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LETTER

Explaining Andean megadiversity: the evolutionary and ecological causes of glassfrog elevational richness patterns

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Abstract

The Tropical Andes are an important global biodiversity hotspot, harbouring extraordinarily high richness and endemism. Although elevational richness and speciation have been studied independently in some Andean groups, the evolutionary and ecological processes that explain elevational richness patterns in the Andes have not been analysed together. Herein, we elucidate the processes underlying Andean richness patterns using glassfrogs (Centrolenidae) as a model system. Glassfrogs show the widespread mid-elevation diversity peak for both local and regional richness. Remarkably, these patterns are explained by greater time (montane museum) rather than faster speciation at mid-elevations (montane species pump), despite the recency of the major Andean uplift. We also show for the first time that rates of climatic-niche evolution and elevational change are related, supporting the hypothesis that climatic-niche conservatism decelerates species' shifts in elevational distributions and underlies the mid-elevation richness peak. These results may be relevant to other Andean clades and montane systems globally.

Keywords

Centrolenidae, climatic-niche evolution, montane museum, montane species pump, niche conservatism, time-for-speciation effect.

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INTRODUCTION

Explaining species richness patterns in montane regions is a critical issue for ecologists and conservationists. Despite a smaller total area, intermediate elevations within montane regions often have higher species richness than adjacent lowlands, with many narrowly distributed species that are increasingly threatened by habitat modification and climate change (e.g. Rahbek 1995; Myers *et al.* 2000; La Sorte & Jetz 2010). Therefore, understanding richness patterns in highly diverse tropical montane regions are particularly important. Nevertheless, the causes of these elevational richness patterns remain poorly understood.

Recently, some studies have considered both the evolutionary and ecological causes of elevational richness patterns (e.g. Smith *et al.* 2007; Li *et al.* 2009; Kozak & Wiens 2010a). Three processes are ultimately responsible for the accumulation of species in a biogeographical region: speciation, extinction and dispersal (Ricklefs 1987). In terms of these processes, two non-exclusive hypotheses have been proposed to explain elevational species richness patterns: the montane species-pump hypothesis and the montane museum hypothesis (Smith *et al.* 2007; Wiens *et al.* 2007). The montane species-pump hypothesis predicts that clades occurring at mid-elevations (where richness is highest) will have higher net diversification rates (speciation minus extinction), presumably caused by increased diversification rates at mid-elevations relative to the lowlands due to habitat heterogeneity. Under the montane museum hypothesis (based on the time-for-speciation effect; Stephens & Wiens 2003), clades colonised intermediate elevation habitats early in their history, leaving more time for speciation and species accumulation in these habitats, relative to those in other elevational zones (similar to the tropical museum hypothesis, which typically focuses on lineage ages across latitudes). Given that climatic zones and their associated habitats may shift up and down mountain slopes over time, the montane museum hypothesis does not require that mid-elevations be colonised first, but rather that habitats presently at mid-elevations be colonised before others. Support for the montane museum hypothesis includes analyses of Middle American salamanders (Wiens *et al.* 2007) and treefrogs (Smith *et al.* 2007), North American salamanders (Kozak & Wiens 2010a) and Asian fish (Li *et al.* 2009), whereas the montane species-pump hypothesis is supported in Middle American treefrogs (Smith *et al.* 2007). However, no study has explicitly tested these hypotheses in the Andes Mountains of South America, the most species-rich mountain range in the world (Myers *et al.* 2000).

These two hypotheses may be closely related to climatic-niche divergence and conservatism, and rates of climatic-niche evolution. Under climatic-niche divergence, species depart from their ancestral climatic-niche to occupy different climatic regimes. Frequent niche divergence among species in a clade may drive speciation and increase the rate of climatic-niche evolution. Climatic-niche divergence and rapid niche evolution may underlie the montane species-pump hypothesis, as colonisation of new elevations and new climatic regimes may increase speciation in montane regions (Kozak & Wiens 2007). In plethodontid salamanders, accelerated rates of climatic-niche evolution are associated with increased diversification rates (Kozak & Wiens 2010b), especially in an Andean clade with

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high richness at mid-elevations (Wiens *et al.* 2007). Under niche conservatism, species tend to retain similar climatic-niches over time (i.e. low rates of climatic-niche evolution), which may result in increased montane diversification through allopatric speciation from range fragmentation during climatic changes (Kozak & Wiens 2007). Thus, both niche divergence and conservatism could underlie the montane species-pump hypothesis. Furthermore, niche conservatism may underlie the montane museum hypothesis, as retention of ancestral climatic tolerances may cause a mid-elevation peak in species richness due to the inability of lineages to disperse into different climatic regimes at lower and higher elevations (e.g. Kozak & Wiens 2010a). However, the connections between climatic-niche divergence, conservatism and elevational distributions remain poorly explored. Specifically, no studies have tested how rates of climatic-niche evolution are related to rates of change in species elevational distributions.

The Tropical Andes have the highest density of species per area in the world, making this region one of the most important global biodiversity hotspots, with 6.7% of the world's plant and 5.7% of the world's vertebrate species (Myers *et al.* 2000). Several studies have shown high richness at intermediate elevations in the Andes (e.g. Rahbek 1997; Kessler *et al.* 2001; Kattan *et al.* 2004), a pattern seen in many montane regions globally (see above). Others have focused on speciation modes in Andean groups (mice: Patton & Smith 1992; frogs: Graham *et al.* 2004; birds: García-Moreno & Fjeldså 2000; Bonaccorso 2009). Furthermore, recent studies also suggest that several groups have accelerated speciation rates in the Andes (birds: Weir 2006; butterflies: Hall 2005; Rosser *et al.* 2012; frogs: Santos *et al.* 2009; plants: Hughes & Eastwood 2006). However, no studies have investigated the ecological and evolutionary causes of elevational richness patterns in any Andean clade.

