ORIGINAL ARTICLE

Andean uplift drives diversification of the bothriurid scorpion genus Brachistosternus

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INTRODUCTION

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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

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; Garzione *et al.*, 2008; Hoorn *et al.*, 2010). Andean uplift had a profound effect on South American geology, climate and biodiversity (Graham, 2009; Fjeldså *et al.*, 2012; Giarla & Jansa, 2014) by changing the flow of major rivers (Turner *et al.*, 2005; Zemlak *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

http://wileyonlinelibrary.com/journal/jbi doi:10.1111/jbi.12760 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-Affilastro *et al.*, 2016). The genus contains 43 described species (Kovařík & Ojanguren-Affilastro, 2013; Ojanguren-Affilastro & Pizarro-Araya, 2014) and an unknown number awaiting description (A.A. Ojanguren-Affilastro *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-Affilastro & Ramírez, 2009; Kovařík & Ojanguren-Affilastro, 2013). Samples of *Brachistosternus mattonii* Ojanguren-Affilastro, 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-Affilastro *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 Scorpionidea 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 basepairs (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-Affilastro *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. Cercophonius, endemic to Australia and Tasmania, is the only bothriurid genus from Oceania (Prendini, 2000). Urophonius, 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 Cercophonius and Urophonius, 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-Affilastro 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 lognormal 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 ($\varepsilon = 0$) and high rates of extinction ($\varepsilon = 0.9$). 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 BIODI-VERSE 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 \times 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 ($\varepsilon = 0.9$) 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 ($\varepsilon = 0$) 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 Δ AICrc value, obtained by running five models, indicates that a rate variable rather than constant-rate model, specifically a Yule-2-rate model (Δ AICrc = 9.66), best fits the

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data. Based on the gamma statistic, diversification rates for *Brachistosternus* have not decreased over time (γ -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 dispersalextinction-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 presentday 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: $1 \ge PP > 0.95$; white circles: $0.95 > PP \ge 0.90$), 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

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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; Garzione *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.



Andean uplift drives Brachistosternus scorpion diversification

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 presentday 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; Rodriguez-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 (Hernandez 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 presentday 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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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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