

ORIGINAL
ARTICLE

Andean uplift drives diversification of the bothriurid scorpion genus *Brachistosternus*

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ABSTRACT

Aim One of the planet’s most imposing geomorphological features, the Andes, played an important role in the evolution of South America’s flora and fauna. The bothriurid scorpion genus *Brachistosternus* Pocock, 1893 comprises more than 40 species with high diversity and endemism in the Andes. The present contribution investigates the biogeographical history of this genus using molecular phylogenetics and dating, to determine the role of Andean uplift on the distribution and diversification of its species.

Location South America.

Methods A dated species tree was obtained for 55 putative species based on two nuclear and three mitochondrial gene loci. Ancestral ranges and biogeographical events were estimated on the species tree, diversification rates and rate shifts calculated, and areas with high phylogenetic diversity (PD) and evolutionary distinctiveness identified.

Results *Brachistosternus* diversified at a steady rate during the main Andean uplift. The central Andean and western slope/Pacific coastal biogeographical provinces played important roles as ancestral areas. Coastal areas of central Chile and southern Peru exhibit high levels of PD in *Brachistosternus*, suggesting they experienced a relatively long period of ecological stability, while the Andes continued to rise.

Main conclusions Andean uplift created new habitats and climate regimes, favouring speciation in genera such as *Brachistosternus*. Coastal areas to the west of the Andes continued to harbour older lineages while accommodating more recently diverged lineages from the nearby Andes.

Keywords

Andes, aridification, Bothriuridae, historical biogeography, Humboldt Current, Scorpiones, South America

INTRODUCTION

The Andes are among the planet’s most impressive geomorphological features, the longest continental mountain chain, extending over 7000 km from north to south, and including the highest peak in the Southern and Western Hemispheres. The orogeny of the Andes was mostly caused by subduction of the Nazca Plate below the oceanic ridge of the South American Plate, which in turn was being pushed upward while itself moving westward (Husson *et al.*, 2012). This process has continued since the Palaeogene, albeit at different rates (Ghosh *et al.*, 2006; Garzone *et al.*, 2008; Hoorn *et al.*,

2010). Andean uplift had a profound effect on South American geology, climate and biodiversity (Graham, 2009; Ejelds *et al.*, 2012; Giarla & Jansa, 2014) by changing the flow of major rivers (Turner *et al.*, 2005; Zemplak *et al.*, 2008) and altering the climate, leading to the hyper-aridification of regions such as the Atacama Desert (Amundson *et al.*, 2012), while creating humid climates elsewhere, for example, the Amazon Basin, the Valdivian forests of southern Chile, and the montane rain forests of the Yungas. These changes in climate regimes in turn favoured the diversification of flora and fauna, producing the world’s most species-rich ecosystem, the Amazon rain forest. Formation of the Andes also

created new valleys, slopes and highlands, each with a unique micro-climate and providing abundant new habitat for flora and fauna to adapt and diversify (Graham, 2009), as proposed in the 'vertical ecotone' hypothesis (Guarnizo *et al.*, 2009).

Fourteen biogeographical provinces, regions containing high levels of biodiversity and endemism, are currently recognized as part of the Andes in the South American transition zone and the Andean region *sensu* Morrone (2006, 2014). Andean uplift was suggested as a driver of diversification in taxa such as the angiosperm genera *Heliotropium* L. (Luebert *et al.*, 2011) and *Hedyosmum* Sw. (Antonelli & Sanmartín, 2011), scorpions (Mattoni *et al.*, 2012), grasshoppers (Pocco *et al.*, 2013), glassfrogs (Hutter *et al.*, 2013), and brush-finches (Sanchez-Gonzalez *et al.*, 2014). Furthermore, Andean orogeny caused the separation of habitats and subsequent speciation of their respective faunas, especially in freshwater fishes (Albert *et al.*, 2006). Connecting geological and evolutionary events can be speculative, but historical biogeographical hypotheses have gained credibility by incorporating a temporal dimension to phylogenies (Ronquist & Sanmartín, 2011; Sanmartín, 2012; Wen *et al.*, 2013).

The bothriurid scorpion genus *Brachistosternus* Pocock, 1893 is endemic to South America and distributed mainly in the Andean region, and the arid areas of the southern and eastern part of the continent (Ojanguren-Affilastró *et al.*, 2016). The genus contains 43 described species (Kovářík & Ojanguren-Affilastró, 2013; Ojanguren-Affilastró & Pizarro-Araya, 2014) and an unknown number awaiting description (A.A. Ojanguren-Affilastró *et al.*, unpublished data). As with other scorpions, *Brachistosternus* species are habitat specific, range-restricted, and have limited vagility and relatively long generation times (1–2 years) compared to most invertebrates, providing a model system for phylogeographical and historical biogeographical studies (Prendini, 2001; Gantenbein & Largiadèr, 2003; Parmakelis *et al.*, 2006; Bryson *et al.*, 2013).

The present contribution investigated the biogeographical history of *Brachistosternus* to determine the role of Andean uplift on the distribution and diversification of its species. In particular: (1) Did Andean uplift promote speciation in *Brachistosternus* and if so, what mechanisms were involved? (2) Where did the genus originate and what processes shaped its present distribution? (3) Which areas harbour the greatest diversity of *Brachistosternus* and is the distribution of this diversity consistent with historical geoclimatic events? To answer these questions, diversification rate analyses with lineage age estimates and geological information were used to detect possible congruence between orogeny and the diversification of *Brachistosternus*; divergence time and ancestral area estimations to understand historical biogeographical processes; and hotspots of diversity for the genus identified using the phylogenetic diversity (PD) metric (Faith, 1992).

MATERIALS AND METHODS

Taxon sample

The ingroup comprised 120 individuals of *Brachistosternus*, 99 representing 40 of the 43 nominal species of *Brachistosternus* (five of which contain deeply divergent populations which probably represent new species) and 21 representing another 10 potential new species, based on morphology in most cases corroborated by DNA sequence data. *Brachistosternus castroi* Mello-Leitão, 1941 and *Brachistosternus holmbergi* Carbonell, 1923 were excluded because they are of dubious taxonomic validity (Ojanguren-Affilastró & Ramírez, 2009; Kovářík & Ojanguren-Affilastró, 2013). Samples of *Brachistosternus mattonii* Ojanguren-Affilastró, 2005 could not be obtained for DNA isolation. New sequences, generated from 22 samples, were added to the sequences previously generated from 98 samples by Ojanguren-Affilastró *et al.* (2016).

The outgroup comprised exemplar species of four bothriurid genera, selected based on phylogenetic analyses of family Bothriuridae Simon, 1880 (Prendini, 2000, 2003a; Mattoni & Prendini, 2007): *Thestylus aurantiurus* Yamaguti & Pinto-da-Rocha, 2003; *Bothriurus flavidus* Kraepelin, 1911; *Cercophonius sulcatus* Kraepelin, 1908; and *Urophonius brachycentrus* (Thorell, 1876). The tree was rooted on *Scorpio fuscus* (Ehrenberg, 1829), an exemplar species of family Scorpionidae Latreille 1802, representing superfamily Scorpionoidea Latreille 1802, the putative sister-group of Bothriuridae (Prendini, 2000). A complete list of the taxa sampled is provided as Table S1.1 of Appendix S1 in the Supporting Information.

DNA sequencing and alignment

Five gene loci were sequenced to reconstruct the phylogeny and biogeographical history of *Brachistosternus*, following methods described by Prendini *et al.* (2003): c. 513 base-pairs (bp) of the D3 region of the large-subunit ribosomal RNA (28S rDNA), and c. 1761 bp of the small-subunit ribosomal RNA (18S rDNA) genes from the nuclear genome, and c. 485 bp of the large-subunit ribosomal RNA (16S rDNA), c. 335 bp of the small-subunit ribosomal RNA (12S rDNA) and 1078 bp of the cytochrome *c* oxidase subunit I (COI) genes, from the mitochondrial genome. DNA sequences were aligned and tested as described by Ojanguren-Affilastró *et al.* (2016).