Frogs exemplify the importance of the Andes to global biodiversity patterns. Among major continental regions, South America has the greatest species richness of frogs (Duellman 1999). Furthermore, the Andes contain more endemic frog species and overall richness than any other region in South America, with over twice as many species as the Amazon lowland rainforests (Duellman 1999). Many Andean frogs are also highly endangered, facing threats from deforestation, climate change and disease (IUCN 2012). Numerous authors have proposed that high Andean species richness for frogs and other organisms is explained by rapid speciation associated with the Andean uplift and historical climatic change (e.g. Lynch & Duellman 1997; Kattan *et al.* 2004; Rosser *et al.* 2012). This hypothesis is similar to the montane species-pump hypothesis, but has not been explicitly tested in the Andes.

Herein, we focus on species richness patterns of glassfrogs (Centrolenidae). Glassfrogs are arboreal, stream-dwelling frogs having transparent venters. There are 150 described species, with ~70% occurring in the Andes (Guayasamin *et al.* 2009; this work). Almost all are South American, with only two Middle American endemics.

In this study, we test the ecological and evolutionary origins of elevational richness patterns in the Andes, using glassfrogs as a model system. We estimate a time-calibrated phylogeny, elevational richness patterns and climatic distributions of species, and then integrate these data to test the montane species-pump and montane museum hypotheses. As expected, we find the highest regional and local richness at mid-elevations, but surprisingly, we strongly support the montane museum hypothesis and not the montane species-pump hypothesis. To reveal the ecological factors underlying this pattern, we test for climatic-niche conservatism. We also test if rates of climatic-niche evolution are related to rates of change in elevational distributions. The results suggest that niche conservatism slows changes in elevational distributions and underlies the montane museum hypothesis.

MATERIAL AND METHODS

Time-calibrated phylogeny

We combined data from three nuclear and three mitochondrial genes for 95 species from all 12 glassfrog genera to estimate a Bayesian time-calibrated phylogeny (Fig. 1; methods in Appendix S1). The phylogeny is generally well-supported and consistent with previous hypotheses (Appendix S1).

Elevational richness patterns

To estimate regional richness patterns, we compiled species' elevational ranges from the Global Amphibian Assessment (GAA: http://www.iucnredlist.org). We supplemented this information with a thorough literature search for range extension accounts; these included well-supported observations (photographs, specimens or calls; see Appendix S2 for elevational ranges and references). For these analyses, we used all 154 known (which included seven well-supported undescribed species) South American species, with 112 species occurring in the Andes (> 1000 m). We excluded two Middle American endemics and the high elevational distributions (> 1000 m) of 10 species from non-Andean montane regions.

We estimated elevational richness by summing the species present in each elevational band. We used 500 m bands (from 0 to 3500 m), following standard practice (e.g. Rahbek 1997; Kozak & Wiens 2010a). Doubling band size left too few bands for statistical analysis, whereas decreasing band size gave similar results (see Appendix S3 for 200 m band results). Previous studies have noted that the subfamilies Centroleninae and Hyalinobatrachinae are more diverse in the Andes and Amazonia respectively (e.g. Guayasamin *et al.* 2009); therefore, we also estimated the elevational richness of each subfamily separately. Prior to testing the species-pump and museum hypotheses, we tested the mid-domain hypothesis (McCain 2004; Appendix S4).

We also considered local richness patterns (all patterns are regional unless otherwise noted). To estimate local richness, we used the R package RASTER (Hijmans & van Etten 2012; R Development Core Team 2012) to find the greatest number of species present in the same 1 × 1 km grid-cell for each elevational band from georeferenced localities (see Climatic-niche evolution section). This approach may underestimate local richness; however, our species richness estimates are similar to those found in extensively surveyed nearby sites, supporting the validity of this approach (Appendix S2). We did not use the mean richness of sites within bands as it likely underestimates local richness due to unequal sampling effort across elevational zones. In addition, using range overlap data may overestimate local richness as most points of overlap lack direct observations.

Montane species-pump hypothesis

To test the montane species-pump hypothesis, we first used a clade-based method that allows inclusion of all species (in the phy-

