Biogeographical patterns and processes in the genus group Scotussae (Acrididae: Melanoplinae): an integrative approach

MARÍA CELESTE SCATTOLINI[1,2*], ANDRÉS LIRA-NORIEGA[3], VIVIANA ANDREA CONFALONIERI[4], SILVIA PIETROKOVSKY[4] and MARÍA MARTA CIGLIANO[1,2]

[1]*Centro de Estudios Parasitológicos y de Vectores, CEPAVE, CONICET-CCT La Plata, Argentina*
[2]*Museo de La Plata, División Entomología, FCNyM-UNLP, La Plata, Argentina*
[3]*Catedrático CONACyT, Instituto de Ecología, A.C., Red de Estudios Moleculares Avanzados, Veracruz, Xalapa, México*
[4]*Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (EGE–FCEN–UBA) and IEGEBA (UBA–CONICET), Ciudad Autónoma de Buenos Aires, Argentina*

A biogeographical study of the genus group Scotussae, a clade of grasshoppers endemic to the subtropical temperate region of the La Plata Basin, South America, was performed within a phylogenetic context to test whether wing reduction reflects evolutionary and ecological processes within the clade. We used an integrative biogeographical approach to determine the role of geohistorical events, geography, ecology and phylogenetic niche conservatism on the distribution and diversification processes of the group. We performed a total evidence phylogenetic analysis and tested the phylogenetic signal of ecological niche traits (niche optimum and niche breadth). We also assessed the degree to which phylogenetic distance is correlated with geographical and ecological niche traits and we used BioGeoBEARS to estimate ancestral ranges. The results provided evidence for phylogenetic niche conservatism as well as a significant association between phylogeny and both geographical and, more strongly, ecological traits. Two main clades were clearly associated with wing development, and evidence points to the evolutionary and ecological processes within these two groups being different. The Brachypterous clade shows evidence that allopatric speciation was the main source of diversification, while for the Macropterous clade sympatric speciation seems more likely.

ADDITIONAL KEYWORDS: brachyptery – diversification – ecological niche modelling – endemism – grasshopper – phylogenetic niche conservatism – phylogeny – South America.

INTRODUCTION

Understanding the historical and ecological processes that determine species distributions is a central goal of biogeography (Gaston, 1996). The roles of historical events and contemporary environments are not independent or mutually exclusive, because most of the 'historical' hypotheses ultimately rely, implicitly and explicitly, on 'ecology' (Brown, 2014). Phylogenetic niche conservatism (PNC) has recently been shown to be highly relevant for addressing many important issues in ecology and evolution, from species distributions and adaptations to understanding the biogeography of speciation (Peterson *et al.*, 1999; Losos *et al.*, 2003; Wiens, 2004; Hamlin *et al.*, 2017). Most PNC studies have focused on realized climatic niches as characterized by the geographical distributions of species (Colwell & Rangel, 2009), suggesting that PNC may be a widespread biogeographical pattern (Wiens & Graham, 2005; Wiens *et al.*, 2010; Peterson, 2011). However, the incorporation of temporal scale via phylogenetic hypotheses is also necessary given that

*Corresponding author. E-mail: celestescattolini@yahoo.com.ar

the use of temporal dimension allows the connection of evolutionary and geological events within historical biogeographical hypotheses (Ronquist & Sanmartín, 2011; Sanmartín, 2012; Ceccarelli *et al.*, 2016). Moreover, rates of niche evolution and consequently degree of PNC can vary depending on the extent of phylogenetic inclusiveness of a study (Losos, 2008; Peterson, 2011; Peixoto *et al.*, 2017; Smith *et al.*, 2019). Thus, it has been suggested that the analysis of niche similarity should be conducted within a phylogenetic framework and if possible at different phylogenetic scales (Diniz-Filho *et al.*, 2010a). Those approaches include tests of 'phylogenetic signal' (i.e. the 'tendency for related species to resemble each other more than they resemble species drawn at random from the tree'; Blomberg & Garland, 2002: 905) and comparisons of the relative fit of evolutionary models to the data (Wiens *et al.*, 2010; Münkemüller *et al.*, 2015).

The subtropical–temperate region of the La Plata Basin (Schivo *et al.*, 2019), which includes north-eastern Argentina, south-eastern Brazil, southern Paraguay, south-eastern Bolivia and Uruguay, is the fifth largest basin in the world and is the second largest in South America, next to the Amazon Basin. This region has undergone important historical events that have shaped its biota. During the Late Miocene–Early Pliocene (~11–3 Mya) the 'Paranean Sea' retreated, giving rise to the 'the Age of the Southern Plains', a widespread surface dominated by dry grasslands extending from northern Patagonia, and reaching central and northern Argentina, Uruguay and the eastern slopes of the rising Andes of Bolivia (Pascual & Bondesio, 1982; Marshall *et al.*, 1983; Pascual *et al.*, 1996; Ortiz-Jaureguizar & Cladera, 2006). In addition, during this period the first evidence of a glacial advance was recorded in southern South America (Mörner & Sylwan, 1989; Rabassa, 1999), generating a cooler climate, and more marked seasonality (Pascual *et al.*, 1996). During the Pleistocene, the Last Glacial Maximum (LGM) cyclical advance and retreat of glaciers produced a marked and concurrent expansion and retraction of arid (savannas, steppes) and humid (tropical and subtropical forests) biomes (Haffer, 1969; Brown & Ab'Sáber, 1979; Werneck *et al.*, 2011; Fregonezi *et al.*, 2013). This pattern permitted alternating opportunities for animals and plants living in these habitats (Hoorn *et al.*, 2010). All of these geohistorical events have generated distinctive ecoregions as a result of the combination of highly heterogeneous topographies, environmental conditions and soil characteristics. La Plata Basin is mainly constituted by the Pampas, the Humid Chaco, Alto Parana Atlantic Forest, the Cerrado and Atlantic Rainforest ecoregions. The Cerrado and Atlantic Rainforest are part of the Brazilian Highlands and can reach up to ~1800 m elevation, providing a wide variety of

environments determined by elevation that permitted altitudinal shifts (Hewitt, 2000; Lorenz-Lemke *et al.*, 2010). In the Atlantic Rainforest, several studies have shown that lowland clades have evidence of moderate to high levels of sympatry and lack of geographical phylogenetic structuring (Lavin, 2006; Pennington *et al.*, 2009; Pennington & Dick, 2010; Hughes *et al.*, 2013). For grassland species that inhabit highlands, the main driver of speciation would be isolation by distance (allopatric speciation) as a consequence of the glacial and interglacial periods (Fregonezi *et al.*, 2013). The Cerrado's complex landscape provides evidence for two distinctive diversification patterns. The phylogenetic structure of open vegetation species groups associated with the more ancient plateaus are expected to be characterized by high genealogical structure and genetic diversity, consistent with their older diversification ages (Werneck, 2011). Moreover, other studies have shown that the relatively high proportion of endemism in elevated plateaus compared with depressions indicates that ancient elevational gradients clearly affected faunal interchange and speciation in the Cerrado, at least for more sedentary organisms (Nogueira *et al.*, 2009). The Humid Chaco and Alto Parana Atlantic Forest ecoregions are considered as one unit (Misiones Nucleous) of the Seasonally Dry Tropical Forests (SDTFs) (Werneck, 2011). These forests have been considered as a unique relict biome left over from a formerly extensive uninterrupted biome present during the late Pleistocene (Prado & Gibbs, 1993; Pennington *et al.*, 2000; Prado, 2000). The consequences of the LGM cyclical vegetation expansions and retractions in this biome have not been studied (Pennington *et al.*, 2000, 2004, 2006) except for a few studies that point to Humid Chaco and Alto Parana Atlantic Forest as being historically stable refugia for different taxa (Nogueira *et al.*, 2011; Werneck *et al.*, 2011; Acosta & Vaschetto, 2017; Costa *et al.*, 2018). The Pampas is one of the world's richest grasslands in terms of size and biodiversity (Chen *et al.*, 2010). Recent studies considered it as an ecoregion of high stability (Costa *et al.*, 2018), and there is some evidence that the LGM has not strongly affected the diversification processes of its biota (Fregonezi *et al.*, 2013).

The La Plata Basin harbours the highest species richness values for melanopline grasshoppers in South America and it is characterized by a similar proportion of apterous/brachypterous (short-winged) and macropterous (long-winged) species (Scattolini *et al.*, 2020). A good example of this variability is found in the genus group Scotussae, consisting of 48 species encompassed within seven genera, which has diversified within this main region (Dinghi *et al.*, 2009; Cigliano *et al.*, 2020). The group is known to inhabit open grasslands and forest ecotones within the subtropical region

(Cigliano *et al.*, 1996). Previous analyses on dispersal abilities and distributional data suggested a clear and consistent association between wing development and biogeographical patterns in South American melanopline grasshoppers (Scattolini *et al.*, 2020) with short-winged grasshoppers having smaller distributions than long-winged grasshoppers. Nonetheless, these analyses did not consider phylogenetic aspects.

Here we performed a biogeographical analysis of the genus group Scotussae within a phylogenetic context to test if wing reduction is linked to the evolutionary and ecological processes of the clade. We follow an integrative approach to determine the role of several variables, including geohistorical events, geography, ecology and PNC on the distribution and diversification processes of the group.