Divergence time estimation and species tree reconstruction

A time-calibrated tree was reconstructed for 120 individuals of *Brachistosternus* and the five outgroup taxa, using BEAST 1.8.2 (Drummond *et al.*, 2012). Details of tree-building, priors and parameters for the runs are provided in Appendix S2.

The outgroup *C. sulcatus* was used to calibrate the divergence between *Cercophonius* Peters, 1861 and *Urophonius*

Pocock, 1893). *Cercophoni*us, endemic to Australia and Tasmania, is the only bothriurid genus from Oceania (Prendini, 2000). *Urophoni*us, on the other hand, is endemic to southern South America. Both genera are remarkably similar morphologically, and form a well supported monophyletic group (Prendini, 2000, 2003a,b). In the absence of bothriurid fossils and/or reliable substitution rates for the family, a geological calibration, the separation of South America from the landmass comprising Antarctica and Australia, was used, assuming that transoceanic dispersal was unlikely to have occurred after the landmasses separated. A truncated normal distribution (mean = 60, SD = 10, max. = 500, min. = 33.9 Ma) was applied to the divergence of *Cercophoni*us and *Urophoni*us, considering a complete separation of Australia, Antarctica and South America by the late Eocene (Sanmartín & Ronquist, 2004; Almeida *et al.*, 2012). The plausibility of the node age estimates were checked by comparing the clock rates obtained to those known for other scorpions.

A multispecies coalescent analysis was also conducted to obtain a species tree of *Brachistosternus*, using *BEAST 1.8.2 (Heled & Drummond, 2010). Individuals were assigned to species based on conservative species limits determined by previous morphological and molecular evidence as well as additional molecular species delimitation analyses as described in Ojanguren-Affilastró *et al.* (2016). The priors applied for the multispecies coalescent tree reconstruction were the same as for the dated tree reconstruction in BEAST 1.8.2. To obtain node age estimates for the species tree, an uncorrelated log-normal relaxed clock prior with a mean rate of 0.008735 was set for the mitochondrial DNA, based on the molecular clock rates obtained by the BEAST analyses (see Results) and the program set to estimate the rates for the nuclear DNA. Both the dated and species tree reconstructions were analysed via the CIPRES Science Gateway 3.3 (Miller *et al.*, 2010).

Diversification rates

The dated *Brachistosternus* species tree was used to investigate patterns of diversification using the R 3.2.3 (R Core Team, 2015) packages 'LASER' 2.4-1 (Rabosky & Schliep, 2013) and 'APE' 3.1-4 (Paradis *et al.*, 2004). Given the uncertainty concerning the exact number of *Brachistosternus* species in the phylogeny, analyses were conducted assuming low ($n = 5$) and high ($n = 50$) numbers of missing species, on which basis overall diversification rates were calculated with no extinction ($\epsilon = 0$) and high rates of extinction ($\epsilon = 0.9$). Five models of diversification (two constant-rate, 'pure birth' and 'birth-death', and three rate-variable models, exponential and linear density-dependent models 'DDX' and 'DDL', and a two-rate Yule model) were then compared, calculating the difference in the Akaike information criterion values (ΔAIC) of said models, as well as of the variable speciation 'SPVAR', variable extinction 'EXVAR', and variable speciation and extinction 'BOTHVAR' models (Rabosky & Lovette, 2008). Ten thousand simulated pure-birth trees were generated for comparison (P -value). The gamma statistic (Pybus & Harvey,

2000) was calculated, although it assumes complete sampling, and must therefore be interpreted with caution. Additionally, a lineage-through-time (LTT) plot was generated to visualize the accumulation of lineages in the genus. Bayesian analysis of macroevolutionary mixtures (Rabosky, 2014), implemented in BAMM 2.5.0, was used to assess diversification rate shifts, by running four Markov chains for 10 million generations and setting initial lambda and mu priors chosen by the R package 'BAMMTOOLS' 2.1.0 (Rabosky *et al.*, 2014), which was also used for post-analysis visualization of the results. The significance of the diversification rate shifts were tested with Bayes factors (Kass & Raftery, 1995).

Ancestral ranges and biogeographical events

Ancestral ranges and speciation events were estimated with the R package 'BioGeoBEARS' 0.2.1 (Matzke, 2012, 2013). 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). In addition, an extra parameter ('j') was considered for each method, modelling 'jump dispersal' or founder events (Matzke, 2014). The *Brachistosternus* species tree was used for the analyses and the species' distributions partitioned into 13 areas based on Morrone's (2006, 2014) biogeographical regions of Latin America (see Table S1.3 in Appendix 1). Further details of ancestral area and event estimations are provided in Appendix S3.

Phylogenetic diversity and evolutionary distinctiveness

The R package 'PICANTE' (Kembel *et al.*, 2010) was used to calculate Faith's PD (Faith, 1992) for the areas of *Brachistosternus* distribution. Ten thousand randomizations were conducted under the 'trialswap' null model (Miklós & Podani, 2004), which randomizes the data to obtain significance values for the PD indices of each area with regard to the expected values considering species occurrence frequencies and sampled species richness.

The evolutionary distinctiveness (ED) of each species was measured by 'equal splits' (Redding & Mooers, 2006) and 'fair proportions' (Isaac *et al.*, 2007). The PD and ED values were visualized across the entire distribution using BRODIVERSE 1.1 (Laffan *et al.*, 2010), setting the cell size to one unit for the GPS coordinates.

RESULTS

DNA sequences

The DNA sequences used in the study contained no missing data. The aligned mitochondrial 12S, 16S and COI sequences, respectively, comprised 103, 112 and 123

haplotypes, with 164/324, 179/463 and 449/1077 variable and 134/324, 133/463 and 375/1077 parsimony-informative sites. The aligned nuclear 18S and 28S markers, respectively, comprised 11 and 44 haplotypes, with 31/1761 and 57/517 variable and 13/1761 and 29/517 parsimony-informative sites. This diversity of nucleotide variation permitted resolution at different taxonomic levels, as indicated by the trees for the different partitions (see Figs S1.1–S1.6 in Appendix S1).

Divergence times and species trees

The divergence time analyses estimated a rate of nucleotide substitution of 8.73×10^{-3} substitutions/site/Myr for the mitochondrial DNA (9.25×10^{-3} for COI and 5.05×10^{-3} for 16S), comparable to rates obtained in previous studies for scorpion-specific mitochondrial markers, that is, 7×10^{-3} substitutions/site/Myr for COI (Gantenbein *et al.*, 2005) and 5×10^{-3} substitutions/site/Myr for 16S (Gantenbein & Largiadèr, 2003). Based on the calibrated Bayesian tree generated by *BEAST (Fig. 1), the most recent common ancestor (mrca) of *Brachistosternus* diverged *c.* 30.53 Ma, during the Oligocene (24.08–38.41, 95% highest posterior density). Most subsequent diversification occurred during the Neogene (late Miocene and Pliocene). The topology was mostly congruent with previous molecular phylogenetic reconstructions of *Brachistosternus* as regards the taxa common to both studies (Ojanguren-Affilastro *et al.*, 2016) and with the tree obtained by BEAST for the divergence time estimates (see Fig. S1.7 in Appendix S1). As in Ojanguren-Affilastro *et al.* (2016), the time-calibrated and species trees recovered subgenera *Brachistosternus* and *Ministernus* Francke, 1985 as well as four main clades within subgenus *Brachistosternus*: the Pacific Coastal Desert Clade (PCDC), the Atacama Desert Clade (ADC), the Andean-Pacific Clade (AndPC) and the Argentine Plains Clade (ArgPC) (Figs 1 & 2). The mrca of the PCDC appears to have speciated earliest, during the early Miocene. The mrca of the remaining clades diverged during the mid-Miocene, separating the ADC from the clade comprising the AndPC and ArgPC. The divergence and diversification of the AndPC and ArgPC appears to have occurred relatively rapidly during the mid- to late Miocene.