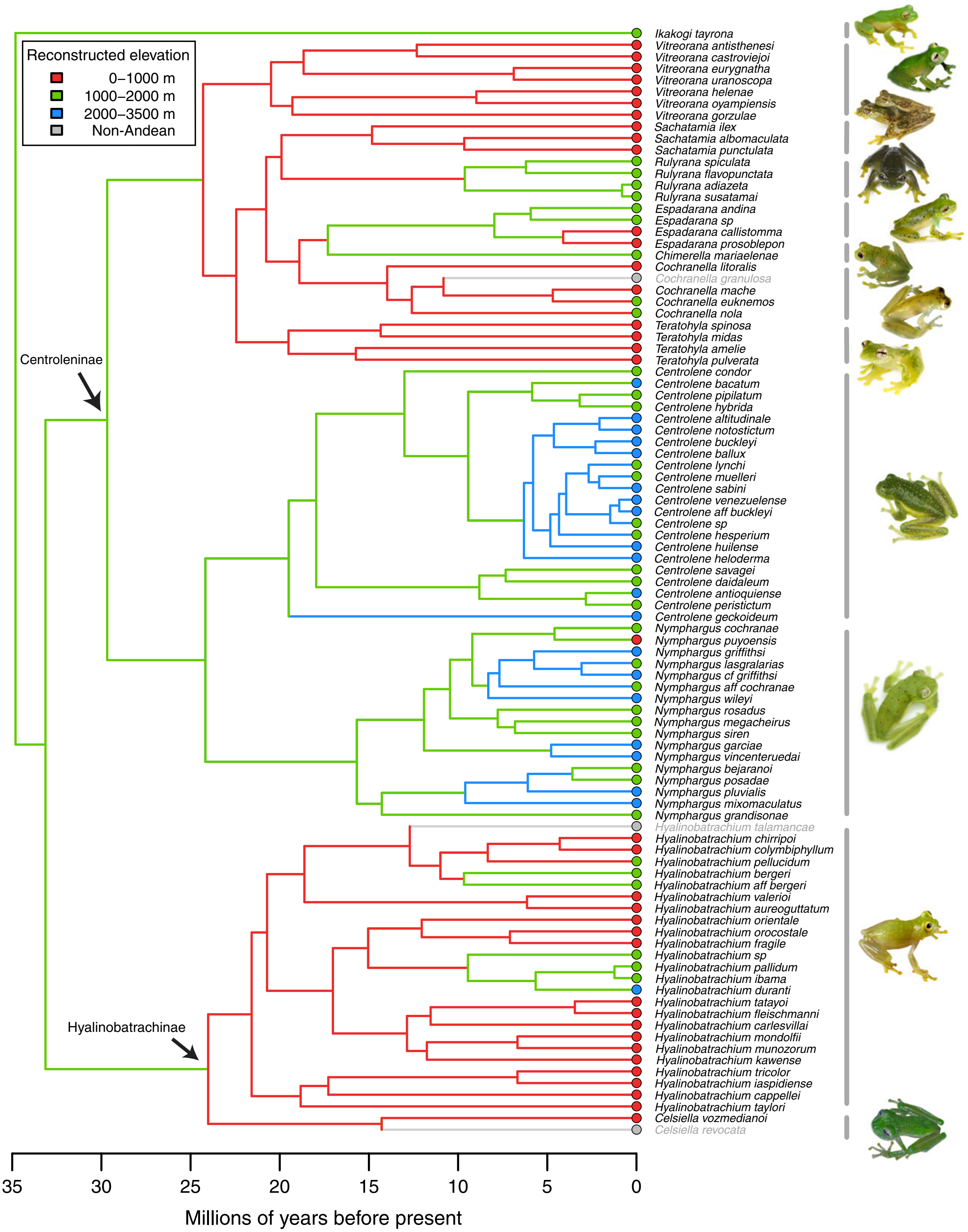

Figure 1 Phylogeny, estimated divergence times and ancestral elevational distributions for glassfrogs. Coloured branches show the general elevational categories for reconstructed nodes for illustrative purposes (analyses are based on reconstructions of continuous trait values; see Appendix S1 for confidence intervals on divergence times and posterior probabilities for nodes, Appendix S2 for elevational distributions and midpoints, and Appendix S8 for confidence intervals on ancestral reconstructions). Coloured circles at the tips represent the general elevational categories for each species' current elevational midpoint. Species' distributions coloured grey as 'Non-Andean' are those endemic to Central America or high elevation non-Andean regions. Photo credits: *Sachatamia albomaculata, Teratohyla pulverata, Chimerella mariaelenae, Cochranella liotoralis,* and *Hyalinobatrachium fleischmanni*, L.A. Coloma; *Espadarana prosoblepon* and *Rulyrana flavopunctata*, A. Arteaga; *Centrolene heloderma* and *Nymphargus grandisonae*, C.R. Hutter; *Celsiella vozmedianoi,* J.M. Guayasamin; *Ikakogi tayrona*, S. Sanchez; and *Vitreorana uranoscopa*, R. Pontes.

logeny or not) and visualisation of how specific clades contribute to diversity patterns. We divided glassfrogs into clades (i.e. genera) and tested for a relationship between their elevational distributions and diversification rates. We first calculated the mean elevational midpoint from all species within each clade. Next, we estimated a diversification rate for each clade using the method-of-moments estimator for stem-group ages (Magallón & Sanderson 2001) with a moderate relative extinction rate (epsilon = 0.45), using GEIGER (Harmon *et al.* 2008). To evaluate the relationship between diversification rate and elevation, we used phylogenetic generalised least-squares regression (PGLS; Martins & Hansen 1997), with CAPER (Orme *et al.* 2012). For this analysis, we created a 'clade tree', pruning the chronogram to include a single, arbitrary species from each genus (the time-span to the genus root is the same for all species). We also used crown-group ages, differing relative extinction rates, and excluded genera with a single species (results remained consistent; Appendix S5). Furthermore, we assessed how species richness patterns within clades might be affected by varying diversification rates over time and if rates are decoupled from richness patterns (details in Appendix S5).

We also estimated the influence of elevational distribution on speciation and extinction rates across the chronogram using the Quantitative State Speciation and Extinction likelihood approach (QuaSSE; FitzJohn 2010) with DIVERSITREE (FitzJohn 2009). We coded each species as its elevational midpoint. To account for species missing from the chronogram, we included a parameter for the proportion of species sampled (60%), which assumes missing taxa have randomly distributed trait values. The elevational richness patterns of sampled species are strongly correlated with those for all species (Spearman's Rank: $r_s = 0.995$; $P < 0.001$), supporting this assumption. Additionally, we used BiSSE and MuSSE (FitzJohn 2009) to address (1) alternative codings of elevational distributions, (2) unsampled taxa and their elevational ranges and (3) varying diversification rates through time (Appendix S5).

We constructed likelihood functions representing different evolutionary models for the relationship between elevational distributions and diversification rate. We tested constant, linear, sigmoidal and modal (hump-shaped) speciation and extinction models that describe this relationship. To support the montane species-pump hypothesis, the modal model should have the greatest support, with the highest diversification at mid-elevations (where richness is highest). Models were compared using the Akaike Information Criterion (AIC); AIC differences ≥ 4 strongly support a given model (Burnham & Anderson 2002). Finally, we directly tested for a relationship between the species richness of each elevational band (using various bin sizes) and that band's diversification rate estimated from the best-fitting QuaSSE models (see Appendix S5). A positive relationship would support the montane species-pump hypothesis.

