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

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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.


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

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the use of temporal dimension allows the connection of evolutionary and geological events within historical biogeographical hypotheses (Ronquist & Sanmartin, 2011; Sanmartin, 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 ‘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 sympathy and lack of geographical phylogenetic structuring (Lavin, 2006; Pennington et al., 2009; Pennington & Dick, 2010; Hughes et al., 2013). For grassland species that inhabit highland, 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, 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 framshifts 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) (Akaile, 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 & Roneros, 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 BEAUtil 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 Paris, 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 ‘phylodist’ 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 (Roe 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).

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 Ma 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). Hellinger'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.
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 *Leiotettix* 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

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

<table>
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<th></th>
<th>Chlorus</th>
<th>Dichromatos</th>
<th>Eurotettix</th>
<th>Atrachelacris</th>
<th>Ronderosia</th>
<th>Leiotettix</th>
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<td>0.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scotussa</td>
<td>0.35</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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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 (LnL), dispersal (d) and extinction (e) values

<table>
<thead>
<tr>
<th>Model</th>
<th>LnL</th>
<th>No. of parameters</th>
<th>d</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEC</td>
<td>−116.73</td>
<td>2</td>
<td>0.069</td>
<td>0.00000</td>
</tr>
<tr>
<td>DIVALIKE</td>
<td>−122.42</td>
<td>2</td>
<td>0.081</td>
<td>0.00000</td>
</tr>
<tr>
<td>BAYAREALIKE</td>
<td>−100.81</td>
<td>2</td>
<td>0.028</td>
<td>0.14551</td>
</tr>
</tbody>
</table>

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

heterogeneity generates 'hard allopatri' (Hickerson & Meyer, 2008; Pyron & Burbrink, 2010; Pyron et al., 2015). Hard allopatri 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 Nucleous 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 (Coynne & Orr, 2004). Recent studies in Ronderosia suggest that chromosome rearrangements (such as various systems for determining neo-sexual chromosomes and autosoma–autosome 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 favored 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 Nucleous of the SDTFs, as...
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.

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### SUPPORTING INFORMATION

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**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.