MATERIAL AND METHODS

PHYLOGENETIC ANALYSIS

A total evidence approach was performed in this study where we included 55 specimens that represented 29 species of Melanoplinae; 25 species belonged to the Scotussae genus group, while the remaining four species were selected as outgroups (*Orotettix colcaensis*, *O. hortensis*, *O. paucartambensis* and *Neopedies brunneri*) (Supporting Information, Table S1). Most of the genera are represented by more than half of their constituent species (Table S1; Cigliano *et al.*, 2020), and only one genus, *Eurotettix*, is represented by one out of 12 species. This genus is morphologically homogeneous and almost exclusively distributed in the Cerrado, where the species are endemic and with small distributions (Cigliano, 2007). Because the focus of this study is to establish the relationships among genera, we consider that our taxon sampling, although not complete at the species level, can still help us to elucidate the diversity patterns of the group. Extraction and amplification of two mitochondrial gene fragments [NADH dehydrogenase subunit 2 (*ND2*) and cytochrome *c* oxidase subunit I (*COI*)] and one nuclear gene fragment [histone 3 (*H3*)] were performed using the methods of Husemann *et al.* (2013). Previous studies have demonstrated that these markers are useful for comparisons among grasshopper species (Husemann *et al.*, 2013; Guzman *et al.*, 2017; Pocco *et al.*, 2018; Scattolini *et al.*, 2018). Sequences for the three gene regions were examined, trimmed and aligned using Geneious 6.1.6 (http://www.geneious.com; Kearse *et al.*, 2012)). To avoid the possibility of working with *COI* pseudogene amplifications (Bensasson *et al.*, 2000), sequences were translated according to the invertebrate mitochondrial genetic code and examined, using as a reference amino

acid sequences obtained for several insect orders (Lunt *et al.*, 1996). A copy was assumed to be mitochondrial if it contained no frameshifts or stop codons (Sorenson & Fleischer, 1996; Zhang & Hewitt, 1996). JModelTest v.2.1.7 (Posada, 2008) was used to infer the most appropriate model of molecular evolution for each dataset (*COI*: GTR+I+G; *ND2*: TPM2uf+I+G, *H3*: F81+I) based on the Akaike information criterion (AIC) (Akaike, 1973).

Morphological data were obtained from a direct examination of multiple specimens collected and from materials of several institutions (Museo de La Plata; Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'; Facultad de Ciencias, Uruguay). The list of characters was based on the revised material and on characters described elsewhere (Cigliano & Ronderos, 1994; Cigliano *et al.*, 1996; Cigliano, 1997; Cigliano & Lange, 2001). The 30 morphological characters defined for the 29 species of Melanoplinae included general morphology (characters 0–17), external and internal male genitalia (8–27) and ovipositor valves (28 and 29) (Supporting Information, Figs S1, S2; Tables S2, S3).

The posterior distribution of the phylogenetic trees was explored using the Metropolis-coupled Markov chain Monte Carlo (MC3) algorithm as implemented in BEAST v.2.6.0 (Bouckaert *et al.*, 2014). An input file for BEAST was generated using the program BEAUti v.2.6.0 (Bouckaert *et al.*, 2014). All three genetic regions and the morphological characters were simultaneously analysed; each partition was treated as unlinked for substitution models but as linked for clock models and trees. The analysis was run for 10 000 000 generations to ensure convergence; trees were sampled every 1000 generations and the Yule process was chosen as the tree prior. Convergence was inferred through the effective sample sizes, which were all above 200. The first 1 000 000 trees (10%) were discarded as burn-in after ensuring the likelihood scores reached a plateau, which was determined with the program Tracer 1.7.1 (Rambaut *et al.*, 2014). We calculated the maximum clade credibility species tree with TreeAnnotator v.2.4.8 (Drummond & Rambaut, 2007). Trees were visualized in FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/), displaying posterior probabilities as branch support. Node ages were estimated with BEAST on a restricted dataset, taking into account only one individual to represent each species, using a generalized *COI* substitution rate of 1.6% substitutions per site per lineage/Myr, estimated from cave crickets (Allegrucci *et al.*, 2011).

OCCURRENCE AND ENVIRONMENTAL DATA

Occurrence records were obtained from specimens collected during field surveys, museum preserved

specimens (Museo de La Plata, Academy of Natural Sciences de Philadelphia, Muséum National d'Histoire Naturelle París, Museu de Zoologia da Universidade de São Paulo, Museu Nacional do Rio Janeiro, Facultad de Ciencias, Universidad de la República), literature and records available in the Orthoptera Species File (http://orthoptera.speciesfile.org/, Cigliano *et al.*, 2020). Records closer than 2.5 arc-minutes for each species were represented by a single and unique record and species with a high number of records were filtered at 5 arc-minutes (~10 km) in order to reduce risks of model overfitting (see below). The final database consists of 764 records for the 48 species of the genus group Scotussae and maps with these geographical species distributions are available in Orthoptera Species File (http://orthoptera.speciesfile.org/, Cigliano *et al.*, 2020).

To characterize the climatic niches for Scotussae grasshoppers, 19 bioclimatic variables and four summer solar radiation layers (December, January, February, March) were downloaded from WorldClim 2 (http://www.worldclim.org; Hijmans *et al.*, 2005; Fick & Hijmans, 2017) at a resolution of 2.5 arc-minutes (~5 km at the equator). Before conducting ecological niche modelling (ENM) to estimate areas of potential distribution, we selected individual background areas according to Barve *et al.* (2011). These areas were estimated based on the ecoregions (Olson *et al.*, 2001) where each species is distributed adding a 100-km buffer around the estimated area using QGIS 2.18.11 (http://qgis.osgeo.org). As climatic surrogates and to avoid issues with multi-collinearity (Dormann *et al.*, 2013), we used a principal component analysis (PCA) of the 19 bioclimatic variables and another PCA for the four solar radiation layers. Principal components (PCs) were produced for the geographical extent that encompasses all the ecoregions inhabited by the genus group Scotussae (i.e. all the species background areas), but models were calibrated across each taxon background area. We used the first three PCs for the bioclimatic variables and the first PC of the solar radiation, which explained 83.5% and 84.5% of the variance, respectively. In the environmental variables, PC1 corresponds mostly to a temperature axis, PC2 was dominated by precipitation, and PC3 represents the diurnal, seasonal and annual variation in temperatures.

ECOLOGICAL ANALYSES

We considered five occurrence records as a minimum to perform the ecological analyses (Pearson *et al.*, 2007; Schwallier *et al.*, 2016). We conducted the studies at two taxonomic levels: genus and species. The species-level analyses were conducted for 17 of the 21 ingroup species of the phylogenetic analysis for which we had more than five records, excluding *Chlorus chiquitensis*, *C. vittatus*, *Ronderosia gracilis*, *R. cinctipes* and *R. dubia*. For the genus-level analyses, we considered records from all 48 species: seven species of *Chlorus* (35 records), four species of *Dichromatos* (49 records), 12 species of *Eurotettix* (39 records), two species of *Atrachelacris* (51 records), 10 species of *Ronderosia* (166 records), five species of *Leiotettix* (52 records) and eight species of *Scotussa* (including *Leiotettix pulcher*, which resolved within this genus in the tree, see Results below) (418 records). Thus, we were able to evaluate the entire environmental variability for each genus.

NICHE SIMILARITY

To evaluate differences in the realized niches we implemented background similarity tests following Broennimann *et al.* (2012), using the first two PCs of the bioclimatic variables (which accounted for 71% of the overall variance) as the environmental dimensions. Presence data were used to perform niche similarity tests between taxa (at species and genus level) using the R package 'ecospat' (Broennimann *et al.*, 2012). We evaluated the degree of niche overlap using Hellinger's *I* distances (Van der Vaart, 1998) following the methods of Warren *et al.* (2008). Background similarity tests allowed us to assess for niche conservatism between each pair of taxa. The way these tests work is by comparing the observed niche overlap value between two taxa against the overlap between one taxon and the density of occurrences randomly shifted in the background area of the other taxon, thus allowing to tell whether two taxa are more similar than expected by chance.

NICHE MODELLING

We performed ENM using the first three PCs of the environmental variables and the first PC of the solar radiation variables. We followed two methodologies to generate niche models. We used the algorithm of maximum entropy as implemented in Maxent v.3.4.1 (Phillips *et al.*, 2006), to estimate a measure related to the environmental suitability of the species based on the Gibbs probability distribution. ENMeval was used to generate Maxent models using the logistic format, with a maximum of 500 iterations. Model calibration was performed evaluating models created with running models with beta-regularization multiplier values from 0.5 to 5, with increments of 0.5 and 29 feature classes (i.e. all combinations of linear, quadratic, product, threshold and hinge response types). Model performance was evaluated considering Training AUC, Test AUC and

AUC_diff, selecting the one that presented the best performance following Radosavljevic & Anderson (2014).

The second methodology was based on minimum volume ellipsoids as implemented by Osorio-Olvera *et al.* (2016), with the R package 'nichetoolbox' (Osorio-Olvera *et al.*, 2018). This is a simpler way to represent the *n*-dimensional hypervolume originally proposed by Hutchinson (1957). It considers a convex shape on environmental conditions within which suitable conditions for the species are higher towards the centroid of the ellipse (niche optimum) and less favourable towards the periphery, while the volume of the ellipse represents the niche breadth of the taxon being analysed. Because there were few records for most taxa, we performed the analysis using the covariance matrix of the input data.

Models with fewer than 14 records were evaluated with the delete-one jackknife optimization approach (Peterson *et al.*, 2011) using the pValueCompute program (Pearson *et al.*, 2007), as it has proven to perform well in studies with small sample sizes (Pearson *et al.*, 2007; Shcheglovitova & Anderson, 2013). Models for taxa with 14 records or more were evaluated with partial ROC (Peterson *et al.*, 2007) with an omission rate of 5%, 50% random points and 1000 iterations using the 'nichetoolbox' program (Osorio-Olvera *et al.*, 2018; http://shiny.conabio.gob.mx:3838/nichetoolb2/). For illustration purposes, ellipsoids were plotted using the first and second PCs of the environmental variables and the first PC of the solar radiation.

PHYLOGENETIC SIGNAL

To test for the phylogenetic signal of ecological niche traits, we considered both the centroid (niche optimum) and the volume of the ellipse (niche breadth) obtained from the ellipsoids model applying the *K* statistic (Blomberg *et al.*, 2003). This metric quantifies the 'phylogenetic signal relative to the expected signal for a given character under the Brownian evolution model according to a determined topology and length of branches' (Blomberg *et al.*, 2003). When $K = 1$, the variable exhibits the expected phylogenetic signal under the Brownian evolution model (null model or trait divergence proportional to time); when $K < 1$, the variable is considered to have a low phylogenetic dependence; and $K > 1$ indicates higher similarity than expected under the Brownian evolution model. The analyses were performed in R using the 'phylosignal' function of the 'picante' package, with significance estimated by 9999 permutations.