Montane museum hypothesis

To assess the montane museum hypothesis, we tested for a relationship between the time that each elevational band was first colonised and the species richness of that band, where a positive relationship supports the hypothesis. We estimated colonisation times by reconstructing ancestral elevational midpoints using PGLS. We acknowledge considerable uncertainty in clade ages (Appendix S1), but we are primarily interested in the relative colonisation time of different elevational zones, not absolute timing. Furthermore, we are interested in testing the influence of time on species richness patterns across elevational zones rather than analysing species accumulation within clades over time (these may be unrelated).

Prior to reconstructions, we compared models of trait evolution for elevational distributions using GEIGER. We transformed the chronogram to the best-fitting model (random walk with strong phylogenetic signal; estimated Pagel's lambda = 0.756; Appendix S6). We then used PGLS ancestral reconstruction in APE (Paradis *et al.* 2004) to estimate ancestral elevational midpoints. When reconstructing ancestral elevational zones, we assume that we are estimating the general habitats that ancestral species inhabited (rather than ancestral elevations), and that the occurrence of species in these habitats depends on traits that can be genetically (and phylogenetically) heritable, such as behaviour (e.g. habitat selection) and physiology (e.g. climatic tolerances). This latter assumption is indirectly supported by strong phylogenetic signal in elevational distributions (see above) and by climatic-niche conservatism (see RESULTS).

Given these reconstructions, we recorded the age of the oldest node with an ancestral midpoint occurring in each band (e.g. for the band 500–1000 m, we recorded the age of the oldest node with a midpoint in this range). In some cases, an extant species represented the oldest colonisation of a band. In these instances, we calculated the colonisation time as half the species' age, assuming that the elevational zone was not colonised in the present time or when the species diverged (results were insensitive to alternative times). If a lineage was the first to colonise two successive bands, we only counted the first, as the second would not be independent. We also conducted separate analyses summing the colonisation times for each elevation band (Appendix S7), but simulations suggest this approach may be problematic (Rabosky 2012).

Next, the relationship between species richness and the first colonisation time of a band was examined using linear regression, using both regional and local richness. We also used both raw and log-transformed richness values but did not log-transform time (following Rabosky 2012).

We conducted several analyses to test the robustness of these results (details in Appendix S8). First, to address possible artefacts of reconstructing species-rich elevational zones as ancestral, we simulated species' elevational ranges and tested whether the observed time-richness relationship is stronger than expected given stochastic sampling of species' elevational ranges (following Kozak & Wiens 2010a). Second, to address uncertainty at the root, we performed reconstructions after adding 175 outgroup taxa. Third, we tested the time-richness relationship after arbitrarily assigning alternative elevations to the root and adjacent nodes.

Climatic-niche evolution

We obtained climatic data for species on the chronogram using georeferenced localities and the WorldClim data set (Hijmans *et al.* 2005). We collected occurrence records from the literature (Appendix S2), HerpNet (http://www.herpnet2.org), museum collections and individuals (see ACKNOWLEDGEMENTS). We used 2495 georeferenced localities (mean = 23.8 ± 46.3 unique locations/species; range = 1–317 locations/species). We and other experts on glassfrog taxonomy (see ACKNOWLEDGEMENTS) verified locality and specimen identification whenever possible.

Each locality was also examined to ensure that it fell within the species' known distributional range reported in the literature and the GAA. Georeferenced localities are available from CRH upon request.

Next, we calculated the multivariate rate of climatic-niche evolution for each clade using the first two axes from a principal components analysis of the 19 WorldClim climatic variables following Fisher-Reid *et al.* (2012) (details in Appendix S9; climatic data in Appendix S10). Many temperature and precipitation variables load strongly onto both axes (Appendix S9), including the annual mean and seasonality for temperature and precipitation. Then, we calculated the rate of elevational change (i.e. the evolution of species' elevational midpoints) for each clade. Rates could not be calculated for clades with a single species; therefore, *Ikakogi* and *Chimerella* were excluded. We created a 'clade tree' (explained above), and used PGLS regression to test whether rates of climatic-niche evolution are related to (1) diversification rates and (2) rates of elevational change. A positive relationship between rates of climatic-niche evolution and diversification would suggest that climatic divergence drives speciation. In addition, a positive relationship between rates of elevational change and climatic-niche evolution would suggest that slow rates of climatic-niche evolution constrain elevational distributions (we acknowledge that constrained elevational changes could slow climatic-niche evolution, but it is unclear what would constrain elevational shifts apart from climate). Given that elevation and climate might be strongly related (regardless of rate), we removed climatic variables strongly correlated with elevation across all species ($r_s \geq 0.75$), and ensured that PC scores were not strongly correlated with species' elevational midpoint. We then examined whether a relationship remained between rates of climatic-niche evolution and elevational change.

Climatic-niche conservatism

To test if climatic-niche conservatism influences richness patterns, we examined whether high richness at mid and low elevations are each due to clades having limited dispersal out of these habitats. The first hypothesis was tested using the *Centrolene-Nymphargus* clade, which has peak richness at mid-elevations. For the second hypothesis we used *Hyalinobatrachium*, which has high richness at low elevations. We used PC1–2 (see above) and compared log-likelihoods of six models of climatic-niche evolution, testing for niche conservatism (i.e. evolutionary stasis). We tested models for white noise, Brownian motion, a single global climatic regime, and separate climatic optima for low, middle and high elevations, testing whether species in each clade are constrained to these optima. To support the hypothesis that niche conservatism underlies richness patterns, models representing the global climatic optima or each clade's current climatic optima would receive the strongest support (details in Appendix S11). Analyses were conducted with OUCH (Butler & King 2004).