Diversification rates

The estimated rate of diversification in *Brachistosternus* is 0.062 Myr^{-1} assuming high extinction rates ($\epsilon = 0.9$) and few (5) missing species, 0.078 Myr^{-1} assuming high extinction rates and many (50) missing species, 0.111 Myr^{-1} assuming no extinction ($\epsilon = 0$) and few missing species, and 0.130 Myr^{-1} assuming no extinction and many missing species. Patterns of diversification in *Brachistosternus* were best explained by a model of variable speciation rather than variable extinction (Table S1.4 in Appendix S1). Furthermore, the positive ΔAICrc value, obtained by running five models, indicates that a rate variable rather than constant-rate model, specifically a Yule-2-rate model ($\Delta\text{AICrc} = 9.66$), best fits the

data. Based on the gamma statistic, diversification rates for *Brachistosternus* have not decreased over time ($\gamma\text{-stat.} = -1.640$; $P = 0.051$; Fig. 3b,c). No significant rate shifts were identified by the Bayesian analyses of macroevolutionary mixtures (BAMM), although increased rates were detected during the early divergence of the clade comprising species of subgenus *Brachistosternus* excluding the PCDC as well as during the last 2 Myr for species belonging to the AndPC inhabiting the Puna Province (Fig. 3a).

Ancestral ranges and biogeographical events

The likelihoods and probabilities obtained by dispersal-extinction-cladogenesis (BGB-D), DIVALIKE (BGB-V) and BAYAREALIKE (BGB-B) analyses in BioGeoBEARS (BGB), as well as by DEC in Lagrange are summarized in Table S1.5 in Appendix S1. When the three methods were applied in BGB, the AIC ratio test always preferred the model allowing founder-effect dispersal. The BGB-D+j model returned the highest global likelihood (results are shown in Fig. 4). All other results are presented as Figs S1.8–S1.19 in Appendix S1.

The area presently corresponding to the Puna and Coquimbo Provinces (G & J; Fig. 4, event 1) in the South American transition zone, which was at least 4 km lower in elevation during the Eocene than at present (Canavan *et al.*, 2014), was estimated to be the ancestral range for the mrca of genus *Brachistosternus*, based on the BGB-D+j model, albeit with a low relative probability (36.98%). The ancestral range for the mrca of *Ministernus* during the mid-Miocene was also estimated to be a widespread area including the Chaco, Monte and Puna Provinces (46.43% relative probability). Vicariance (Fig. 4, event 2) then separated the ancestral areas of *Brachistosternus andinus* Chamberlin, 1916 and *B. peruvianus* Piza, 1974, now inhabiting the Puna Province, from *B. ferrugineus* (Thorell, 1876) found in the Chaco, Pampa and Monte provinces, and *B. simoneae* Lourenço, 2000 inhabiting the Cerrado province.

In contrast to the species-poor subgenus *Ministernus*, subgenus *Brachistosternus* is highly diverse and its mrca appears to have diverged earlier (Oligo-Miocene boundary) while distributed in what is presently regarded as the Coquimbo Province (J; 39.56% relative probability; Fig. 4, event 3). The mrcas of its four major clades were distributed in present-day Coquimbo Province (mrca of PCDC, with 67.12% relative probability; mrca of ADC, with 94.16% relative probability), Central Patagonian Province (mrca of ArgPC, with 53.53% relative probability) and Prepuna Province (mrca of AndPC, with 45.40% relative probability). The northward range expansion of the mrca of the PCDC enabled this clade to diversify along the Pacific coast (Fig. 4, event 4), while a similar pattern occurred later for the ADC (Fig. 4, event 5). The ancestral range of the AndPC+ArgPC was established by the separation of the Coquimbo and Prepuna Provinces during the mid-Miocene. Subsequently, a founder-effect dispersal event to the Central Patagonian Province established the

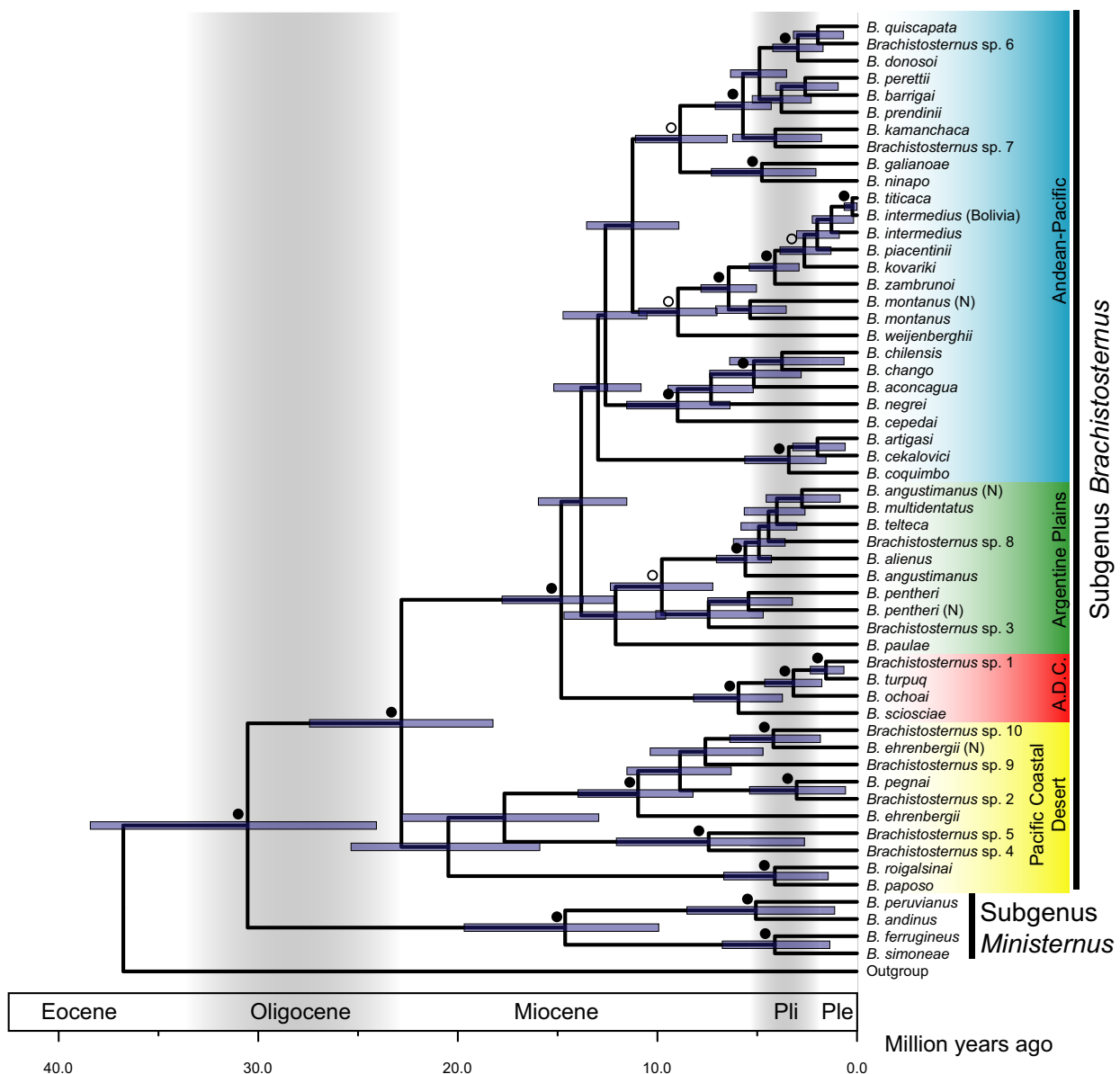


Figure 1 Species tree for the bothriurid scorpion genus *Brachistosternus* Pocock, 1893 with node age estimates inferred by *BEAST (Heled & Drummond, 2010). Bayesian posterior probabilities (PP) represented by circles at nodes (black circles: $1 \geq PP \geq 0.95$; white circles: $0.95 > PP \geq 0.90$), 95% highest posterior density of node heights by blue bars. Major clades are indicated on the right margin (ADC, Atacama Desert Clade). Geological epochs are indicated below the tree (Pli, Pliocene; Ple, Pleistocene).

ancestral range of the ArgPC (Fig. 4, event 6), which later colonized and diversified in the Monte and Pampa provinces (Fig. 4, event 7). The mrca of the AndPC diversified southward from the Prepuna Province (Fig. 4, event 8) and into the Central Andes (Fig. 4, event 9). Speciation, following founder-effect dispersal events, appears to have been common during the mid- to late Miocene and Pliocene (rate of 'j' for BGB-D+j: 0.139 Myr^{-1}). Range contractions or extinctions in certain areas and range expansions or anagenetic dispersal appear to have occurred less frequently, that is, 0.0073 and 0.0001 Myr^{-1} , respectively, than founder-effect dispersals (Table S1.5 in Appendix S1) and only three vicariance events were detected, all mid-Miocene or earlier.