To evaluate if there was a correlation between the calculated phylogenetic relatedness with the ecological and geographical diversification, we performed Mantel's tests (Knouft *et al.*, 2006; Warren *et al.*, 2008; Diniz-Filho *et al.*, 2010b; Peixoto *et al.*, 2017). We calculated the phylogenetic distance matrix between pairs of species using the package 'ape' v.4.11 (Paradis *et al.*, 2004). For the geographical position of a species, we used the geographical centre (mean latitude and longitude) of each species' geographical range estimated from unique occurrences, and for the species niche optimum we used the ecological niche centroid from the ellipsoidal ecological niche models. Distances between species' geographical centres and ecological optima were calculated using Euclidean pairwise distances with the 'dist2' function. We also generated an environmental niche overlap matrix using the observed Hellinger's *I* values between taxon pairs. Because these values indicate degrees of overlap, and Mantel's test is a correlation between two dissimilarity matrices, the logarithm of the inverse of the matrix was calculated to work with data that resemble the variation of the original matrix. Finally, to assess the degree to which phylogenetic distance (patristic distance between taxa) is correlated with the geographical centre, the species niche optimum and the species niche overlap, we performed Mantel's tests with significance estimated by 9999 matrix permutations (Sokal, 1979; Borcard & Legendre, 2012) with the 'vegan' package in R (Oksanen *et al.*, 2019). A significant positive correlation would imply that closely related species are more similar ecologically (and/or geographically) than distantly related species.

ANCESTRAL RANGES AND BIOGEOGRAPHICAL/ DIVERSIFICATION EVENTS

To estimate ancestral ranges and speciation events, we applied probabilistic analysis of the ancestral range estimation implemented in the 'BioGeoBEARS' package (Matzke, 2014). BioGeoBEARS integrates and compares three commonly used data analysis algorithms in historical biogeography: dispersal-extinction-cladogenesis (Ree *et al.*, 2005); DIVALIKE, modified from the DIVA program of Ronquist (1997); and BAYAREALIKE, modified from the BayArea program of Landis *et al.* (2013). The phylogenetic analysis generated in BEAST was pruned to consider only one terminal per species, including external groups. The ecoregions scheme of Olson *et al.* (2001) was used to divide the species distributions into seven main areas (Fig. 1). These seven areas were the Cerrado, Dry Chaco, Pampas, Atlantic Rainforest and the Southern Biomes, which were all of the habitats in the south, and from the SDTFs (Werneck's, 2011) the Misiones Nucleous (Humid Chaco and Alto Paraná Atlantic Forest ecoregions) and a Western Nucleous (Chiquitano and Yungas ecoregions).
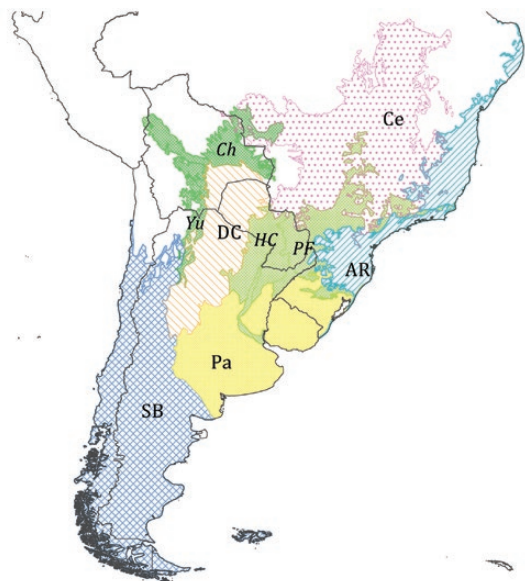
Figure 1. Map of the study area showing the South American ecoregions scheme (Olson *et al.* 2001). Acronyms in bold correspond to Cerrado (Ce), Dry Chaco (DC), Pampas (Pa), Atlantic Rainforest (AR) and Southern Biomes (SB). Acronyms in italics belong to ecoregions of the Seasonally Dry Tropical Forests (SDTFs), such as Humid Chaco (HC), Alto Paraná Atlantic Forest (PF), Chiquitano (Ch) and Yungas (Yu).

RESULTS

PHYLOGENETIC ANALYSIS

Amplification and sequencing of the three marker fragments (*COI*, *ND2*, *H3*) resulted in a total of 1390 base pairs (bp), of which 1067 bp corresponded to the two mitochondrial DNA sequences and 323 bp to nuclear DNA sequences. Supporting Information Table S1 provides the GenBank accession numbers for sequences generated for these analyses as well as those of previous publications.

The total evidence analysis applied herein recovered the monophyly of the Scotussae genus group, of all genera (if *Leiotettix pulcher* is included as a member of *Scotussa*) and of all species with high support (Fig. 2). Two main clades were recovered, one that included those genera with exclusively brachypterous species (*Chlorus*, *Eurotettix* and *Dichromatos*) and the other that encompasses the remaining genera, composed mainly of macropterous species. Within this clade, we recovered a close relationship between the genera *Atrachelacris* and *Ronderosia*, and between *Scotussa* and *Leiotettix*. The topology was mostly congruent with previous morphological (Cigliano, 2007; Cigliano & Lange, 2007; Dinghi *et al.*, 2009) and molecular (Castillo *et al.*, 2019) partial phylogenetic reconstructions for the group.

Based on the calibrated Bayesian tree generated in BEAST (Fig. 2), the most common recent ancestor of the genus group Scotussae is hypothesized to have diverged *c.* 4.59 Mya during the Pliocene and subsequent diversification occurred mostly during the late Pliocene and Pleistocene.

ECOLOGICAL ANALYSES

Niche similarity

Values for realized niche overlap between species are presented in Supporting Information Table S4. As there were species with very wide distributions, only those with significant overlap in both directions are indicated in bold (Table S4). Hellingers's *I* index showed that of the 136 comparisons between species, 47 species pairs showed significant niche overlap.

At the genus level, the genera within the Brachypterous clade (*Chlorus*, *Eurotettix* and *Dichromatos*) did not show significant overlap among each other (Table 1; Fig. 3), while most of the remaining genera, all within the Macropterous clade, had significant niche overlap (except for *Atrachelacris–Leiotettix*).

Niche modelling

Results from ENM performed on species with Maxent and ellipsoids yielded similar geographical distributions. Adequate models were generated for all species, except for *C. spatulus* and *R. paraguayensis* with both methodologies and for *D. schrottkyi* with Maxent (Supporting Information, Table S5). Because both methodologies gave similar distributional results but one species model was not validated with Maxent, thereafter we used the ellipsoid-based models as in Scattolini *et al.* (2018).

Species within the Brachypterous clade showed low values of niche breadth while species within the Macropterous clade had higher values (Supporting Information, Table S6). Genera within the Brachypterous clade showed low values of environmental and geographical overlap (Fig. 3). Within this group we observed the highest niche breadth values in the genus *Chlorus* (Fig. 3), which is distributed in several ecoregions of the SDTFs such as Humid Chaco, Alto Paraná Atlantic Forest, Chiquitano and Yungas, as well as in the northern region of Dry Chaco. The sister genera *Eurotettix* and *Dichromatos* had very narrow niche breadths (Fig. 3), but they were clearly differentiated in their environmental and geographical spaces. *Dichromatos* inhabits the Alto Paraná Atlantic Forest and the southern regions of the Atlantic Rainforest (Fig. 3), while *Eurotettix* is mainly distributed in the southern portion of the Cerrado ecoregion, barely occupying the boundary area between
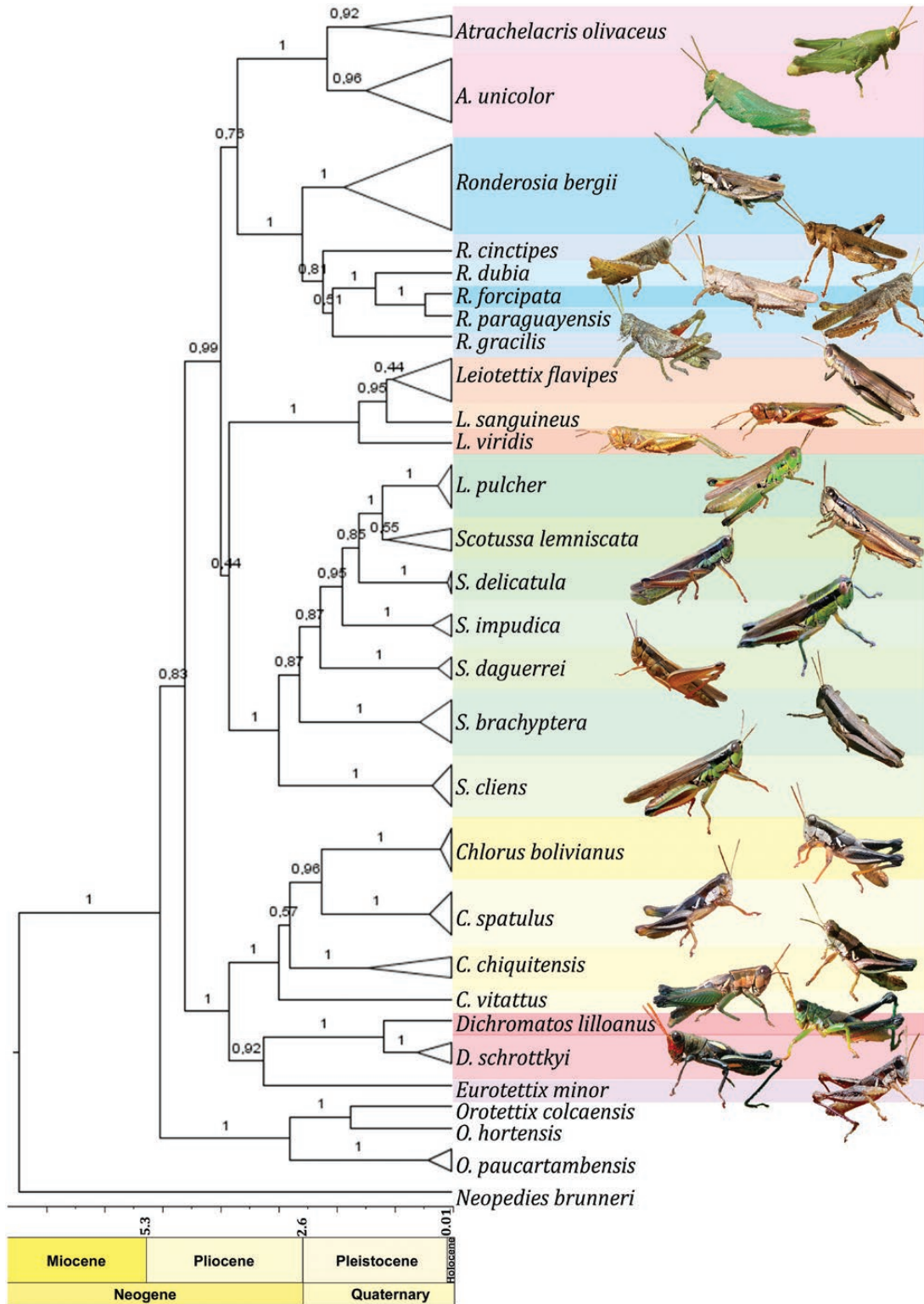
Figure 2. Total evidence consensus tree for 25 species of the genus group Scotussae and four outgroup species obtained from the Bayesian analysis of 30 morphological characters and the *COI*, *ND2* and *H3* dataset. Numbers above branches indicate posterior probabilities. Major geological epochs are indicated below the tree. Each genus was assigned a respective colour palette.