RESULTS

Elevational richness patterns

Regional richness has a hump-shaped pattern (Fig. 2a), with the highest richness at intermediate elevations (1000–2000 m) and high richness at lower elevations. Above 2000 m, species richness decreases markedly (Fig. 2a). The subfamily Centroleninae has high richness at mid-elevations (driving the hump-shaped pattern in the family), whereas richness for Hyalinobatrachinae peaks at lower elevations (0–1000 m) (Fig. 2b). Local elevational richness gives a similar hump-shaped pattern (Fig. 2c), with peak richness at mid-elevations. However, lower elevations have relatively high maximum local richness (six species), seemingly an artefact of high richness in the Chocóan region. The maximum richness at lower elevations outside this region is three species (Appendix S2). Despite this, local species richness is strongly related to regional species richness ($R^2 = 0.921$; $P < 0.001$). We also find that regional richness patterns are not explained by the mid-domain hypothesis (Fig. 2a; Appendix S4).

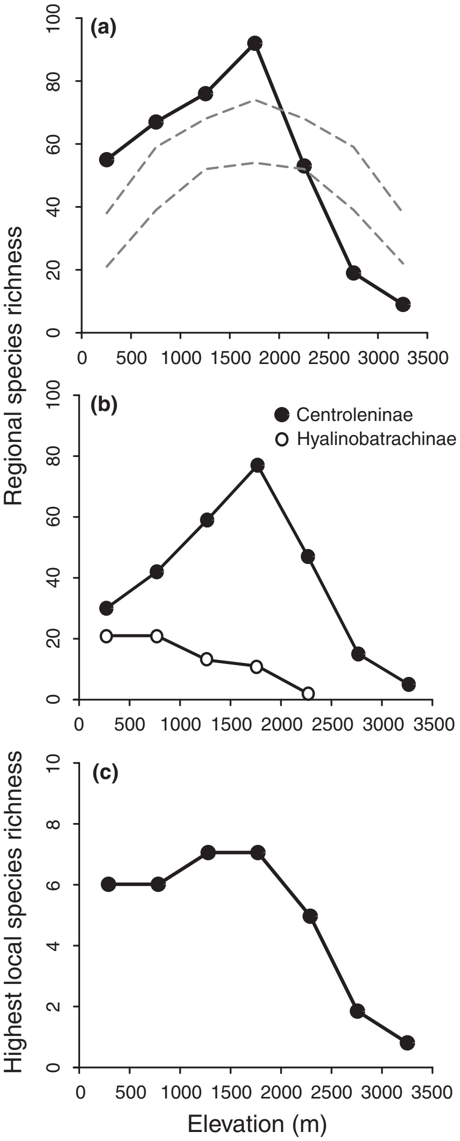

Figure 2 Species richness for glassfrogs in the Andes Mountains plotted for each 500 m elevational band. (a) Regional species richness for all glassfrogs, with dotted lines representing the expected range of richness values under the mid-domain null model (i.e. the 95% confidence intervals; see Appendix S4). (b) Regional species richness plotted separately for both glassfrog subfamilies (Centroleninae and Hyalinobatrachinae). (c) Local species richness for glassfrogs, representing the maximum observed local richness within any given 1 × 1 km grid-cell of plotted georeferenced localities (for a given elevational band).

Montane species-pump hypothesis

We find no relationship between the elevational distribution of clades (genera) and their diversification rates (Table 1; Fig. 3a), across all metrics (range of PGLS results: $R^2 = 0.053–0.246$; $P = 0.096–0.899$; Appendix S5). We find no evidence that varying diversification rates over time causes rates to be decoupled from richness patterns or are otherwise problematic (Appendix S5). QuaSSE analyses do not support modal models (i.e. highest diversification at mid-elevations) and instead strongly support models where diversification rates increase with elevation (Table 2). Furthermore, BiSSE/MuSSE analyses suggest the same pattern (rapid diversification in low richness, high elevation regions), while incorporating elevational range uncertainty, phylogenetically unsampled taxa and varying rates through time (Appendix S5). Finally, we find no relationship or a significant negative relationship between species richness and QuaSSE-estimated diversification rates for each elevational zone (range of results: $R^2 = 0.433–0.820$; $P = < 0.001–0.108$; Fig. 3b; Appendix S5). Together, these results do not support the idea that diversification rates explain elevational richness patterns, as predicted by the montane species-pump hypothesis.

Montane museum hypothesis

We find a positive relationship between the species richness of elevational zones and their first colonisation time (raw richness: $R^2 = 0.836$; $P = 0.004$; log-transformed richness: $R^2 = 0.819$; $P = 0.005$; Fig. 3c), strongly supporting the montane museum hypothesis. Additionally, we find a strong positive relationship between the highest observed local richness of an elevational band and the colonisation time of that band ($R^2 = 0.906$; $P < 0.001$; Fig. 3d). Ancestral reconstructions suggest that glassfrogs were present in mid-elevation habitats ancestrally, and that lower and higher elevation habitats were colonised more recently (Fig. 1).

Multiple lines of evidence support the robustness of these conclusions (Appendix S8). First, we reject the hypothesis that the observed time-for-speciation effect is an artefact of biased reconstructions, given that the observed relationship is stronger than in stochastic simulations ($P = 0.032$). Second, using additional outgroups, we estimate a similar ancestral elevation for glassfrogs (1074 m vs. 1180 m). Third, the time–richness relationship is robust to alternative ancestral elevations at the root. Fourth, local richness patterns show a strong time-for-speciation effect that is concordant with that for regional richness. Fifth, the sister taxon to all other glassfrogs (*Ikakogi*) is Andean, also supporting an Andean origin of glassfrogs.

Climatic-niche evolution

We find no significant relationship between rate of climatic-niche evolution and diversification rate (Fig. 3e), for all measures tested (range of PGLS results: $R^2 = 0.001–0.049$; $P = 0.667–0.909$). However, rates of climatic-niche evolution are positively related to rates of elevational change (PGLS: $R^2 = 0.774$; $P < 0.001$; Fig. 3f). Additionally, the relationship is robust to elimination of climatic variables strongly correlated with elevation (PGLS: $R^2 = 0.875$; $P < 0.001$). Together, these results suggest that shifts in elevation are related to shifts in climatic-niche, but shifts in climatic-niche are not a major driver of speciation.