Phylogenetic diversity and evolutionary distinctiveness

The highest PD for *Brachistosternus* is found in the Coastal Peruvian Desert Province (F), followed by the Coquimbo (J), Monte (E) and Puna (G) provinces, whereas the lowest PD is found in the Tumbes-Piura (A), Cerrado (B), Santiago+Maule (K) and Central Patagonia (L) provinces (Table S1.6 in Appendix S1). The observed PD values were not significantly different than expected based on 10,000 randomizations considering species occurrence and richness for those areas. In other words, the areas harbouring more species also exhibited higher PD values. The only exception was

the Puna Province (G), the observed PD value of which was significantly ($P < 0.05$) lower than expected. Although the PD of this area was relatively high overall, certain regions within it contained few species of *Brachistosternus*, as illustrated in Fig. 5, which reveals the fine scale areas which contributed to the overall values for each province.

The distribution map illustrating the evolutionary distinctiveness (ED) of *Brachistosternus* is similar to that of the PD. Per species ED was highest among the species of subgenus *Ministernus*, and among the species of subgenus *Brachistosternus* distributed along the Pacific coast (Fig. 5).

DISCUSSION

Origins and ancestral areas of *Brachistosternus*

The results presented here provide a plausible explanation for the origin and diversification of the bothriurid scorpion genus *Brachistosternus* based on dated geo-climatic events. The ancestral habitat of bothriurid scorpions was mesic and probably forested (Prendini, 2003a). *Brachistosternus* presently inhabits semi-arid to hyper-arid habitats and exhibits adaptations to loose, sandy soil, which may be highly developed in some species (Ojanguren-Affilastro *et al.*, 2016). The putative Eocene origin of the most recent common ancestor of this genus, at c. 30.53 Ma, is consistent with the origin of the oldest arid areas in western South America (Amundson *et al.*, 2012) which developed as a consequence of the proto-Humboldt current, after the South American continent separated completely from Antarctica and Oceania in the late Eocene (Zachos *et al.*, 2001; Dunai *et al.*, 2005). This cold Humboldt Current flows northward from Antarctica, along the Pacific coast of South America, and inhibits rainfall by cooling the air, thereby promoting aridification of the Pacific coast of central and north-central South America, considered the ancestral range of *Brachistosternus*.

Around the same time as the formation of the proto-Humboldt current (Lamb & Davis, 2003; Dunai *et al.*, 2005), the Andean mountain chain began uplifting, creating a rain shadow which exacerbated aridification on the western side of the Andes, as well as on the eastern side, in Patagonia. According to the analyses presented here, a marked shift in diversification rates of *Brachistosternus* occurred between 25 to 5 Ma (especially for the Andean clades), the period characterized by elevation of the Andes, and subsequent expansion of the arid zones of the continent, consistent with the suggestion by Prendini (2003a) that the increase in diversity of South American Bothriuridae occurred after the aridification of South America, in the late Miocene.

Andean uplift and diversification of *Brachistosternus*

This study indicates that diversification rates for *Brachistosternus* remained high and constant until the present, although differences were evident between subgenera *Brachistosternus* and *Ministernus*, the former being more species-rich

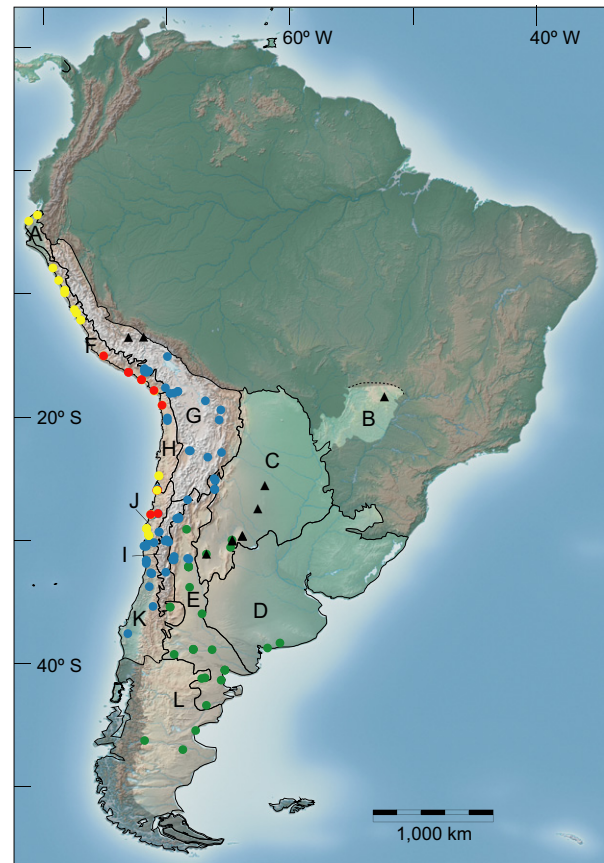


Figure 2 Map illustrating geographical delimitation of areas, based on Morrone (2006, 2014; see Table S1.3 in Appendix S1), for biogeographical study of the bothriurid scorpion genus *Brachistosternus* Pocock, 1893, and collection localities of species in subgenus *Ministernus* Francke, 1985 (black triangles) and the Andean-Pacific Clade (blue circles), the Argentine Plains Clade (green circles), the Atacama Desert Clade (red circles) and the Pacific Coastal Desert Clade (yellow circles) of subgenus *Brachistosternus*. Province 'B' (Cerrado) was reduced (dotted line) to the area inhabited by *Brachistosternus*.

(especially in the Andes), with higher rates of diversification than the latter, reflecting higher rates of speciation in *Brachistosternus* or higher rates of extinction in *Ministernus*. The general concordance between the timing of the main phase of Andean uplift and the steepest part of the species accumulation curve for *Brachistosternus* suggests that Andean uplift promoted speciation in this genus, as in other Andean taxa which diversified during the last 10 Myr (e.g. Chaves *et al.*, 2011; Jabaily & Sytsma, 2013; Fernandes *et al.*, 2014). Therefore, the diversification of *Brachistosternus* at high altitudes of the Andes during the past 10 to 5 Ma coincides with the last phase of uplift, when the mountain chain increased by an altitude of c. 3000 m in a relatively short time period (Ghosh *et al.*, 2006; Garzone *et al.*, 2008).