Table 1. Results from the similarity tests for niche conservatism between genera niches based on Hellinger's *I* distance

	Chlorus	*Dichromatos*	*Eurotettix*	*Atrachelacris*	*Ronderosia*	*Leiotettix*	*Scotussa*
Chlorus							
Dichromatos	0.09						
Eurotettix	0.18	0.07					
Atrachelacris	**0.32**	0.26	0.17				
Ronderosia	0.28	**0.37**	**0.18**	**0.65**			
Leiotettix	0.17	**0.29**	0.12	0.41	**0.65**		
Scotussa	0.25	**0.35**	0.09	**0.55**	**0.84**	**0.64**	

Bold indicates that niches are more similar than expected by chance ($P < 0.05$).

the Humid Chaco and Alto Paraná Atlantic Forest (Fig. 3).

The Macropterous clade showed high values of niche breadth and of environmental and geographical overlap (Fig. 3). The sister genera *Atrachelacris* and *Ronderosia* are distributed in the Pampas, Humid Chaco and Alto Paraná Atlantic Forest, with the latter genus having a slightly wider distribution (Fig. 3). The genus *Scotussa* inhabits the Pampas, some regions of the Alto Paraná Atlantic Forest and the southern portion of the Humid Chaco (Fig. 3). The sister genus *Leiotetix* had the narrowest niche breadth within the Macropteros clade, having a northern distribution: in the Alto Paraná Atlantic Forest, Humid Chaco and the northern portion of the Pampas (Fig. 3).

Phylogenetic signal

The phylogenetic signal in the niche breadth and in the niche centroid values of the PCs was evaluated by means of the *K* statistic (Blomberg *et al.*, 2003). We found that PC1–environment (which mainly represents temperature) and PC1–radiation were the only components with a significant phylogenetic signal and both were more similar than expected under a Brownian motion model (i.e. $K > 1$; Table 2).

Mantel's tests showed a significant positive correlation between phylogenetic distance with the geographical centre, species niche optimum and species niche overlap (Table 3). We also found that phylogenetic distance had a higher positive association with distance to the environmental centroid ($R = 0.31$) than to the geographical centre ($R = 0.22$). Thus, phylogeny had a greater association with environmental distance, compared to geographical distances and with the logarithm of the inverse of the superposition matrix (Table 3).

Ancestral ranges and biogeographical events

BioGeoBEARS analysis indicated that the genus group Scotussae would have originated in the SDTFs and Pampas (Fig. 4) and BAYAREALIKE was recovered

as the most probable model (Table 4). The ancestor of the Macropterous clade would have retained this distribution as well as the ancestor of two of its genera (*Scotussa* and *Leiotettix*) and most of its descendants. The *Ronderosia–Atrachelacris* ancestor also maintained this distribution, but *Atrachelacris* would have remained in the Misiones Nucleous and Pampas while the *Ronderosia* ancestor inhabited these areas, also invading the Cerrado region (Fig. 4). The ancestor of the Brachypterous clade would have remained exclusively in the SDTFs, as well as the genus *Chlorus*. The ancestor of *Dichromatos* remained only within the Misiones Nucleous and dispersed into the Atlantic Rainforest and *Eurotettix* also remained within the Misiones Nucleous and dispersed into the Cerrado.

DISCUSSION

PNC has been widely discussed to explain diversity patterns (Wiens & Graham, 2005; Losos, 2008; Wiens *et al.*, 2010; Peterson, 2011; Pyron *et al.*, 2015). Under PNC, closely related species should be more similar ecologically than those that are distantly related (Holt, 2009; Losos, 2008). The similarity can be measured as the overlap in climatic conditions associated with the geographical distributions of species (Broennimann *et al.*, 2012). A study conducted in bats on a global scale indicated that although more than 50% of the species had significant realized niche overlap, it was not possible to associate these trends with phylogenetic relationships (Peixoto *et al.*, 2017). Similar results were observed in the closely related species of the Andean grasshopper genus *Orotettix* (Scattolini *et al.*, 2018). However, our results here show that while less than 35% of the species had significant realized niche overlap, a phylogenetic signal was found with values higher than expected under a Brownian motion model ($K > 1$), suggesting that closely related species are more similar in their realized niches than distantly related ones. Also, our results show a significant
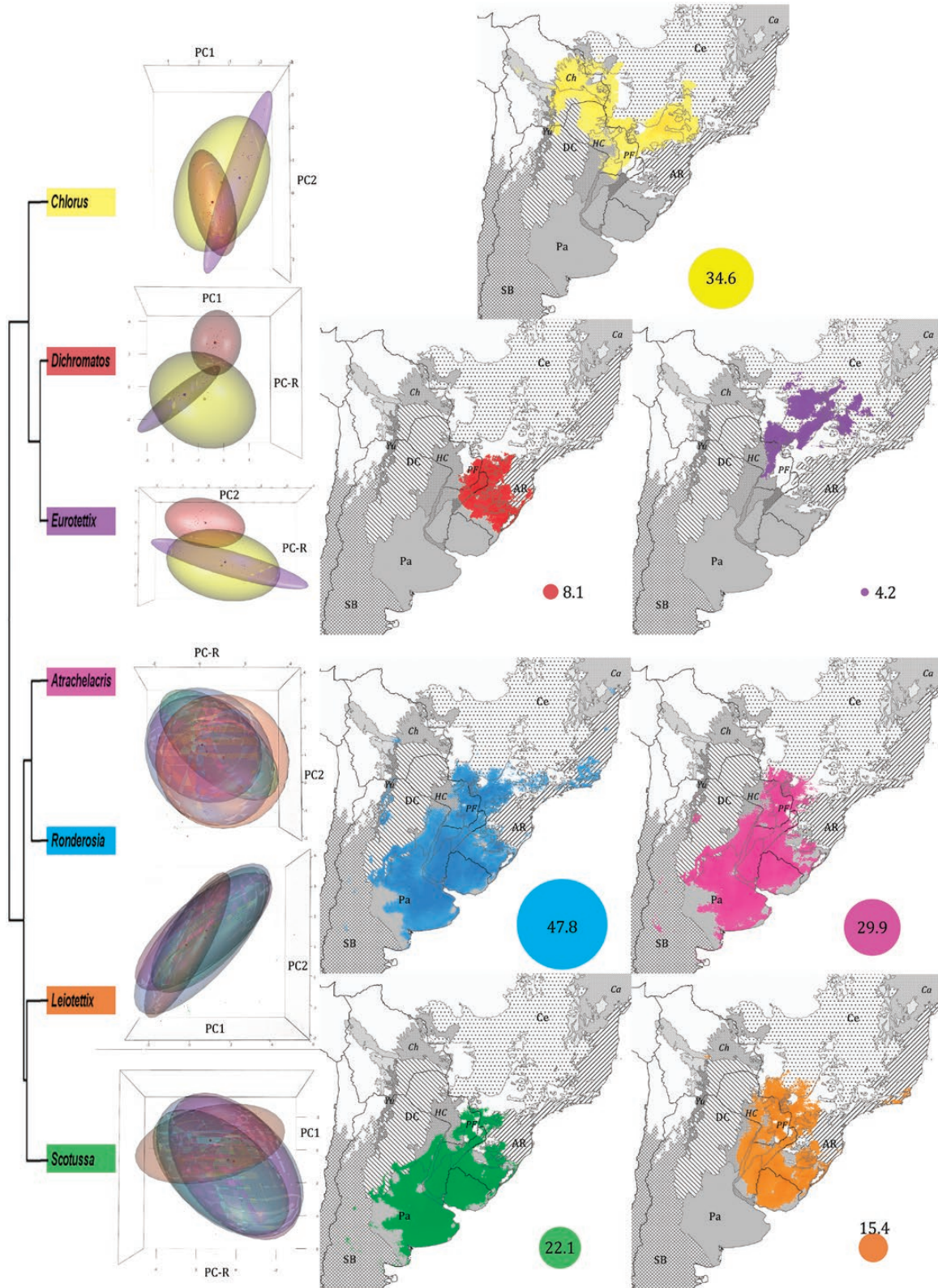
Figure 3. Niche models generated with Ellipsoid methodology for the genera of the Scotussae group, following the phylogenetic relationships obtained. The ellipsoids were plotted on the first and second principal components (PCs) of the

Table 2. Results from the phylogenetic signal performed with the *K* statistic in the niche parameters from the Ellipsoids models; niche breadth (NB) and niche centroid values of the four principal components (PC1_rad, PC1, PC2 and PC3)

Variable	NB	PC1_Rad	PC1	PC2	PC3
K	0.9	**1.02**	**1.2**	0.85	0.79

Significant values are shown in bold (*P* < 0.05).

association between the phylogenetic relationships and geographical centre, as well as realized niche overlap and niche optimum, with the latter variable having the strongest association of all.