Climatic-niche conservatism

For PC1, we find significant support for models that have separate adaptive optima for each clade's current elevational zone (Table 3). For *Centrolene-Nymphargus*, the best-fit models for PC1 are the intermediate and high elevation climatic models, suggesting niche conservatism associated with ancestral climatic-niches at the present elevations of these lineages. For *Hyalinobatrachium*, the best-fit model for PC1 is the global climatic model, suggesting niche conservatism of ancestral climatic-niches at their present elevations. For PC2, the white noise and global models are strongly supported for both clades, but PC2 accounts for little climatic variation (25.2%) relative to PC1 (48.8%).

DISCUSSION

The Tropical Andes are one of the most species-rich regions in the world (Myers *et al.* 2000), and this is especially true for frogs (Duellman 1999). Recently, several studies have examined patterns of

Table 1 Information on glassfrog clades (i.e. genera), including the total number of species sampled in the phylogeny and those missing from the phylogeny, the clade stem-group age (in millions of years), elevation midpoint and elevational range of the clade, and estimated diversification rates. Diversification rates were calculated from the stem-group age with a relative extinction rate of 0.45 (thus sister clades have equal age). The rate of climatic-niche evolution is calculated from PC1–2 from 19 bioclimatic variables. The rate of elevational change uses the midpoint of each species' elevational distribution

Genus	Included species (missing)	Stem age	Clade midpoint	Elevational range (m)	Diversification rate	Niche evolution rate	Elevational change rate
Celsiella	2 (0)	24.02	1137.5	750–1800	0.018	0	0
Centrolene	22 (11)	24.17	2100.2	675–3500	0.120	2.184	54827.6
Chimerella	1 (1)	17.31	1357.5	605–1715	0.025	–	–
Cochranella	5 (11)	18.90	705.0	30–2630	0.118	1.115	22160.6
Espadarana	4 (4)	19.90	1153.8	30–2500	0.079	9.267	82275.8
Hyalinobatrachium	25 (5)	24.02	876.5	0–2850	0.118	0.911	43157.6
Ikakogi	1 (0)	34.82	1385.0	980–1790	0	–	–
Nymphargus	17 (22)	24.17	1971.2	500–3030	0.128	1.546	29089.9
Rulyrana	4 (5)	19.90	1280.1	400–2060	0.085	8.172	121654.8
Sachatamia	3 (1)	19.90	756.7	0–1500	0.049	0.270	37.4
Teratohyla	4 (1)	22.44	432.5	0–1100	0.052	1.665	2011.7
Vitreorana	7 (3)	24.30	709.7	0–1700	0.065	0.548	4947.2

