2.24	
20	1.34	0.95–1.77	
21	5.73	4.80–6.68	3.69–6.68
22	1.47	1.00–1.98	
23	3.20	2.43–3.97	
24	2.02	1.52–2.56	
25	0.92	0.61–1.23	
26	1.08	0.75–1.43	
27	0.65	0.42–0.92	

Central America disjunction

The main phylogeographical break identified within *S. mexicanus sensu stricto* is associated with the Nicaragua Depression, the biogeographical barrier that defines the limits between the CAN and CAS. This barrier is important for a number of unrelated taxa (e.g. Duellman, 1999; Marshall & Liebherr, 2000; Pérez-Emán, 2005). The estimated divergence time between CAN and CAS lineages of *S. mexicanus* was substantial (from 3.09 to 5.49 Ma; node 14; Fig. 3, Table 1), placing the differentiation of *S. m. mexicanus* (CAN) and *S. m. pullus* (CAS) in the Late Miocene–Early Pliocene. Narrow landmasses (Kirby & MacFadden, 2005) and marine gaps occurred in this region during most of the Miocene (Coates & Obando, 1996). The estimated divergence time between the two Central American *S. mexicanus* lineages show a great overlap with that estimated for *Cerropidion* snakes (from 3.1 to 6.0 Ma; Castoe *et al.*, 2009), but is more recent than estimates for other snakes, *Bothriechis* and *Atropoides* (5.7–9.9 and 6.8–10.6, respectively; Castoe *et al.*, 2009), suggesting more than one vicariance event across the Nicaraguan Depression.
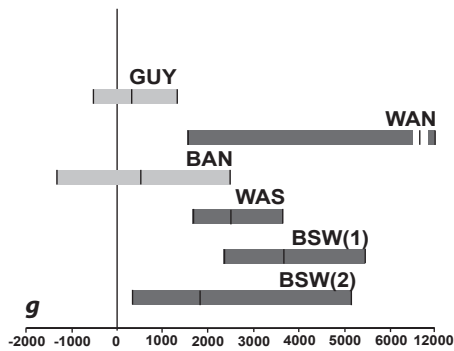
Table 2 Results of summary statistical tests applied to populations of *Sclerurus* leaftossers associated with distinct regions of Neotropics, for mitochondrial DNA (mtDNA; cyt *b*, *ND2* and *ND3*), and to *Fib7*.

Region	Species	Marker	*n*	Tests D	F_S	R_2
Base of the Andes (BAN)	*S. peruvianus*	mtDNA	5	−1.16 ns	1.21 ns	0.33 ns
		Fib7	10	1.32 ns	−0.63 ns	0.22 ns
Guiana shields (GUY)	*S. rufigularis*	mtDNA	6	−0.53 ns	1.10 ns	0.20 ns
		Fib7	10	0.05 ns	−0.91 ns	0.16 ns
Western Amazonia – north (WAN)	*S. rufigularis*	mtDNA	8	−1.60 ns	−8.19**	0.06**
		Fib7	8	−0.43 ns	−11.67**	0.11 ns
Western Amazonia – south (WAS)	*S. caudacutus*	mtDNA	11	−0.45 ns	−0.48 ns	0.19 ns
		Fib7	16	−0.41 ns	−1.75 ns	0.16 ns
Brazilian shields – west (BSW)	*S. caudacutus*	mtDNA	5	−0.68 ns	−0.75 ns	0.14*
		Fib7	10	−0.85 ns	−5.99**	0.11*
	S. rufigularis	mtDNA	7	−1.18 ns	−2.94*	0.12*
		Fib7	14	0.36 ns	−1.12 ns	0.17 ns

n, number of sequences; *D*, Tajima's (1989) *D*-test; F_S, Fu's (1997) F_S-test; R_2, Ramos-Onsins & Rozas' (2002) R_2-test.
ns, not significant; *$P < 0.05$; **$P < 0.01$.