We found greatest niche overlap for the group in the Misiones Nucleous of the SDTFs. Historical biogeographical analyses have indicated that the genus group Scotussae would have originated during the Pliocene, a period known as 'the Age of the Southern Plains' given the new and extremely widespread grasslands habitats that dominated this region at that time (Pascual & Bondesio, 1982; Marshall *et al.*, 1983; Pascual *et al.*, 1996; Ortiz-Jaureguizar, 1998). During this period, a cooler climate with a much more marked seasonality appeared (Pascual *et al.*, 1996). These conditions marked an optimal scenario for the appearance of this group of subtropical grasshoppers adapted to open grasslands and forest ecotones. Furthermore, the subsequent diversification occurred mainly during the Pleistocene in the SDTFs, when SDTFs were much greater in extent (Prado & Gibbs, 1993; Pennington *et al.*, 2000; Prado, 2000). Moreover, the strong association between phylogeny and environmental conditions, together with the high degree of environmental overlap found in the Misiones Nucleous, allows us to infer that the Scotussae ancestor would have populated open SDTFs with similar environmental characteristics to those currently found in this region.

According to Pyron *et al.* (2015), studies that relate PNC to patterns of speciation should consider additional factors that may promote this process, such as physical heterogeneity (e.g. topography) and environmental heterogeneity (e.g. temperature, precipitation) (Coyne & Orr, 2004). In addition, intrinsic characteristics related to the mobility of species can also influence

Table 3. Results of Mantel's tests for analyses of correlation between phylogenetic distances with niche overlap, geographical centroids and ecological niche optimum

| Mantel's tests		N.O.	G.C.	I.I.
Phylogeny	*R*	0.31	0.22	0.20
	P	**0.005**	**0.032**	**0.019**

Phylogeny refers to phylogenetic distance, N.O. to niche optimum, G.C. to geographical centroids, I.I. to the inverse logarithm of Hellinger's *I* overlap values, *R* to Mantel's correlation coefficient and *P* to the associated probability.

biogeographical and diversification patterns. Recent studies have shown that wing development can be a key factor associated with these patterns in several groups of insects (Malmqvist, 2000; Ikeda *et al.*, 2012; Homburg *et al.*, 2013; McCulloch *et al.*, 2017), including South American melanopline grasshoppers (Scattolini *et al.*, 2020). The two main clades recovered in the present study are clearly associated with wing development and they represent a perfect scenario for analysing their PNC patterns. Our findings support different patterns of niche overlap for these clades, at both the species and the genus level, which would imply a differing evolution of these two clades.

BRACHYPTEROUS CLADE

The ancestor of the Brachypterous clade would have inhabited the SDTFs, as would the genus *Chlorus*. The ancestor of *Eurotettix* would have remained in the Misiones Nucleous and dispersed into the Cerrado, where most of its species are currently distributed, while the ancestor of *Dichromatos* would have dispersed into the Atlantic Rainforest. These latter two ecoregions form the Brazilian Highlands, which provide a wide variety of environments determined by elevation patterns that permitted altitudinal shifts (Hewitt, 2000). The cyclical changes in temperature during the LGM would have generated contractions and expansions of the SDTFs, Cerrado and Atlantic Rainforest (Haffer, 1969; Brown & Ab'Sáber, 1979; Werneck *et al.*, 2011) favouring the existence of refugia and of multiple microrefugia (Werneck *et al.*, 2011; Perez *et al.*, 2016; Costa *et al.*, 2018). A PNC pattern in regions with high physical and low environmental

environmental variables and the first PC of the solar radiation variables. Each genus was assigned a respective colour that is consistent across the cladogram, the ellipsoid and the map representation. The geographical representations of the ellipsoids are plotted onto the South American ecoregions scheme (Olson *et al.* 2001). Acronyms in bold correspond to Cerrado (Ce), Dry Chaco (DC), Pampas (Pa), Atlantic Rainforest (AR) and Southern Biomes (SB). Acronyms in italics belong to ecoregions of the Seasonally Dry Tropical Forests (SDTFs), such as Humid Chaco (HC), Alto Paraná Atlantic Forest (PF), Chiquitano (Ch) and Yungas (Yu). A proportional circle representing the niche breadth value for each genus is drawn in the lower right corner of each map.
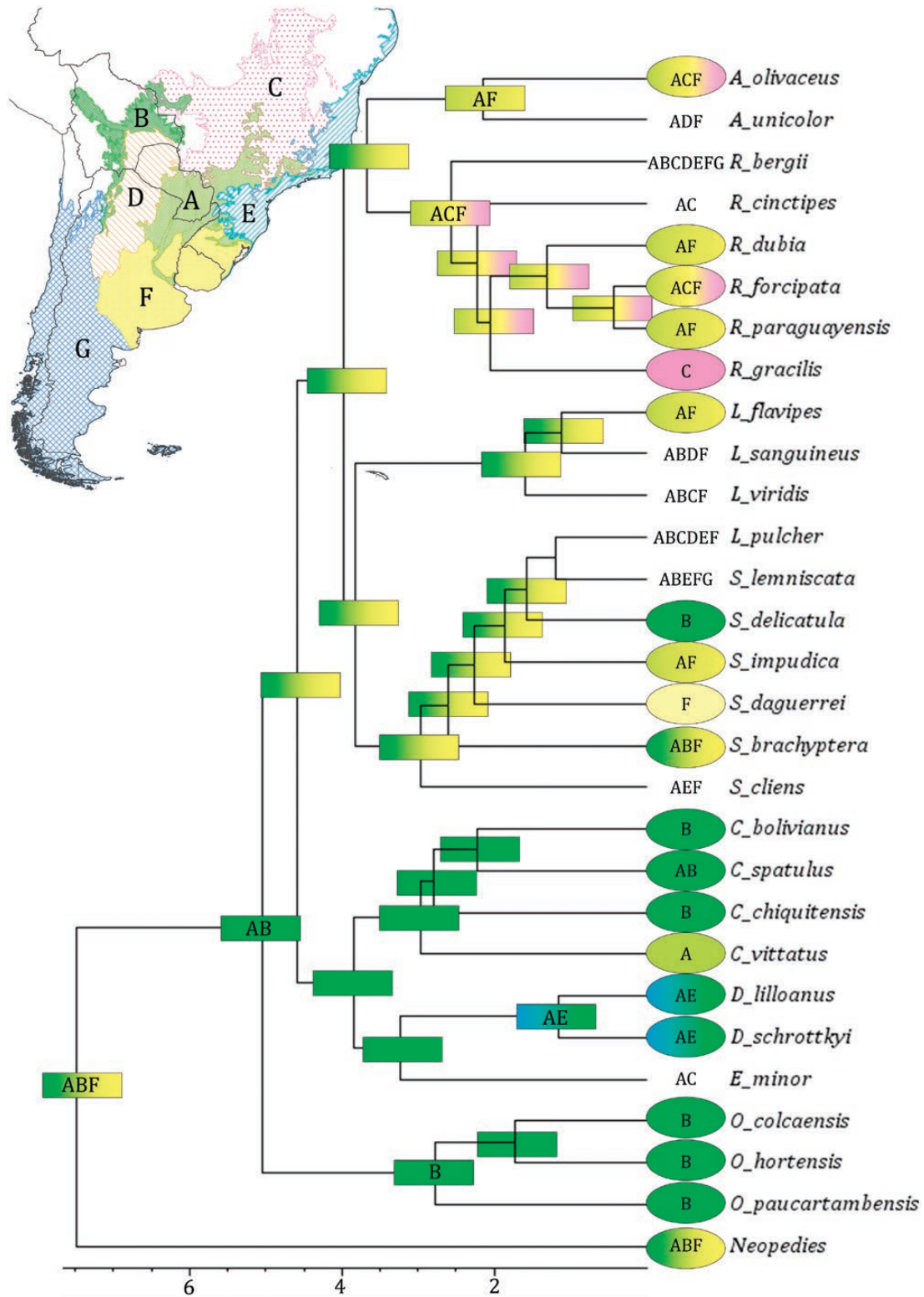
Figure 4. Ancestral range estimations for the Scotussae genus group obtained with 'BioGeoBEARS'. States at the nodes represent the possible geographical range just before and after each speciation event of the most probable model in each clade. Main biogeographical areas are shown on the map as different colours and letters. Seasonally Dry Tropical Forests (SDTFs) are coded as two shades of green, represented as light green (A) = Misiones Nucleous and dark green (B) = Western

Table 4. Comparison between models estimated in 'BioGeoBEARS' for the Scotussae genus group showing log-likelihood (Ln*L*), dispersal (*d*) and extinction (*e*) values

Model	Ln*L*	No. of parameterss	*d*	*e*
DEC	−116.73	2	0.069	0.00000
DIVALIKE	−122.42	2	0.081	0.00000
BAYAREALIKE	**−100.81**	2	0.028	0.14551

The model selected as the best fit for the ancestral area estimation by 'BioGeoBEARS' is in bold.

heterogeneity generates 'hard allopatry' (Hickerson & Meyer, 2008; Pyron & Burbrink, 2010; Pyron *et al.*, 2015). Hard allopatry is caused by physical barriers to gene flow that disrupt an ecologically homogeneous habitat; geographical barriers limit gene flow so that ecological selection may be relatively unimportant for driving speciation. This evidence is consistent with results found herein, where most species within the Brachypterous clade showed low values of niche breadth and of environmental and geographical overlap, with no evidence of significant realized niche overlap. Similar drivers of diversification have been proposed for several taxonomic groups that inhabit these regions (Behling & Pillar, 2007; Lorenz-Lemke *et al.*, 2010; Fregonezi *et al.*, 2013; Perez *et al.*, 2016). The historical biogeographical analysis rendered BAYAREALIKE as the most probable model, which would suggest sympatric speciation. However, while integrating the results obtained by the different analyses conducted herein we consider that allopatry seems to be the most probable diversification event within this clade.

It could be argued that the lower values of niche breadth found within the Brachypterous clade could be related to sampling bias. However, we believe that this should not be the case because extensive collections (from 1960) have been made as a result of acridological surveys led by R. A. Ronderos, C. A. Carbonell, C. Amédégnato, M. L. Monné and our research group throughout most of the distribution area of the clade (see Cigliano *et al.*, 2020 for an exhaustive list of references). Most of the brachypterous species within Scotussae are rare with highly endemic, restricted distributions. This pattern has also been found in a recent study on South American melanopline grasshoppers, where the niche breadth of brachypterous species is significantly narrower than for macropterous ones (Scattolini *et al.*, 2020).