The inferred divergence times are supported when diversification rates are examined more closely. Diversification rates among *Brachistosternus* species inhabiting the Andes are greater than among the species inhabiting non-Andean areas,

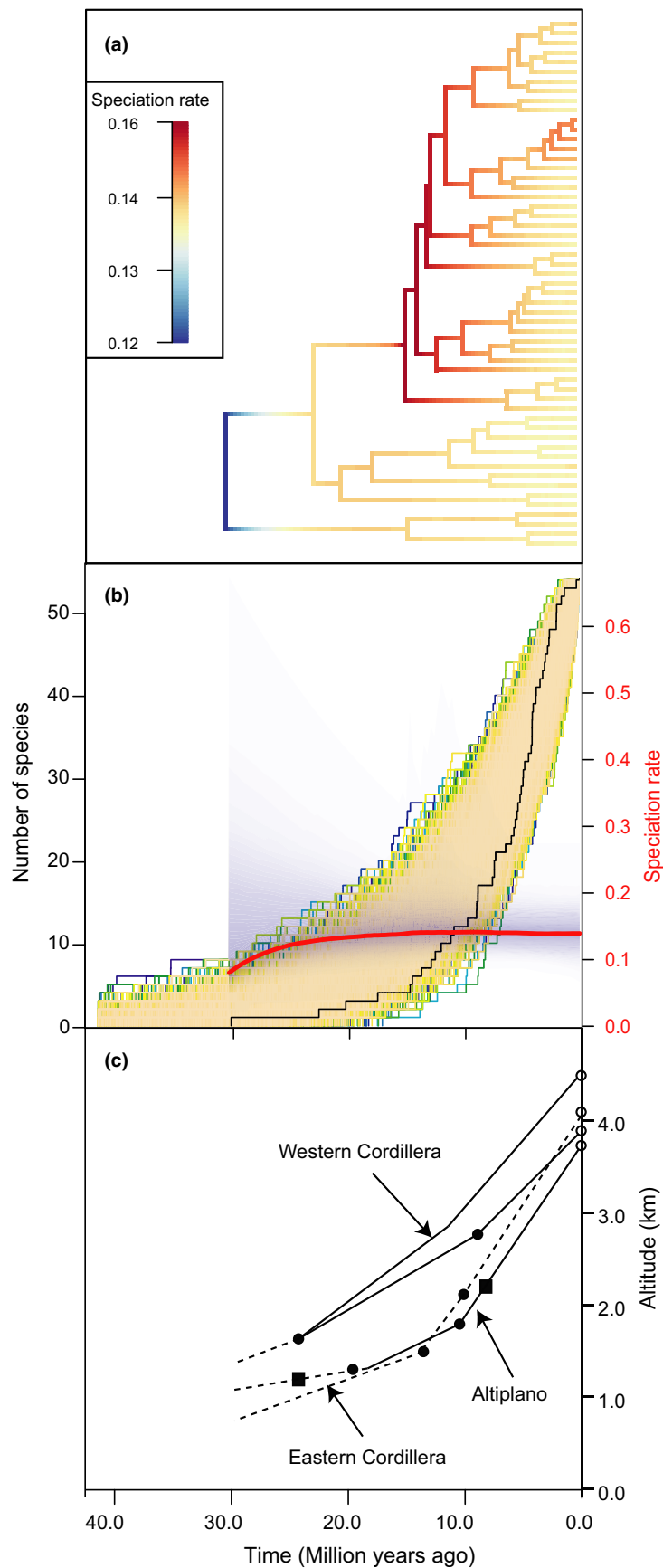


Figure 3 (a) Species tree for the bothriurid scorpion genus *Brachistosternus* Pocock, 1893 with speciation rates colour-coded along branches and values of colour gradient indicated in legend. (b) Lineage-through-time (LTT) plot for species tree in 3a (black line) not significantly different ($P = 0.999$) from 10,000 Yule-process-simulated trees (coloured lines), indicating speciation rate (red line) and confidence intervals (blue shading around red line). (c) Altitude-through-time plot for different parts of the Andes (modified from Hartley, 2003) plotted on same time-scale as LTT and speciation rate plots in b.

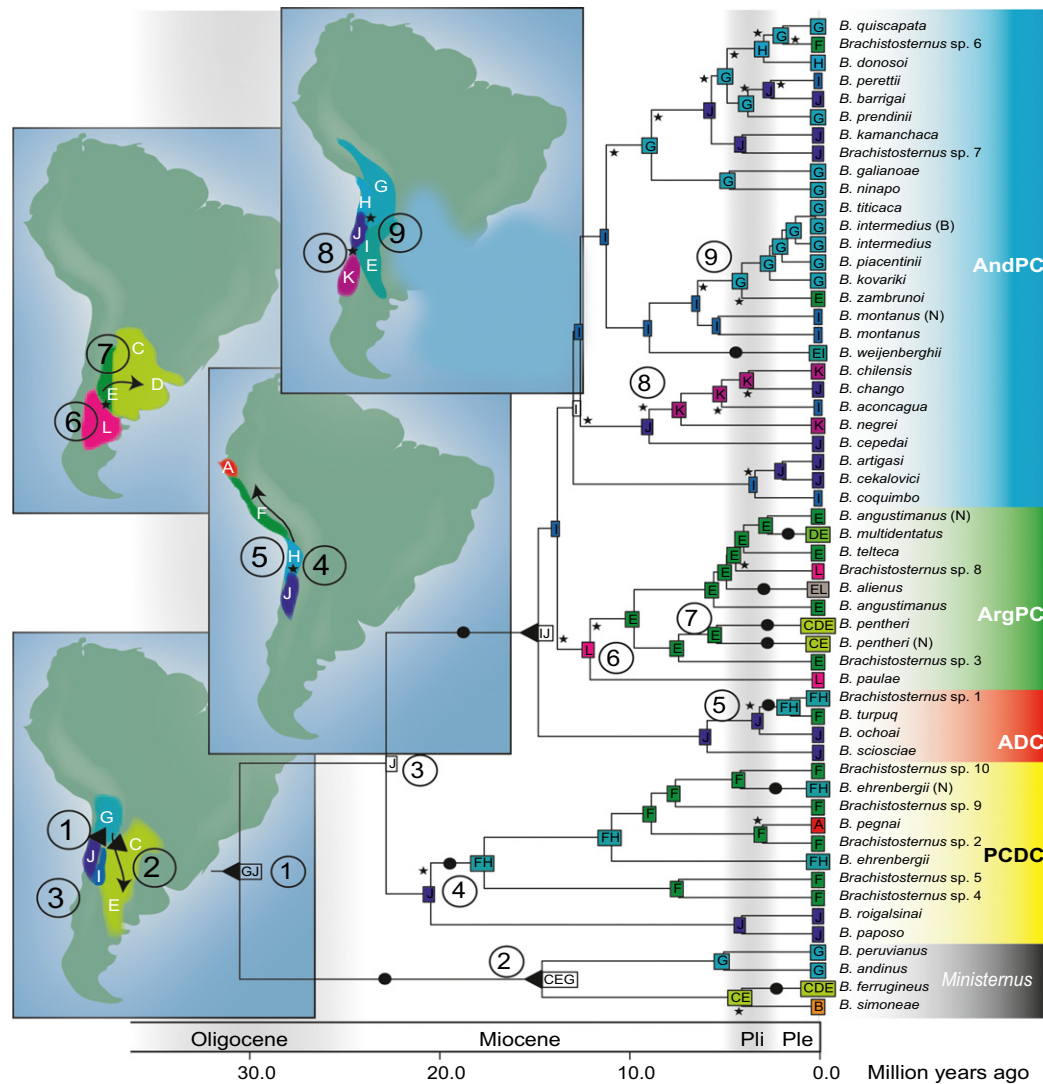


Figure 4 Ancestral range and historical biogeographical event estimates for the bothriurid scorpion genus *Brachistosternus* Pocock, 1893 in South America, based on DEC+j model implemented in BioGeoBEARS (Matzke, 2013). The four maps are schematic representations of ancestral areas and events, with numbers in circles cross-referencing selected events discussed in the text. Boxed letters at the tips and nodes of the tree represent current and ancestral ranges of distribution, respectively, with letters corresponding to areas indicated on the larger map, coloured when the relative probability (P) was > 0.5 and white when $0.3 < P < 0.5$. Symbols at nodes and on branches represent events (black circle = dispersal/anagenetic range expansion; black star = 'jump' dispersal/cladogenetic founder-effect speciation; black triangle = vicariant speciation event). Geological epochs are indicated below the tree (Pli = Pliocene, Ple = Pleistocene) and clades are coloured as in Figs 1 and 2: Pacific Coastal Desert Clade (PCDC), Atacama Desert Clade (ADC), Andean-Pacific Clade (AndPC), Argentine Plains Clade (ArgPC).

for which the following explanations, which are not mutually exclusive, may apply.