Figure 5 Values of exponential population growth rate (*g*) of *Sclerurus* lineages from distinct Amazonian biogeographical regions. Light grey bars represent populations for which 95% confidence intervals of *g* include zero, hence demographic stability is inferred; dark grey bars represent populations that have experienced demographic expansion. GUY: Guiana Shield (*S. rufigularis*); WAN: western Amazonia – north (*S. rufigularis*); WAS: western Amazonia – south (*S. caudacutus*); BAN: base of the Andes (*S. peruvianus*); BSW: Brazilian shields – west (1, *S. rufigularis*; 2, *S. caudacutus*).

Disjunctions across the Andes

The importance of the Andes in the differentiation of the Neotropical biotas is unquestionable, but their function as a primary (Chapman, 1917) or secondary (Haffer, 1967) barrier has been the subject of debate (e.g. Brumfield & Capparella, 1996; Ribas *et al.*, 2007).

The sister species pair *S. caudacutus–S. guatemalensis*, as well as *S. obscurior–S. macconnelli/peruvianus*, are isolated by the Andes (Fig. 2b,c, respectively). Divergence time estimates between these two cross-Andes pairs are largely similar, overlapping between 4.8 and 6.0 Ma (Fig. 3; nodes 21 and 4). The estimates of divergence time between cis- and trans-Andean lineages of lowland species in these *Sclerurus* taxa (Fig. 3, Table 1) and *Pyrilia* (6.8–8.8 Ma; Ribas *et al.*, 2005), suggest that those lineages diverged between the Late Miocene and Early Pliocene. On the other hand, pairs of montane lineages, i.e. *S. andinus* and *S. peruvianus* (up to 2200 m) diverged during the Late Pliocene (1.9–3.3 Ma; node 5; Fig. 3, Table 1) or, in the case of the *Pionus corallinus–Pionus mindoensis* group (up to 3000 m; Ribas *et al.*, 2007) during the Pleistocene. These results are congruent with the expected differential effect that the Andean uplift may have had in species with distinct elevational ranges. Additionally, the divergence times are compatible with the temporal evolution of the Andes (Gregory-Wodzicki, 2000).

In contrast, Ribas *et al.* (2007) estimated the divergence time between cis- and trans-Andean lineages of lowland lineages of *Pionus* as being between 0.34 and 1.41 Ma, indicating dispersal of populations after the Andes had already reached high elevations.

Large variation among divergence times across barriers could arise for a number of reasons, such as the stochasticity of

the coalescent processes, selection, demographic processes and ecological traits. Also, differential rates of molecular evolution between lowland and montane lineages (Gillman *et al.*, 2009) could potentially affect our interpretation of the influence of the Andean uplift on the diversification of *Sclerurus*. Such a pattern is suggested in our data, especially in the South American clade formed by *S. obscurior* (lowlands), *S. andinus* (montane), *S. macconnelli* (lowlands) and *S. peruvianus* (montane), where there appears to be a gradient of branch lengths that decrease with increasing elevation (see Fig. 2c).

Elevational zonation in the trans-Andean foothills

Along the continuum of humid forests from the Chocó lowlands to the slopes of the western Andes, two lineages seem to be segregated elevationally: *S. obscurior* restricted to the Chocó lowlands (M09 and M10, Fig. 2c), and *S. andinus* found locally from about 1000 m (often up to 2000 m) along the western slopes of the Andes from Ecuador to Venezuela (M11–M14, Fig. 2c). The two lineages are potentially syntopic at an intermediate point of the elevational and ecological gradient, where no obvious physical barrier is in place. Furthermore, our results indicate that the lowland Chocó (i.e. *S. obscurior*) and the Andean foothill species (i.e. *S. andinus*) have been evolutionarily isolated for a substantial length of time; they last shared a common ancestor in the Early Pliocene, between 3.6 and 6.0 Ma (node 4; Fig. 3, Table 1). The Andean foothill lineage originated during the Late Pliocene–Early Pleistocene, between 1.9 and 3.3 Ma (node 5; Fig. 3, Table 1). In addition, another case of elevational parapatry is observed between two divergent, non-sister lineages of the South American *mexicanus* group: *S. macconnelli* and *S. peruvianus*. These two species are in close geographical proximity in southern Peru and Bolivia but seem to occupy different elevations along the cis-Andean foothills (M25–M33 versus M15–M24; Fig. 2c). Although elevational zonation is a prominent characteristic of the Andean avifauna (Terborgh, 1977; Graves, 1985), so far only a few studies have documented ecological replacement of closely related, ecologically equivalent species along elevational gradients (Dingle *et al.*, 2006; Cadena, 2007).