MACROPTEROUS CLADE

The Macropterous clade had quite different patterns from the Brachypterous clade. Most species have high values of niche breadth and environmental and geographical overlap, with significant values of realized niche overlap. These results are congruent with the model obtained by BioGeoBEARS, which gave sympatric speciation as the most probable diversification event. The ancestor of the group would have inhabited the SDTFs and the Pampas, where all genera are currently still distributed, specifically in the Misiones Nucleus of the SDTFs.

The Pampas ecoregion has mainly flat or slightly undulating topographies, with some low-elevation mountains (<1200 m) (Cabrera & Willink, 1973). It was not strongly affected by the expansion of forest over the grasslands during glacial and interglacial periods (Fregonezi *et al.*, 2013) so it is considered an ecoregion of high stability (Costa *et al.*, 2018). According to Pyron *et al.* (2015), PNC in regions with low physical and environmental heterogeneity will not act strongly to promote population divergence, and speciation must occur either by intrinsic mechanisms (e.g. autopolyploidy) or by sexual selection (Coyne & Orr, 2004). Recent studies in *Ronderosia* suggest that chromosome rearrangements (such as various systems for determining neo-sexual chromosomes and autosoma–autosoma central fusion) may have played a key role during speciation processes (Castillo *et al.*, 2010, 2019). In addition, long-term stability of biomes has been proposed to generate complex biotic interaction networks and, eventually, co-evolutionary processes (e.g. Jansson & Dynesius, 2002; Dalsgaard *et al.*, 2011; Costa *et al.*, 2018). *Scotussa* is another genus with many species among the group, characterized by morphological adaptations in the ovipositor structure that allowed the species to exploit highly diversified habitats for oviposition (Cigliano *et al.*, 1996). In this case, we consider that sympatric speciation would be the main diversification event within this group and that the modification of the ovipositor could have given *Scotussa* species adaptive advantages, generating opportunities that have favoured their ecological diversification. Similar diversification patterns were found in the Pampas for Solanaceae, where sympatric speciation would have been the main diversification event, leading to evidence of gametic isolation and ecological speciation (Fregonezi *et al.*, 2013).

Even though the Macropterous clade has a more southern distribution than the Brachypterous clade, they both inhabit Misiones Nucleus of the SDTFs, as

Nucleous; pink (B) = Cerrado; orange (C) = Dry Chaco; turquoise (D) = Atlantic Rainforest; yellow (E) = Pampas; light blue (F) = Southern Biomes. Some of the coloured rectangles do not match with one of the seven pre-defined colours for the biogeographical areas, as they represent the combination of two or more areas.

well as the Cerrado and the Atlantic Rainforest. These regions have been described as historically stable refugia for different taxa including invertebrates, vertebrates and plants (Nogueira *et al.*, 2011; Werneck *et al.*, 2011; Acosta & Vaschetto, 2017; Costa *et al.*, 2018). The existence of multiple microrefugia gives a pattern of functional diversity characterized by a mixture of communities with a high proportion of specialists and other, more widespread communities with a stronger dominance of generalists and good dispersers (Jansson & Dynesius, 2002; Costa *et al.*, 2018). These results are consistent with our findings as these regions are not only inhabited by these brachypterous species with reduced ranges, but also by widespread species of the Macropterous clade.

In conclusion, our findings highlight the usefulness of applying an approach that integrates the historical and ecological factors that shape the biogeography of the taxa. Ecology would have a major role in the diversification of the genus group Scotussae, because environmental patterns show a greater association with phylogeny than with geographical distribution. Moreover, we found that the evolution of climatic niches is not constant across the two main clades depicted in the phylogeny. Wing development is associated with different patterns of niche overlap, implying that the diversification process took different pathways between these two clades of South American grasshoppers.

ACKNOWLEDGEMENTS

REFERENCES

Acosta LE, **Vaschetto LM. 2017.** Palaeoclimatic distribution models predict Pleistocene refuges for the Neotropical harvestman *Geraeocormobius sylvarum* (Arachnida: Opiliones: Gonyleptidae). *Journal of Natural History* **51:** 17–32.

Akaike H. 1973. Information theory and an extension of the maximum likelihood principle. In Petrov BN, Csaki BF, eds. *Second International Symposium On Information Theory.* Budapest: Academiai Kiado, 267–281.

Allegrucci G, **Trucchi E**, **Sbordoni V. 2011.** Tempo and mode of species diversification in *Dolichopoda* cave crickets (Orthoptera, Rhaphidophoridae). *Molecular Phylogenetics and Evolution* **60:** 108–121.

Barve N, **Barve V**, **Jiménez-Valverde A**, **Lira-Noriega A**, **Maher SP**, **Peterson AT**, **Soberón J**, **Villalobos F. 2011.** The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling* **222:** 1810–1819.

Behling H, **Pillar VP. 2007.** Late Quaternary vegetation, biodiversity and fire dynamics on the southern Brazilian highland and their implication for conservation and management of modern Araucaria forest and grassland ecosystems. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* **362:** 243–251.

Bensasson D, **Zhang D**, **Hewitt GM. 2000.** Frequent assimilation of mitochondrial DNA by grasshopper nuclear genomes. *Molecular Biology and Evolution* **17:** 406–415.

Blomberg SP, **Garland T. 2002.** Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *Journal of Evolutionary Biology* **15:** 899–910.

Blomberg SP, **Garland TJ**, **Ives AR. 2003.** Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57:** 717–745.

Borcard D, **Legendre P. 2012.** Is the Mantel correlogram powerful enough to be useful in ecological analysis? A simulation study. *Ecology* **93:** 1473–1481.

Bouckaert R, **Heled J**, **Kühnert D**, **Vaughan T**, **Wu CH**, **Xie D**, **Suchard MA**, **Rambaut A**, **Drummond AJ. 2014.** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10:** 1–6.

Broennimann O, **Fitzpatrick MC**, **Pearman PB**, **Petitpierre B**, **Pellissier L**, **Yoccoz NG**, **Thuiller W**, **Fortin MJ**, **Randin C**, **Zimmermann NE**, **Graham CH**, **Guisan A. 2012.** Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology and Biogeography* **21:** 481–497.

Brown JH. 2014. Why are there so many species in the tropics? *Journal of Biogeography* **41:** 8–22.

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

Cabrera A., **Willink A. 1973.** *Biogeografía de América Latina*. Washington: Monografías OEA.

Castillo ERD, **Bidau CJ**, **Martí DA. 2010.** Neo-sex chromosome diversity in Neotropical melanopline grasshoppers (Melanoplinae, Acrididae). *Genetica* **138:** 775–786.

Castillo ERD, **Martí DA**, **Maronna MM**, **Scattolini MC**, **Cabral-de-Mello DC**, **Cigliano MM. 2019.** Chromosome evolution and phylogeny in Ronderosia (Orthoptera, Acrididae, Melanoplinae): clues of survivors to the challenge of sympatry? *Systematic Entomology* **44:** 61–74.

Ceccarelli FS, **Ojanguren-Affilastro AA**, **Ramírez MJ**, **Ochoa JA**, **Mattoni CI**, **Prendini L. 2016.** Andean uplift drives diversification of the bothriurid scorpion genus *Brachistosternus*. *Journal of Biogeography* **43:** 1942–1954.

Chen JL, **Wilson CR**, **Tapley BD**, **Longuevergne L**, **Yang ZL**, **Scanlon BR. 2010.** Recent la Plata basin drought conditions observed by satellite gravimetry. *Journal of Geophysical Research Atmospheres* **115:** 1–12.

Cigliano MM. 1997. *Ronderosia*, a new genus of South American Melanoplinae (Orthptera, Acrididae). *Journal of Orthopeta Research* **6:** 1–19.

Cigliano MM. 2007. Review of the South American genus *Eurotettix* Bruner (Orthoptera, Acridoidea, Melanoplinae). *Systematic Entomology* **32:** 176–195.

Cigliano MM, **Braun H**, **Eades DC**, **Otte D. 2020.** *Orthoptera species file. Version 5.0/5.0*. Available at: http://Orthoptera.SpeciesFile.org

Cigliano MM, **Lange CE. 2001.** Revision of the South American genus *Atrachelacris* Giglio-Tos (Orthoptera, Acridoidea, Melanoplinae). *Transactions of the American Entomological Society* **127:** 359–370.

Cigliano MM, **Lange CE. 2007.** Systematic revision and phylogenetic analysis of the South American genus *Chlorus* (Orthoptera, Acridoidea, Melanoplinae). *Zoologica Scripta* **36:** 241–254.

Cigliano MM, **Ronderos RA. 1994.** Revision of the South American grasshopper genera *Leiotettix* Bruner and *Scotussa* Giglio-Tos (Orthoptera, Acrididae, Melanoplinae). *Transactions of the American Entomological Society* **120:** 145–180.

Cigliano MM, **Ronderos RA**, **Kemp WP. 1996.** Phylogenetic relationships of *Scotussa* and *Leiotettix* (Orthoptera: Acrididae). *Cladistics* **12:** 125–138.

Colwell RK, **Rangel TF. 2009.** Hutchinson's duality: the once and future niche. *Proceedings of the National Academy of Sciences USA* **106:** 19651–19658.

Costa GC, **Hampe A**, **Ledru MP**, **Martinez PA**, **Mazzochini GG**, **Shepard DB**, **Werneck FP**, **Moritz C**, **Carnaval AC. 2018.** Biome stability in South America over the last 30 kyr: inferences from long-term vegetation dynamics and habitat modelling. *Global Ecology and Biogeography* **27:** 285–297.

Coyne JA, **Orr HA. 2004.** *Speciation*. Sunderland: Sinauer Associates Inc.

Dalsgaard B, **Magård E**, **Fjeldså J**, **González AMM**, **Rahbek C**, **Olesen JM**, **Ollerton J**, **Alarcón R**, **Araujo AC**, **Cotton PA**, **Lara C**, **Machado CG**, **Sazima I**, **Sazima M**, **Timmermann A**, **Watts S**, **Sandel B**, **Sutherland WJ**, **Svenning JC. 2011.** Specialization in plant–hummingbird networks is associated with species richness, contemporary precipitation and quaternary climate-change velocity. *PLoS One* **6:** e25891.