Orogeny promoted speciation

As the Andes uplifted, more habitats and transitional ecosystems became available to *Brachistosternus* species. The present evidence can be seen at high altitude (above 3000 m) where several hitherto undescribed species of *Brachistosternus* were discovered during the past decade (A.A. Ojanguren-Affilastro *et al.*, unpublished data). These areas represent extremely

harsh environments, to which the species inhabiting them are highly adapted and usually restricted to narrow zones of no more than 1500 m between the upper and lower altitudes. Among these specialized *Brachistosternus* species inhabiting high-altitude areas on adjacent mountains, each mountain represents an 'island'. The intervening lowlands presumably acted as agents of vicariance, isolating ancestral populations as they were carried upwards by the rising mountains. According to this scenario, the distributions of ancestral populations encompassed the distributions of all descendent populations presently isolated on different mountains.

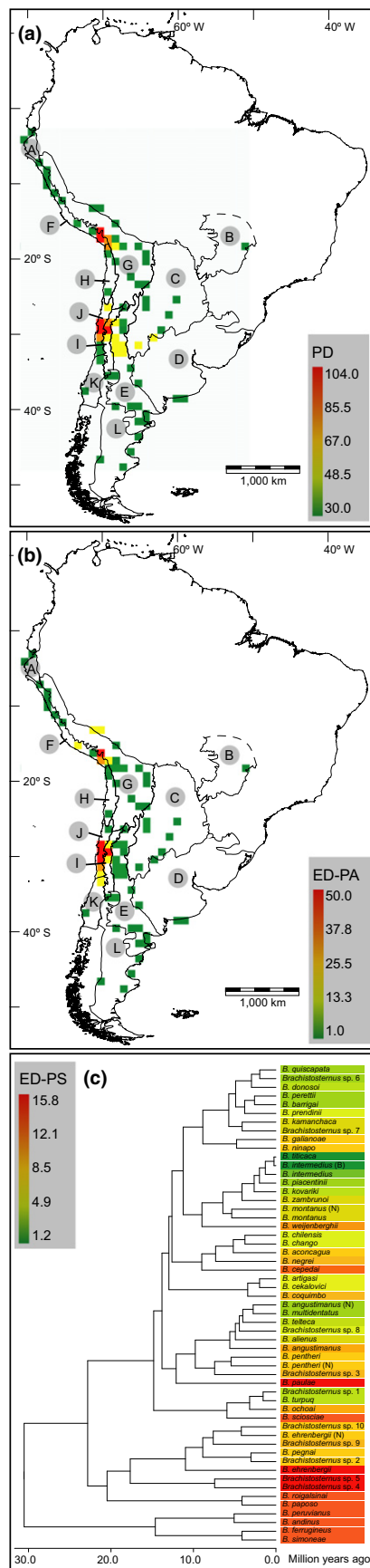


Figure 5 (a, b) Distribution of the bothriurid scorpion genus *Brachistosternus* Pocock, 1893 representing the phylogenetic diversity (PD) and evolutionary distinctiveness (ED-PA) in each area (letters represent provinces based on Morrone, 2006, 2014; see Table S1.6 in Appendix S1). (c) The evolutionary distinctiveness of each species (ED-PS) in the species tree is coloured accordingly.

Alternatively, populations from smaller ancestral areas may have dispersed into new microhabitats before becoming separated by distance and suboptimal intervening habitat as the mountains rose, ultimately undergoing founder-effect speciation in a manner similar to the 'vertical ecotone' speciation hypothesis (e.g. Guarnizo *et al.*, 2009), in the sense that newly formed intervening or transitional ecosystems may favour parapatric speciation. In the present study, no vicariance events were detected during the phase of accelerated orogeny (last 10 Myr), probably owing to the fact that vicariance events between the areas chosen in the analysis were less likely than founder effects, because the ancestral areas were rarely estimated to span more than one present-day province. Therefore, on a broad scale, Andean uplift does not appear to have created an east–west 'barrier', resulting in speciation by vicariance in *Brachistosternus*, as in fishes (Albert *et al.*, 2006), contrary to the suggestion by Ojanguren-Affilastro & Ramírez (2009) that the Andes presented a barrier separating ancient populations of *Brachistosternus* in the lowlands on either side. However, the few vicariance events detected in this study suggest a separation between lowland and highland populations leading to speciation during the early phases of Andean uplift, similar to the hypothesis put forward by Quintero *et al.* (2012), but at an earlier stage. Fine-scale studies, with more detailed area delimitations, are necessary to test hypotheses concerning localized vicariance events.

Lowland extinctions

There are several possible explanations for the low diversity of *Brachistosternus* in the semi-desert lowlands on the eastern side of the Andes compared to the high diversity in the Andes. The former habitats are less fragmented and cover larger areas compared to the western side of the Andes. For example, *B. ferrugineus*, an abundant species occupying an area of c. 1 million km², encompassing both the Chaco and Espinal provinces, exhibits little intraspecific variation in morphology or cytogenetics across its broad distribution (Ojanguren-Affilastro, 2005; Rodríguez-Gil *et al.*, 2009), supporting the hypothesis of a relatively recent colonization or range expansion. Numerous marine transgressions in the eastern plains of South America, responsible for extinctions or displacements of terrestrial fauna (Hernández *et al.*, 2005; Lima & Ribeiro, 2011; Candela *et al.*, 2012), may have further reduced the diversity.

Ancestral range estimations and phylogenetic diversity of *Brachistosternus*

Based on the biogeographical estimation presented here, the mrca of *Brachistosternus* inhabited the vicinity of the present-day Altiplano, Coquimbo and the Coastal Peruvian Desert, when the area was much lower in altitude, and probably closer to the coast (Canavan *et al.*, 2014). The Altiplano is not currently a hotspot for *Brachistosternus*, despite being part of its ancestral range. Indeed, the Altiplano represents a coldspot for scorpion diversity and endemism, as corroborated by the significantly lower than expected PD value, most of its species occurring on the slopes of mountains surrounding it (Mattoni *et al.*, 2012). This may be due to extinction or dispersal out of the area associated with several flooding events (Fornari *et al.*, 2001).

On the other hand, the Coastal Peruvian Desert and Coquimbo area contain high PD for *Brachistosternus*, as expected by the high species richness observed there. The current hotspots of PD and evolutionary distinctiveness (ED) inferred for *Brachistosternus* coincide with hotspots for other organisms. For example, central Chile is one of the 25 global biodiversity hotspots (Myers *et al.*, 2000) while the southern Peruvian Coastal Desert is an area of high conservation priority (Rodríguez & Young, 2000) because of its high levels of endemism. Although mid-elevation Andean habitats were found to harbour higher species richness in other studies (Hutter *et al.*, 2013), due to a presumably extended period of 'stability' for speciation, that is, the so-called 'montane species-pump' and 'montane museum' hypotheses (Smith *et al.*, 2007; Wiens *et al.*, 2007), this may not apply to *Brachistosternus* with their requirement for drier habitats. Instead, the relative long-term stability of the Peruvian Coastal Desert and the Coquimbo area of central Chile appears to explain the high PD and ED found in this study.

CONCLUSIONS

The formation of one of the planet's most impressive geological landforms, the Andes mountain chain, had an enormous impact on South American hydrogeomorphology, climate and biota. As revealed by this study, the scorpion genus *Brachistosternus* is no exception. The evolution and diversification of this genus is closely linked to the major phase of Andean uplift and its origins lie in the central proto-Andean region. A series of complex geological and climatic events shaped the present distribution of *Brachistosternus*, resulting in heterogeneous clades with different levels of diversification and areas of high PD.