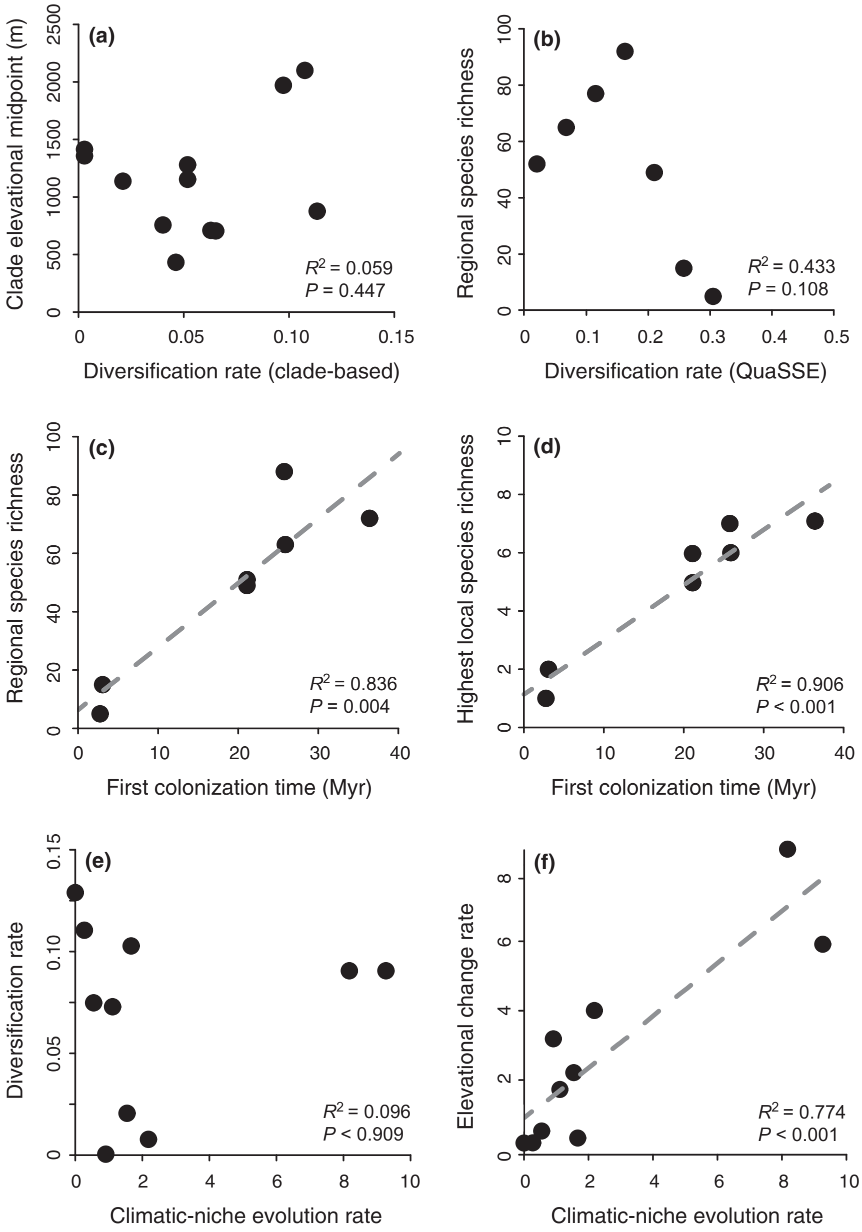

Figure 3 (a) Relationship between diversification rate (clade-based method, stem-group ages; relative extinction rate = 0.45) and clade elevational midpoint, showing no significant relationship using PGLS. (b) Relationship between diversification rate (calculated from the best-fit linear speciation/extinction QuaSSE model) and species richness for 500 m elevational zones, showing no significant relationship. (c) Strong relationship between timing of first colonisation and the regional species richness of elevational zones (from Fig. 2a). (d) Strong relationship between timing of first colonisation and the highest local species richness of an elevational zone (from Fig. 2c). (e) Rates of climatic-niche evolution and diversification rate (clade-based method, stem-group ages; relative extinction rate = 0.45), showing no significant relationship among genera using PGLS. (f) Rates of climatic-niche evolution and rates of elevational change among genera, showing a strong relationship using PGLS.

species richness in the Andes (e.g. Kattan *et al.* 2004) and others have explored diversification in Andean clades (e.g. Hall 2005; Weir 2006; Bonaccorso 2009). However, no study has investigated how the processes of speciation, extinction and dispersal drive elevational richness patterns in the Andes. In glassfrogs, we find a strong peak in richness at intermediate elevations. This pattern occurs in other Andean clades (e.g. Rahbek 1997; Kattan *et al.* 2004), and in clades in many other regions. To address the processes underlying this pattern, we tested the montane species-pump and montane museum hypotheses. We support the montane museum hypothesis and find no evidence for the montane species-pump hypothesis. These results are surprising given previous studies on Andean diversity (see below). We also test, for the first time, how rates of elevational change are related to rates of climatic-niche evolution, and we support the hypothesis that climatic-niche conservatism slows shifts in the elevational distributions of lineages over time. Thus, we demonstrate how climatic-niche conservatism contributes to the mid-elevation peak in species richness under the montane museum hypothesis.

Our support for the montane museum hypothesis is unexpected because the Andes are considered a relatively young mountain range, especially their present height. In the past ~15 million years (Myr), the Northern Andes rapidly increased from an average

Table 2 Summary of results from the Quantitative State Speciation and Extinction (QuaSSE) analyses. The minimal model and the four models with the greatest support are shown (see Appendix S5 for complete results). There was no support for any of the modal models (i.e. highest diversification rates at mid-elevations), which would support the hypothesis that mid-elevations (where richness is highest) have the highest rates.

No.	*P*	Model	*n*	*C*	B_0	B_1	Rate 0	Rate 1	AIC
1	λ	Constant	3	0.172					2065.8
	μ	Constant		1.6×10^{-07}					
2	λ	Linear	5		−0.024	9.9×10^{-05}			2040.5
	μ	Constant		1.1×10^{-04}					
3	λ	Linear	6		0.041	5.7×10^{-05}			2038.9
	μ	Linear			−69386.0	−3826.5			
4	λ	Sigmoid	8				0.085	0.245	2038.2
	μ	Linear			2485.6	−1834.9			
5	λ	Linear	8		−0.012	9.5×10^{-05}			2042.1
	μ	Sigmoid					1406.1	1.5×10^{-13}	

Definitions: (1) *P*, speciation (λ) or extinction (μ) model and parameters; (2) Model, speciation followed by extinction model for each set of models; (3) *n*, number of parameters (not all are shown; Appendix S5); (4) *C*, constant rate; (5) B_0, intercept for linear models; (6) B_1, slope for linear models (positive values are increasing rates with increasing trait values and negative values are the opposite); (7) Rate 0, rate at lowest trait value; (8) Rate 1, rate at highest trait value; (9) AIC, Akaike information criterion.

Table 3 Comparison of AIC values for different models of climatic-niche evolution for two clades of glassfrogs. The *Centrolene-Nymphargus* clade has high richness at mid-elevations and the *Hyalinobatrachium* clade has high richness at low elevations. The clades' climatic distributions are fitted to alternative models of divergence and stasis, compared for the first two principal component axes from an analysis of 19 climatic variables (see Appendix S9 for variable loadings). Models include white noise (random variation), Brownian motion and four models that test for niche conservatism (i.e. evolutionary stasis) associated with ancestral climatic optima. These four climatic models include a global model that tests for niche conservatism to a global climatic optima and three models that test for niche conservatism associated with ancestral climatic optima at low-, mid-, and high elevations. Bolded values are the best-fit model(s) for each clade; AIC differences < 4 were not considered to support one model over another

Clade/variable	White noise	Brownian motion	Global	Low	Mid	High
Centrolene-Nymphargus						
PC1	195.1	214.9	197.2	180.2	**173.9**	**173.6**
PC2	**160.5**	183.6	**162.9**	167.5	167.3	167.3
Hyalinobatrachium						
PC1	129.9	116.2	**111.8**	115.8	**114.4**	115.7
PC2	**105.1**	120.4	**107.7**	112.3	112.3	111.8