Cis-Andean South America disjunctions

Several birds, including *Sclerurus*, exhibit high congruence in the geographical range boundaries of divergent populations across cis-Andean forests. However, our results and the available data strongly suggest that these biogeographical patterns emerged more than once as a result of distinct sequences of cladogenetic events (e.g. Marks *et al.*, 2002; Aleixo, 2004; Ribas *et al.*, 2011).

Recent studies have shed new light on the processes of landscape change in Amazonia since the Miocene (Hoorn *et al.*, 1995, 2010; Rossetti *et al.*, 2005; Campbell *et al.*, 2006). The available data support the idea that different areas of Amazonia experienced distinct rates of landscape change, with WA (see Fig. 3) being the most dynamic (Hoorn *et al.*, 1995;

Rossetti *et al.*, 2005; Campbell *et al.*, 2006). Marine incursions during the Miocene (Räsänen *et al.*, 1995), as well as the development of a mega-wetland system in western Amazonia during the Early to Middle Miocene, caused by the Andean uplift (Hoorn *et al.*, 2010), would have isolated three regions of Amazonia (Bates, 2001; Aleixo & Rossetti, 2007): GUY, BS and BAN (Fig. 3). With the increase in the rate of Andean uplift the sedimentary wedge progressed to the east, promoting the disappearance of the mega-wetland system in western Amazonia and allowing the expansion of *terra firme* environments through this region.

The evolutionary relationships among *Sclerurus* lineages support the geographical predictions that emerge from this scenario. Three species have lineages associated with WA (*S. macconnelli*, *S. caudacutus*, *S. rufigularis*), but no WA lineage occupies basal positions regarding BA, BS or GUY lineages, whereas in *S. macconnelli* and *S. caudacutus*, GUY and BA lineages are basal in relation to WA lineages. In addition, demographic expansion was inferred for populations associated with WA (WAS, *S. caudacutus*; WAN, *S. rufigularis*), whereas the hypothesis of demographic stability was not rejected for populations from BA (*S. peruvianus*) and GUY (*S. rufigularis*) (Fig. 5). The two populations from BSW also exhibit clear signs of population expansion (Fig. 5), unlike that observed for *Xiphorhynchus* (Aleixo, 2004) and *Psophia* (Ribas *et al.*, 2011). These contrasting patterns in BSW are expected considering that this region was partially submerged by the wetland system (Bates, 2001).

Some authors support the hypothesis that the end of the WA mega-wetlands took place during the Miocene (Hoorn *et al.*, 1995; Figueiredo *et al.*, 2009), whereas recent studies indicate that the wetland system may have persisted until the Pliocene or Pleistocene (Rossetti *et al.*, 2005; Campbell *et al.*, 2006; Latrubesse *et al.*, 2010). The estimates of divergence time among WA lineages and the historical demographic results are congruent with the scenario of recent evolution of western Amazonian drainage: the divergence between WA and GUY/BS lineages was estimated at 2.2 Ma (95% CI, 1.5–2.8; *S. rufigularis*, Fig. 3, node 13, Table 1) and 2.02 Ma (1.5–2.6; *S. caudacutus*, Fig. 3, node 24, Table 1); and the divergence time between WAN and WAS lineages of *S. caudacutus* was estimated at 0.9 Ma (0.6–1.2; Fig. 3, node 25, Table 1).

The demographic results obtained for cis-Andean *Sclerurus* could be interpreted as temporally congruent with the mechanism proposed by Haffer (1969), but are highly contrasting with the palaeoenvironmental scenarios inferred for this region (Anhuf *et al.*, 2006), which suggest that forests of GUY and parts of the BS region would have been strongly reduced during glacial periods, while those of WA would have remained stable.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Tissue samples analysed, taxa, collection number and localities.
Appendix S2 Procedures of DNA extraction, amplification, sequencing and alignment.
Appendix S3 Mean pairwise (*p*) distances between and within species of *Sclerurus* leaftossers.

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BIOSKETCH

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Editor: Len N. Gillman