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REFERENCES

- Albert, J.S., Lovejoy, N.R. & Crampton, W.G.R. (2006) Miocene tectonism and the separation of cis- and trans-Andean river basins: evidence from Neotropical fishes. *Journal of South American Earth Sciences*, **21**, 14–27.
- Almeida, E.A.B., Pie, M.R., Brady, S.G. & Danforth, B.N. (2012) Biogeography and diversification of colletid bees (Hymenoptera: Colletidae): emerging patterns from the southern end of the world. *Journal of Biogeography*, **39**, 526–544.
- Amundson, R., Dietrich, W., Bellugi, D., Ewing, S., Nishizumi, K., Chong, G., Owen, J., Finkel, R., Heimsath, A., Stewart, B. & Caffee, M. (2012) Geomorphologic evidence for the late Pliocene onset of hyperaridity in the Atacama Desert. *Bulletin of the Geological Society of America*, **124**, 1048–1070.
- Antonelli, A. & Sanmartín, I. (2011) Mass extinction, gradual cooling, or rapid radiation? Reconstructing the spatiotemporal evolution of the ancient angiosperm genus *Hedyosmum* (Chloranthaceae) using empirical and simulated approaches. *Systematic Biology*, **60**, 596–615.
- Bryson, R.W., Jr, Riddle, B.R., Graham, M.R., Tilston Smith, B. & Prendini, L. (2013) As old as the hills: montane scorpions in southwestern North America reveal ancient associations between biotic diversification and landscape history. *PLoS ONE*, **8**, e52822. doi:10.1371/journal.pone.0052822.
- Canavan, R.R., Carrapa, B., Clementz, M.T., Quade, J., DeCelles, P.G. & Schoenbohm, L.M. (2014) Early Cenozoic uplift of the Puna Plateau, Central Andes, based on stable isotope paleoaltimetry of hydrated volcanic glass. *Geology*, **42**, 447–450.
- Candela, A.M., Bonini, R.A. & Noriega, J.I. (2012) First continental vertebrates from the marine Paraná Formation (Late Miocene, Mesopotamia, Argentina): chronology, biogeography, and paleoenvironments. *Geobios*, **45**, 515–526.

- Chaves, J.A., Weir, J.T. & Smith, T.B. (2011) Diversification in *Adelomyia* hummingbirds follows Andean uplift. *Molecular Ecology*, **20**, 4564–4576.
- Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.
- Dunai, T.J., González-López, G.A. & Juez-Larré, J. (2005) Oligocene-Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. *Geographical Society of America*, **33**, 321–324.
- Faith, D.P. (1992) Systematics and conservation: on predicting the feature diversity of subsets of taxa. *Cladistics*, **8**, 361–373.
- Fernandes, A.M., Wink, M., Sardelli, C.H. & Aleixo, A. (2014) Multiple speciation across the Andes and throughout Amazonia: the case of the spot-backed antbird species complex (*Hylophylax naevius*/*Hylophylax naevioides*). *Journal of Biogeography*, **41**, 1365–2699.
- Fjeldså, J., Bowie, R.C.K. & Rahbek, C. (2012) The role of mountain ranges in the diversification of birds. *Annual Review of Ecology, Evolution and Systematics*, **43**, 249–265.
- Fornari, M., Risacher, F. & Féraud, G. (2001) Dating the paleolakes of the central Altiplano of Bolivia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **172**, 269–282.
- Gantenbein, B. & Largiadèr, C.R. (2003) The phylogeographic importance of the Strait of Gibraltar as a gene flow barrier in terrestrial arthropods: a case study with the scorpion *Buthus occitanus* as model organism. *Molecular Phylogenetics and Evolution*, **28**, 119–130.
- Gantenbein, B., Fet, V., Gantenbein-Ritter, I.A. & Balloux, F. (2005) Evidence for recombination in scorpion mitochondrial DNA (Scorpiones: Buthidae). *Proceedings of the Royal Society B: Biological Sciences*, **272**, 697–704.
- Garzone, C.N., Hoke, G.D., Libarkin, J.C., Withers, S., MacFadden, B., Eiler, J., Ghosh, P. & Mulch, A. (2008) Rise of the Andes. *Science*, **320**, 1304–1307.
- Ghosh, P., Garzone, C.N. & Eiler, J.M. (2006) Rapid uplift of the Altiplano revealed through 13C–18O bonds in paleosol carbonates. *Science*, **311**, 511–515.
- Giarla, T.C. & Jansa, S.A. (2014) The role of physical geography and habitat type in shaping the biogeographical history of a recent radiation of Neotropical marsupials (*Thylamys*: Didelphidae). *Journal of Biogeography*, **41**, 1547–1558.
- Graham, A. (2009) The Andes: a geological overview from a biological perspective. *Annals of the Missouri Botanical Garden*, **96**, 371–385.
- Guarnizo, C.E., Amézquita, A. & Bermingham, E. (2009) The relative roles of vicariance versus elevational gradients in the genetic differentiation of the high Andean tree frog, *Dendropsophus labialis*. *Molecular Phylogenetics and Evolution*, **50**, 84–92.
- Hartley, A.J. (2003) Andean uplift and climate change. *Journal of the Geological Society London*, **160**, 7–10.
- Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.
- Hernandez, R.M., Jordan, T.E., Dalenz Farjat, A., Echavarría, L., Idleman, B.D. & Reynolds, J.H. (2005) Age, distribution, tectonics, and eustatic controls of the Paraneense and Caribbean marine transgressions in southern Bolivia and Argentina. *Journal of South American Earth Sciences*, **19**, 495–512.
- Hoorn, C., Wesselingh, F.P., Ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkinen, T. & Antonelli, A. (2010) Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, **330**, 927–931.
- Husson, L., Conrad, C.P. & Faccenna, C. (2012) Plate motions, Andean orogeny, and volcanism above the South Atlantic convection cell. *Earth and Planetary Science Letters*, **317–318**, 126–135.
- Hutter, C.R., Guayasamin, J.M. & Wiens, J.J. (2013) Explaining Andean megadiversity: the evolutionary and ecological causes of glassfrog elevational richness patterns. *Ecology Letters*, **16**, 1–9.
- Isaac, N.J.B., Turvey, S.T., Collen, B., Waterman, C. & Bailie, J.E.M. (2007) Mammals on the EDGE: conservation priorities based on threat and phylogeny. *PLoS ONE*, **2**, e296. doi:10.1371/journal.pone.0000296.
- Jabaily, R.S. & Sytsma, K.J. (2013) Historical biogeography and life-history evolution of Andean *Puya* (Bromeliaceae). *Botanical Journal of the Linnean Society*, **171**, 201–224.
- Kass, R.E. & Raftery, A.E. (1995) Bayes factors. *Journal of the American Statistical Association*, **90**, 773–795.
- Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P. & Webb, C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.
- Kovářík, F. & Ojanguren-Affilastro, A.A. (2013) *Illustrated catalog of scorpions. Part II. Bothriuridae; Chaeriliidae; Buthidae I., genera Compsobuthus, Hottentotta, Isometrus, Lychas, and Sassanidotus*, p. 400. Jakub Rolčík Publisher, Czech Republic.
- Laffan, S.W., Lubarsky, E. & Rosauer, D.F. (2010) Biodiverse, a tool for the spatial analysis of biological and related diversity. *Ecography*, **33**, 643–647.
- Lamb, S. & Davis, P. (2003) Cenozoic climate change as a possible cause for the rise of the Andes. *Nature*, **425**, 792–797.
- Landis, M.J., Matzke, N.J. & Moore, B.R. (2013) Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology*, **62**, 789–804.
- Lima, F.C.T. & Ribeiro, A.C. (2011) Continental-scale tectonic controls of biogeography and ecology. *Historical biogeography of Neotropical freshwater fishes* (ed. by J.S. Albert and R.E. Reis), pp. 145–164. University of California, Oakland, CA, USA.