Dinghi PA, **Confalonieri VA**, **Cigliano MM. 2009.** Phylogenetic studies in the South American tribe Dichroplini (Orthoptera : Acrididae : Melanoplinae). *Zootaxa* **62:** 51–62.

Diniz-Filho JAF, **de Marco P**, **Hawkins BA. 2010b.** Defying the curse of ignorance: perspectives in insect macroecology and conservation biogeography. *Insect Conservation and Diversity* **3:** 172–179.

Diniz-Filho JAF, **Terribile LC**, **Da Cruz MJR**, **Vieira LCG. 2010a.** Hidden patterns of phylogenetic non-stationarity overwhelm comparative analyses of niche conservatism and divergence. *Global Ecology and Biogeography* **19:** 916–926.

Dormann CF, **Elith J**, **Bacher S**, **Buchmann C**, **Carl G**, **Carré G**, **Marquéz JRG**, **Gruber B**, **Lafourcade B**, **Leitão PJ**, **Münkemüller T**, **Mcclean C**, **Osborne PE**, **Reineking B**, **Schröder B**, **Skidmore AK**, **Zurell D**, **Lautenbach S. 2013.** Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* **36:** 027–046.

Drummond AJ, **Rambaut A. 2007.** BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Fick SE, **Hijmans RJ. 2017.** WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37:** 4302–4315.

Fregonezi JN, **Turchetto C**, **Bonatto SL**, **Freitas LB. 2013.** Biogeographical history and diversification of *Petunia* and *Calibrachoa* (Solanaceae) in the Neotropical Pampas grassland. *Botanical Journal of the Linnean Society* **171:** 140–153.

Gaston KJ. 1996. Species-range-size distributions: patterns, mechanisms and implications. *Trends in Ecology and Evolution* **11:** 197–201.

Guzman NV, **Pietrokovsky SM**, **Cigliano MM**, **Confalonieri VA. 2017.** Unraveling the diversification history of grasshoppers belonging to the "*Trimerotropis pallidipennis*" (Oedipodinae: Acrididae) species group: a hotspot of biodiversity in the Central Andes. *PeerJ* **5:** e3835.

Haffer J. 1969. Speciation in Amazonian forest birds. *Science* **165:** 131–137.

Hamlin JAP, **Simmonds TJ**, **Arnold ML. 2017.** Niche conservatism for ecological preference in the Louisiana iris species complex. *Biological Journal of the Linnean Society* **120:** 144–154.

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

Hickerson MJ, **Meyer CP. 2008.** Testing comparative phylogeographic models of marine vicariance and dispersal using a hierarchical Bayesian approach. *BMC Evolutionary Biology* **8:** 322.

Hijmans RJ, **Cameron SE**, **Parra JL**, **Jones PG**, **Jarvis A. 2005.** Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25:** 1965–1978.

Holt RD. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences USA* **106:** 19659–19665.

Homburg K, **Schuldt A**, **Drees C**, **Assmann T. 2013.** Broad-scale geographic patterns in body size and hind wing development of western Palaearctic carabid beetles (Coleoptera: Carabidae). *Ecography* **36:** 166–177.

Hoorn C, **Wesselingh FP**, **ter Steege H**, **Bermudez MA**, **Mora A**, **Sevink J**, **Sanmartín I**, **Sanchez-Meseguer A**,

Anderson CL, **Figueiredo JP**, **Jaramillo C**, **Riff D**, **Negri FR**, **Hooghiemstra H**, **Lundberg J**, **Stadler T**, **Särkinen T**, **Antonelli A. 2010.** Amazonia through time: andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330:** 927–31.

Hughes CE, **Pennington RT**, **Antonelli A. 2013.** Neotropical plant evolution: assembling the big picture. *Botanical Journal of the Linnean Society* **171:** 1–18.

Husemann M, **Guzman NV**, **Danley PD**, **Cigliano MM**, **Confalonieri VA. 2013.** Biogeography of *Trimerotropis pallidipennis* (Acrididae: Oedipodinae): deep divergence across the Americas. *Journal of Biogeography* **40:** 261–273.

Hutchinson GE. 1957. Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*. **22:** 415–427.

Ikeda H, **Nishikawa M**, **Sota T. 2012.** Loss of flight promotes beetle diversification. *Nature Communications* **3:** 647–648.

Jansson R, **Dynesius M. 2002.** The fate of clades in a world of recurrent climatic change: Milankovitch oscillations and evolution. *Annual Review of Ecology and Systematics* **33:** 741–777.

Kearse M, **Moir R**, **Wilson A**, **Stones-Havas S**, **Cheung M**, **Sturrock S**, **Buxton S**, **Cooper A**, **Markowitz S**, **Duran C**, **Thierer T**, **Ashton B**, **Meintjes P**, **Drummond A. 2012.** Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28:** 1647–1649.

Knouft JH, **Losos JB**, **Glor RE**, **Kolbe JJ. 2006.** Phylogenetic analysis of the evolution of the niche in lizard of the *Anolis sagrei* group. *Ecology* **87:** S29–S38.

Landis MJ, **Matzke NJ**, **Moore BR**, **Huelsenbeck JP. 2013.** Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology* **62:** 789–804.

Lavin M. 2006. Floristic and geographic stability of discontinuous seasonally dry tropical forests explains patterns of plant phylogeny and endemism. In: Pennington RT, Ratter JA, Lewis GP, eds. *Neotropical savannas and seasonally dry forests: plant biodiversity, biogeography and conservation*. Boca Raton: CRC Press, 433–447.

Lorenz-Lemke AP, **Togni PD**, **Mäder G**, **Kriedt RA**, **Stehmann JR**, **Salzano FM**, **Bonatto SL**, **Freitas LB. 2010.** Diversification of plant species in a subtropical region of eastern South American highlands: a phylogeographic perspective on native *Petunia* (Solanaceae). *Molecular Ecology* **19:** 5240–5251.

Losos JB. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* **11:** 995–1003.

Losos JB, **Leal M**, **Glor RE**, **Queiroz K De**, **Lara AC. 2003.** Niche lability in the evolution of a Caribbean lizard community. *Nature* **424:** 542–545.

Lunt DH, **Zhand DX**, **Szymura JM**, **Hewitt GM. 1996.** The insect cytochrome oxidase I gene evolutionary patterns and conserved primers for phylogenetic studies. *Insect MoleclarBiology* **53:** 153–165.

Malmqvist B. 2000. How does wing length relate to distribution patterns of stoneflies (Plecoptera) and mayflies (Ephemeroptera)? *Biological Conservation* **93:** 271–276.

Marshall LG, **Hoffstetter R**, **Pascual R. 1983.** Mammals and stratigraphy: geochronology of the continental mammal-bearing Tertiary of South America. *Palaeovertebrata, Mémoire Extraordinaire* **1983:** 1–93.

Matzke NJ. 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology* **63:** 951–970.

McCulloch GA, **Wallis GP**, **Waters JM. 2017.** Does wing size shape insect biogeography? Evidence from a diverse regional stonefly assemblage. *Global Ecology and Biogeography* **26:** 93–101.

Mörner NA, **Sylwan C. 1989.** Magnetostratigraphy of the Patagonian moraine sequence at Lago Buenos Aires. *Journal of South American Earth Sciences* **2:** 385–389.

Münkemüller T, **Boucher FC**, **Thuiller W**, **Lavergne S. 2015.** Phylogenetic niche conservatism – common pitfalls and ways forward. *Functional Ecology* **29:** 627–639.

Nogueira C, **Colli GR**, **Martins M. 2009.** Local richness and distribution of the lizard fauna in natural habitat mosaics of the Brazilian Cerrado. *Austral Ecology* **34:** 83–96.

Nogueira C, **Ribeiro S**, **Costa GC**, **Colli GR. 2011.** Vicariance and endemism in a Neotropical savanna hotspot: distribution patterns of Cerrado squamate reptiles. *Journal of Biogeography* **38:** 1907–1922.

Oksanen J, **Blanchet FG**, **Friendly M**, **Kindt R**, **Legendre P**, **McGlinn D**, **Minchin PR**, **O'Hara RB**, **Simpson GL**, **Solymos P**, **Stevens MHH**, **Szoecs E**, **Wagner H. 2019.** *vegan: community ecology package. R package version 2.5-6*. Available at: https://CRAN.R-project.org/package=vegan

Olson DM, **Dinerstein E**, **Wikramanayake ED**, **Burgess ND**, **Powell GVN**, **Underwood EC**, **D'amico JA**, **Itoua I**, **Strand HE**, **Morrison JC**, **Loucks CJ**, **Allnutt TF**, **Ricketts TH**, **Kura Y**, **Lamoreux JF**, **Wettengel WW**, **Hedao P**, **Kassem KR. 2001.** Terrestrial ecoregions of the world: a new map of life on earth. *BioScience* **51:** 933.

Ortiz-Jaureguizar E. 1998. Paleoecología y evolución de la fauna de mamíferos de América del sur durante la "Edad de las Planicies Australes" Mioceno Superior–Plioceno Superior. *Estudios Geoloógicos* **54:** 161–169.

Ortiz-Jaureguizar E, **Cladera GA. 2006.** Paleoenvironmental evolution of southern South America during the Cenozoic. *Journal of Arid Environments* **66:** 498–532.

Osorio-Olvera L, **Barve V**, **Barve N**, **Soberón J**, **Falconi M. 2018.** *ntbox: from getting biodiversity data to evaluating species distribution models in a friendly GUI environment, R package version 0.2.5.4*. Available at: https://github.com/luismurao/ntbox

Osorio-Olvera LA, **Falconi M**, **Soberón J. 2016.** Sobre la relación entre idoneidad del hábitat y la abundancia poblacional bajo diferentes escenarios de dispersión. *Revista Mexicana de Biodiversidad* **87:** 1080–1088.

Paradis E, **Claude J**, **Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20:** 289–290.