~2000 m to their present average height of ~4000 m (Graham 2009). This rapid uprising suggests that montane taxa should be relatively young, leaving little time for species richness to accumulate relative to other regions. Further, no previous studies have suggested that greater time at mid-elevations would be an important cause of Andean richness patterns. Despite this, the estimated age for the formation of moderate elevation uplands (~1000–1500 m) in the proto-Andes is during the late Eocene (~40 Myr ago; Graham 2009), and we estimate similar dates and elevations for the origin of glassfrogs (~35 Myr, ~1100 m; Fig. 1). Thus, glassfrogs may have been accumulating richness for ~35 Myr in present-day mid-elevation habitats, long before the Andes reached their current heights. We also find that lineages at the highest elevations have the highest diversification rates (Table 2; Fig. 3b), possibly due to absence of other lineages in these newly formed habitats, and/or speciation driven by glaciation (see below). However, these increased rates have not (so far) generated high species richness at these elevations. This pattern has also been observed in other Andean lineages, where extensive glaciation at high elevations in the Northern Andes in the past ~2 Myr drove vicariance and rapid speciation (e.g. plants: Hughes & Eastwood 2006; hummingbirds: Weir 2006). Supporting this idea, several Northern Andean glassfrog lineages have diverged in the past ~5 Myr (Fig. 1). This is especially true for species in the Eastern Cordilleras of the Northern Andes, which uplifted in the past ~5 Myr, with glaciation occurring in the past ~1–2 Myr (Hooghiemstra & van der Hammen 2004). However, our results do not suggest that high intermediate elevation richness originated recently, as most mid-elevation lineages in the Andes are much older (~15–25 Myr; Fig. 1).

Our results are also surprising given that many authors have suggested that high Andean species richness is explained by rapid montane speciation, as predicted by the montane species-pump hypothesis (e.g. Lynch & Duellman 1997; Kattan *et al.* 2004; Rosser *et al.* 2012). However, these studies did not directly relate diversification to elevational richness patterns or consider the role of time, so it is unknown if the montane species-pump hypothesis would be supported in these groups. Several other studies in other montane regions support the montane museum hypothesis (see INTRODUCTION). Importantly, other phylogenetic studies of Andean clades have found that they are ancestrally montane and subsequently gave rise to lowland lineages (e.g. butterflies: Elias *et al.* 2009; poison-dart frogs: Santos *et al.* 2009), as in our results. Further analyses of these and other systems in the Andes may also support the montane museum hypothesis.

Our lack of support for the montane species-pump hypothesis is particularly unexpected given the high frog diversity of the Andes relative to adjacent lowlands (i.e. Amazonia). Andean frogs are presumably derived from lowland ancestors, as evidence suggests that many major clades of South American frogs originated before the formation of mid-elevations in the Andes ~40 Myr ago (Roelants *et al.* 2007; Graham 2009). Furthermore, the Andes contain roughly twice the frog richness of adjacent lowlands (Duellman 1999). Considering both ideas strongly suggests that increased diversification rates drive high Andean richness. Nevertheless, we acknowledge that diversification rates in glassfrogs may be rapid relative to other Andean and lowland clades, especially considering their young age compared to other frog groups (Roelants *et al.* 2007). Therefore, the time-for-speciation effect and increased diversification in the Andes may both be true.

Interestingly, we also find a mid-elevation species richness peak for local richness, which is also strongly related to the colonisation time of each elevational band. These results contrast with the implicit expectations of the 'ecological limits' hypothesis (Rabosky 2009), which suggests an upper limit on the number of co-occurring species in a particular location (due to limited resources) and implies little or no relationship between local richness and time. Despite these expectations, we show that species accumulation at both the regional and local scale is strongly related to the amount of time glassfrogs have been present in each elevational zone (Fig. 3c–d). Few studies have tested for relationships between local richness and time, but those that have also found strong relationships (e.g. treefrogs: Wiens *et al.* 2011; salamanders: Kozak & Wiens 2012).

Our study offers insights on how speciation might occur in the Andes and its relationship to rates of diversification, niche evolution and elevational change. Several studies predict widespread speciation via niche divergence in Andean frogs (e.g. Lynch & Duellman 1997; Graham *et al.* 2004). Another study shows that higher rates of climatic-niche evolution are associated with higher rates of diversification in an Andean salamander clade, again suggesting speciation via niche divergence (Kozak & Wiens 2010b). However, we do not find support for this relationship in glassfrogs (Fig. 3e). Instead, we suggest that climatic-niche conservatism limits dispersal of montane clades into the lowlands, assuming that behavioural and physiological traits (e.g. habitat selection, climatic tolerances) determining species' distributions are potentially heritable. We show a pattern of niche conservatism in ancestral climatic-niches for each clade tested (Table 3). We also demonstrate that clades with slow rates of climatic-niche evolution have slow rates of elevational change (Fig. 3f). Intuitively, this latter result supports the hypothesis that climatic-niches change largely through elevational shifts and that climatic-niche conservatism slows rates of change in elevational distributions (suggested but not demonstrated by Kozak & Wiens 2010b). However, a relationship between niche shifts and elevational changes may not always be present, as differing climates may be present at similar elevations (e.g. dry interandean valleys vs. wet slopes; Herzog *et al.* 2011).

Our results raise many questions and suggest avenues for future research. First, are there similar causes of richness patterns in other Andean groups, especially when including related clades outside the Andes? Second, what are the precise mechanisms that set elevational range limits in Andean clades? One possibility is that limited physiological tolerances underly climatic-niche conservatism (e.g. low temperatures for lowland clades, and high temperatures or low precipitation for montane clades). Another possibility is that competitive exclusion limits dispersal. Third, our results suggest an important role of the early colonisation time of the Andes and climatic-niche conservatism. These two factors, combined with the topographic complexity and rapid Andean uprising, may promote allopatric speciation and high richness at mid-elevations for other lineages. However, more work is needed to test modes of Andean speciation more directly (e.g. comparing climatic-niches of sister species; Kozak & Wiens 2007). Finally, climatic-niche conservatism may endanger these narrowly distributed lineages as global climate warms rapidly; this is an urgent topic for future research for all Andean lineages.

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AUTHORSHIP

CRH, JMG and JJW conceived and designed the study. CRH and JMG collected the data; JMG provided additional materials. CRH performed the analyses. CRH wrote the manuscript; JMG and JJW contributed significantly to revisions.

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