- Luebert, F., Hilger, H.H. & Weigend, M. (2011) Diversification in the Andes: age and origins of South American *Heliotropium* lineages (Heliotropiaceae, Boraginales). *Molecular Phylogenetics and Evolution*, **61**, 90–102.
- Mattoni, C.I. & Prendini, L. (2007) Phylogeny and biogeography of the family Bothriuridae (Scorpiones). *Darwiniana*, **45**, 96–98.
- Mattoni, C.I., Ochoa, J.A., Ojanguren-Affilastro, A.A. & Prendini, L. (2012) *Orobothriurus* (Scorpiones: Bothriuridae) phylogeny, Andean biogeography, and the relative importance of genitalic and somatic characters. *Zoologica Scripta*, **41**, 160–176.
- Matzke, N.J. (2012) Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in Dispersal-Extinction-Cladogenesis (DEC) analyses. *Frontiers of Biogeography*, **4**, 210.
- Matzke, N.J. (2013) *BioGeoBEARS: Biogeography with Bayesian (and Likelihood) evolutionary analysis in R scripts*. University of California, Berkeley, CA.
- Matzke, N.J. (2014) Model Selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology*, **63**, 951–970.
- Miklós, I. & Podani, J. (2004) Randomization of presence-absence matrices: comments and new algorithms. *Ecology*, **85**, 86–92.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 November 2010, pp. 1–8. IEEE publications, New Orleans, LA.
- Morrone, J.J. (2006) Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. *Annual Review of Entomology*, **51**, 467–494.
- Morrone, J.J. (2014) Biogeographical regionalisation of the Neotropical region. *Zootaxa*, **3782**, 1–110.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.
- Ojanguren-Affilastro, A.A. (2005) Estudio monográfico de los escorpiones de la República Argentina. *Revista Ibérica de Aracnología*, **11**, 75–241.
- Ojanguren-Affilastro, A.A. & Pizarro-Araya, J. (2014) Two new scorpion species from Paposo, in the coastal desert of Taltal, Chile (Scorpiones, Bothriuridae, *Brachistosternus*). *Zootaxa*, **3785**, 400–418.
- Ojanguren-Affilastro, A.A. & Ramírez, M.J. (2009) Phylogenetic analysis of the scorpion genus *Brachistosternus* (Arachnida, Scorpiones, Bothriuridae). *Zoologica Scripta*, **38**, 183–198.
- Ojanguren-Affilastro, A.A., Mattoni, C.I., Ochoa, J.A., Ramírez, M.J., Ceccarelli, F.S. & Prendini, L. (2016) Phylogeny, species delimitation and convergence in the South American bothriurid scorpion genus *Brachistosternus* Pocock 1893: integrating morphology, nuclear and mitochondrial DNA. *Molecular Phylogenetics and Evolution*, **94**, 159–170.
- Paradis, E., Claude, J. & Strimmer, K. (2004) APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.
- Parmakelis, A., Stathi, I., Chatzaki, M., Simaiakis, S., Spanos, L., Louis, C. & Mylonas, M. (2006) Evolution of *Mesobuthus gibbosus* (Brullé, 1832) (Scorpiones: Buthidae) in the northeastern Mediterranean region. *Molecular Ecology*, **15**, 2883–2894.
- Pocco, M.E., Posadas, P.A., Lange, C.E. & Cigliano, M.M. (2013) Patterns of diversification in the high Andean *Ponderacris* grasshoppers (Orthoptera: Acrididae: Melanoplinae). *Systematic Entomology*, **38**, 365–389.
- Prendini, L. (2000) Phylogeny and classification of the superfamily Scorpionoidea Latreille 1802 (Chelicerata, Scorpiones): an exemplar approach. *Cladistics*, **16**, 1–78.
- Prendini, L. (2001) Substratum specialization and speciation in southern African scorpions: the Effect Hypothesis revisited. *Scorpions 2001*. In Memoriam (ed. by G.A. Polis, V. Fet and P.A. Selden), pp. 113–138. British Arachnological Society, Burnham Beeches, Bucks, UK.
- Prendini, L. (2003a) A new genus and species of bothriurid scorpion from the Brandberg Massif, Namibia, with a reanalysis of bothriurid phylogeny and a discussion of the phylogenetic position of *Lisposoma* Lawrence. *Systematic Entomology*, **28**, 149–172.
- Prendini, L. (2003b) Revision of the genus *Lisposoma* Lawrence 1928 (Scorpiones: Bothriuridae). *Insect Systematics and Evolution*, **34**, 241–264.
- Prendini, L., Crowe, T.M. & Wheeler, W.C. (2003) Systematics and biogeography of the family Scorpionidae Latreille, with a discussion of phylogenetic methods. *Invertebrate Systematics*, **17**, 185–259.
- Pybus, O.G. & Harvey, P.H. (2000) Testing macro-evolutionary models using incomplete molecular phylogenies. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 2267–2272.
- Quintero, E., Ribas, C.C. & Cracraft, J. (2012) The Andean *Hapalopsittaca* parrots (Psittacidae, Aves): an example of montane-tropical lowland vicariance. *Zoologica Scripta*, **42**, 28–43.
- R Core Team (2015) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org/>.
- Rabosky, D.L. (2014) Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE*, **9**, e89543.
- Rabosky, D.L. & Lovette, I.J. (2008) Explosive evolutionary radiations: decreasing speciation or increasing extinction through time? *Evolution*, **62**, 1866–1875.
- Rabosky, D.L. & Schliep, K. (2013). *Laser: Likelihood analysis of speciation/extinction rates from phylogenies*. R package version 2.4-1. Available at: <http://CRAN.R-project.org/package=laser>.

- Rabosky, D.L., Grundler, M., Anderson, C., Title, P., Shi, J.J., Brown, J.W., Huang, H. & Larson, J.G. (2014) BAMM-tools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution*, **5**, 701–707.
- Redding, D.W. & Mooers, A.O. (2006) Incorporating evolutionary measures into conservation prioritization. *Conservation Biology*, **20**, 1670–1678.
- Ree, R.H., Moore, B.R., Webb, C.O. & Donoghue, M.J. (2005) A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, **59**, 2299–2311.
- Rodríguez, L.O. & Young, K.R. (2000) Biological diversity of Peru: determining priority areas for conservation. *Ambio*, **29**, 329–337.
- Rodríguez-Gil, S., Ojanguren-Affilastro, A.A., Barral, L., Scioscia, C.L. & Mola, L. (2009) Cytogenetics of three species of *Brachistosternus* Pocock from Argentina (Scorpi- ones, Bothriuridae). *Journal of Arachnology*, **37**, 331–337.
- Ronquist, F. (1997) Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, **46**, 195–203.
- Ronquist, F. & Sanmartín, I. (2011) Phylogenetic methods in biogeography. *Annual Review of Ecology, Evolution and Sys- tematics*, **42**, 441–464.
- Sanchez-Gonzalez, L.A., Navarro-Siguenza, A.G., Krabbe, N.K., Fjeldsø, J. & Garcia-Moreno, J. (2014) Diversification in the Andes: the *Atlapetes* brush-finches. *Zoologica Scripta*, **44**, 135–152.
- Sanmartín, I. (2012) Historical biogeography: evolution in time and space. *Evolution: Education and Outreach*, **5**, 555–568.
- Sanmartín, I. & Ronquist, F. (2004) Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology*, **53**, 216–243.
- Smith, S.A., Nieto Montes de Oca, A., Reeder, T.W. & Wiens, J.J. (2007) A phylogenetic perspective on eleva- tional species richness patterns in Middle American tree- frogs: why so few species in lowland tropical rainforests? *Evolution*, **61**, 1188–1207.
- Turner, K.J., Fogwill, C.J., McCulloch, R.D. & Sugden, D.E. (2005) Deglaciation of the eastern flank of the North Patagonian Icefield and associated continental-scale lake diversions. *Geografiska Annaler Series A: Physical Geogra- phy*, **87A**, 363–374.
- Wen, J., Ree, R.H., Ickert-Bond, S.M., Nie, Z. & Funk, V. (2013) Biogeography: where do we go from here? *Taxon*, **62**, 912–927.
- Wiens, J.J., Parra-Olea, G., Garcia-Paris, M. & Wake, D.B. (2007) Phylogenetic history underlies elevational patterns of biodiversity in tropical salamanders. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 919–928.
- Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. (2001) Trends, rhythms and aberrations in global climate 65 Ma to present. *Science*, **292**, 686–693.
- Zemlak, T.S., Habit, E.M., Walde, S.J., Battini, M.A., Adams, E.D.M. & Ruzzante, D.E. (2008) Across the southern Andes on fin: glacial refugia, drainage reversals and a sec- ondary contact zone revealed by the phylogeographical sig- nal of *Galaxias platei* in Patagonia. *Molecular Ecology*, **17**, 5049–5061.

SUPPORTING INFORMATION

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

Appendix S1 Supplementary tables and figures.

Appendix S2 Supplementary materials and methods.

Appendix S3 Supplementary materials and methods.

BIOSKETCH

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