Pascual R, **Bondesio P. 1982.** Un roedor Cardiatheriinae Hydrochoeridae de la Edad Huayqueriense Mioceno tardío

de La Pampa. Sumario de los ambientes terrestres en la Argentina durante el Mioceno. *Ameghiniana* **19:** 19–36.

Pascual R, **Ortiz-Jaureguizar E**, **Prado JL. 1996.** Land mammals: paradigm of Cenozoic South American geobiotic evolution. In: Arratia G, ed. Contribution of Southern South America to vertebrate paleontology. *Müncher Geowissenschaftliche Abhandlungen A* **30:** 265–319.

Pearson RG, **Raxworthy CJ**, **Nakamura M**, **Townsend Peterson A. 2007.** Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography* **34:** 102–117.

Peixoto FP, **Villalobos F**, **Cianciaruso MV. 2017.** Phylogenetic conservatism of climatic niche in bats. *Global Ecology and Biogeography* **26:** 1055–1065.

Pennington RT, **Dick CW. 2010.** Diversification of the Amazonian flora and its relation to key geological and environmental events: a molecular perspective. In: Hoorn C, Wesselingh F, eds. *Amazonia, landscape and species evolution*, *1st edn*. Oxford: Blackwell Publishing, 373–385.

Pennington RT, **Lavin M**, **Oliveira-Filho A. 2009.** Woody plant diversity, evolution, and ecology in the tropics: perspectives from seasonally dry tropical forests. *Annual Review of Ecology, Evolution, and Systematics* **40:** 437–457.

Pennington RT, **Lavin M**, **Prado DE**, **Pendry CA**, **Pell S**, **Butterworth CA. 2004.** Historical climate change and speciation: Neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359:** 515–538.

Pennington RT, **Prado DA**, **Pendry C. 2000.** Neotropical seasonally dry forests and Pleistocene vegetation changes. *Journal of Biogeography* **27**:261–273.

Pennington RT, **Richardson JE**, **Lavin M. 2006.** Insights into the historical construction of species-rich biomes from dated plant phylogenies, neutral ecological theory and phylogenetic community structure. *New Phytologist* **172:** 605–616.

Perez MF, **Bonatelli IAS**, **Moraes EM**, **Carstens BC. 2016.** Model-based analysis supports interglacial refugia over long-dispersal events in the diversification of two South American cactus species. *Heredity* **116:** 550–557.

Peterson AT. 2011. Ecological niche conservatism: a time-structured review of evidence. *Journal of Biogeography* **38:** 817–827.

Peterson AT, **Papeş M**, **Eaton M. 2007.** Transferability and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. *Ecography* **30:** 550–560.

Peterson AT, **Soberón J**, **Pearson RG**, **Anderson RP**, **Martinez-Meyer E**, **Nakamura M**, **Araujo MB. 2011.** *Ecological niches and geographic distributions*. Princeton: Princeton University Press.

Peterson AT, **Soberón J**, **Sanchez-Cordero VV. 1999.** Conservatism of ecological niches in evolutionary time. *Science* **285:** 1265–1267.

Phillips SJ, **Anderson RP**, **Schapire RE. 2006.** Maximum entropy modeling of species geographic distributions. *Ecological Modelling* **190:** 231–259.

Pocco ME, **Guzmán N**, **Plischuk S**, **Confalonieri V**, **Lange CE**, **Cigliano MM. 2018.** Diversification patterns of the grasshopper genus *Zoniopoda* Stål (Romaleidae, Acridoidea, Orthoptera) in open vegetation biomes of South America. *Systematic Entomology* **43:** 290–307.

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Prado DE. 2000. Seasonally dry forests of tropical South America: from forgotten ecosystems to a new phytogeographic unit. *Edinburgh Journal of Botany* **57:** 437–461.

Prado DE, **Gibbs PE. 1993.** Patterns of species distributions in the dry seasonal forests of South America. *Annals of the Missouri Botanical Garden* **80:** 902–927.

Pyron RA, **Burbrink FT. 2010.** Hard and soft allopatry: physically and ecologically mediated modes of geographic speciation. *Journal of Biogeography* **37:** 2005–2015.

Pyron RA, **Costa GC**, **Patten MA**, **Burbrink FT. 2015.** Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biological Reviews* **90:** 1248–1262.

Rabassa J. 1999. Cuaternario de la cordillera patagónica y Tierra del Fuego. In: Caminos R, ed. *Geología Argentina.* Buenos Aires: Anales del Instituto de Geología y Recursos Minerales, 710–714.

Radosavljevic A, **Anderson RP. 2014.** Making better Maxent models of species distributions: complexity, overfitting and evaluation (M Araújo, Ed.). *Journal of Biogeography* **41:** 629–643.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. *Tracer v1.6*. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

Ree RH, **Moore BR**, **Webb CO**, **Donoghue MJ. 2005.** Alikelihood framework for inferring theevolution of geographic range on phylogenetic trees. *Evolution* **59:** 2299–2311.

Ronquist F. 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46:** 195.

Ronquist F, **Sanmartín I. 2011.** Phylogenetic methods in biogeography. *Annual Review of Ecology, Evolution, and Systematics* **42:** 441–464.

Sanmartín I. 2012. Historical biogeography: evolution in time and space. *Evolution: Education and Outreach* **5:** 555–568.

Scattolini MC, **Confalonieri V**, **Lira-Noriega A**, **Pietrokovsky S**, **Cigliano MM. 2018.** Diversification mechanisms in the Andean grasshopper genus *Orotettix* (Orthoptera : Acrididae): ecological niches and evolutionary history. *Biological Journal of the Linnean Society* **123:** 697–711.

Scattolini MC, **Lira-Noriega A**, **Cigliano MM. 2020.** Species richness, range size, and wing development in South American melanopline grasshoppers (Orthoptera, Acrididae). *Ecological Entomology* **45:** 840–853.

Schivo F, **Baun V**, **Krug P**, **Quintana RD. 2019.** Distribution and richness of amphibians under different climate change scenarios in a subtropical region of South America. *Applied Geography* **103:** 70–89.

Schwallier R, **Raes N**, **de Boer HJ**, **Vos RA**, **van Vugt RR**, **Gravendeel B. 2016.** Phylogenetic analysis of niche divergence reveals distinct evolutionary histories and

climate change implications for tropical carnivorous pitcher plants. *Diversity and Distributions* **22:** 97–110.

Shcheglovitova M, **Anderson RP. 2013.** Estimating optimal complexity for ecological niche models: a jackknife approach for species with small sample sizes. *Ecological Modelling* **269:** 9–17.

Smith AB, **Godsoe W**, **Rodríguez-Sánchez F**, **Wang HH**, **Warren D. 2019.** Niche estimation above and below the species level. *Trends in Ecology and Evolution* **34:** 260–273.

Sokal RR. 1979. Testing statistical significance of geographic variation patterns. *Society of Systematic Biologists* **28:** 227–232.

Sorenson MD, **Fleischer RC. 1996.** Multiple independent transposition of mitochondrial DNA control region sequences to the nucleus. *Proceedings of the National Academy of Sciences of USA* **93:** 15239–15243.

Van der Vaart AW. 1998. *Asymptotic statistics*. Cambridge: Cambridge University Press.

Warren DL, **Glor RE**, **Turelli M. 2008.** Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution; International Journal of Organic Evolution* **62:** 2868–83.

Werneck FP. 2011. The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. *Quaternary Science Reviews* **30:** 1630–1648.

Werneck FP, **Costa GC**, **Colli GR**, **Prado DE**, **Sites JW. 2011.** Revisiting the historical distribution of Seasonally Dry Tropical Forests: new insights based on palaeodistribution modelling and palynological evidencegeb. *Global Ecology and Biogeography* **20:** 272–288.

Wiens JJ. 2004. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution* **58:** 193.

Wiens JJ, **Ackerly DD**, **Allen AP**, **Anacker BL**, **Buckley LB**, **Cornell HV**, **Damschen EI**, **Jonathan Davies T**, **Grytnes JA**, **Harrison SP**, **Hawkins BA**, **Holt RD**, **McCain CM**, **Stephens PR. 2010.** Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters* **13:** 1310–1324.

Wiens JJ, **Graham CH. 2005.** Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics* **36:** 519–539.

Zhang DX, **Hewitt GM. 1996.** Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution* **11:** 247–251.

SUPPORTING INFORMATION

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

Figure S1. External morphology characters. The morphological characters and their states are listed in Table S1. A, *Neopedies brunneri*; B, *Orotettix hortensis*; C, *Atrachelacris unicolor*; D, *Chlorus vittatus*; E, *Leiotettix pulcher*; F, *Scotussa brachyptera*; G, *Leiotettix pulcher*; H, *Dichromatos lilloanus*; I, *Atrachelacris unicolor*; J, *Chlorus spatulus*.
Figure S2. Morphological characters from male and female abdominal terminalia and male genitalia. The morphological characters and their states are listed in Table S1. A, *Leiotettix viridis*; B, *Scotussa cliens*; C and D, *Dichromatos lilloanus*; E, *Scotussa impudica*; F, *Neopedies brunneri*; G, *Eurotettix minor*; H, *Chlorus chiquitensis*; I, *Dichromatos lilloanus;* J, *Leiotettix flavipes*; K, *Leiotettix pulcher*; L, *Neopedies brunneri*; M, *Scotussa impudica*; N, *Neopedies brunneri*; Ñ, *Leiotettix flavipes*; O, *Scotussa cliens*; P, *Neopedies brunneri*; Q, *Leiotettix pulcher*.
Table S1. Information on the locality, wing length (W.L., Wing Length; M, macropterous; B, brachypterous species) and GenBank accession numbers of the specimens used in the phylogenetic analysis.
Table S2. List of morphological characters used in the total evidence analysis (Figs S1, S2).
Table S3. Data matrix for the 30 morphological characters used in the Scotussae genus group total evidence analysis. A dash indicates non-comparable characters.
Table S4. Results from similarity tests for niche conservatism between species niches based on Hellinger's *I* distance. Bold indicates that niches are more similar than expected by chance ($P < 0.05$).
Table S5. Results from niche model evaluations and performances for both methodologies (Maxent and Ellipsoids) at the species and genus levels.
Table S6. Ellipse volumes of each species as a measure of niche breadth.