SYNTHESIS

Neotropical diversification seen through glassfrogs

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ABSTRACT

Aim We used frogs of the clade Allocentroleniae (Centrolenidae + Allophrynidae; *c.* 170 species endemic to Neotropical rain forests) as a model system to address the historical biogeography and diversification of Neotropical rain forest biotas.

Location Neotropical rain forests.

Methods We used an extensive taxon (109 species) and gene (seven nuclear and three mitochondrial genes) sampling to estimate phylogenetic relationships, divergence times, ancestral area distributions, dispersal–vicariance events, and the temporal pattern of diversification rate.

Results The Allocentroleniae started to diversify in the Eocene in South America and by the early Miocene were present in all major Neotropical rain forests except in Central America, which was colonized through 11 late range expansions. The initial uplifts of the Andes during the Oligocene and early Miocene, as well as marine incursions in the lowlands, are coincidental with our estimates of the divergence times of most clades of Allocentroleniae. Clades with broad elevational distributions occupy more biogeographical areas. Most dispersals involve the Andes as a source area but the majority were between the Central and the Northern Andes, suggesting that the Andes did not play a major role as a species pump for the lowlands. The diversification of glassfrogs does not follow a south-to-north pattern of speciation for Andean clades, and the establishment of a transcontinental Amazon drainage system is coincidental in time with the isolation of the Atlantic Forest glassfrogs. Diversification analyses indicated that a model of constantly increasing diversity best fits the data, compatible with the 'evolutionary museum' hypothesis or 'ancient cradle' hypothesis.

Main conclusions Our work illustrates how the different geological and climatic historical events of the Neotropics shaped, at different levels of the phylogeny, the diversity of a species-rich clade, highlighting the importance of studying large evolutionary radiations at a continental scale.

Keywords
Allocentroleniae, Amazon, Andes, biogeography, Centrolenidae, evolutionary radiation, extinction, Guiana Shield, phylogenetics, speciation.

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INTRODUCTION

Current debates regarding diversification of rain forest biotas in the Neotropics can be grouped into three main topics.
1. Tempo of diversification: are species in Neotropical rain forests recent, originating in the Pliocene and Quaternary, or do they date back at least to the Oligocene–Miocene (Rull,

2008, 2011a,b; Hoorn *et al.*, 2010a)? It is also an open question whether diversification rates have been constant or variable through time (e.g. McKenna & Farrell, 2006; Couvreur *et al.*, 2011). Thus, several studies have addressed whether shifts in diversification rates are coincidental in time with major geological and climatic events (Moore & Donoghue, 2007; Santos *et al.*, 2009; Drummond *et al.*, 2012).

2. Mode of diversification: most recurrent questions revolve around the relative influence on speciation of vicariance versus dispersal (Cracraft & Prum, 1988; Brumfield & Edwards, 2007; Bonaccorso, 2009), geographical isolation versus ecological gradients (e.g. Jiggins *et al.*, 2006; Cadena *et al.*, 2011), and the effects of different barriers (e.g. rivers, mountains, valleys) and/or gradients (elevation, vegetation, rainfall, temperature) on speciation (e.g. Gascon *et al.*, 2000; Chaves *et al.*, 2011; Gutiérrez-Pinto *et al.*, 2012).

3. Influence of some areas or habitats in shaping the diversity of others: this third group of questions is related to whether different areas have or have not influenced each other's species diversity. Major topics are the Great American Biotic Interchange (GABI) (Stehli & Webb, 1985), the role of the Andes as a species-pump for lowland diversity and vice versa (Fjeldså, 1994; Sedano & Burns, 2010), the Precambrian Shields as a source area for rain forest diversification (Nores, 1999; Aleixo & Rossetti, 2007), and a south-to-north diversification of high Andean taxa (Doan, 2003).

Despite the large area under study, the high levels of diversity involved, and the complex and intricate historical scenarios where many contributing phenomena (e.g. orogenies, marine incursions, glaciations, and elevational habitat shifts) are linked and virtually impossible to disentangle (Moritz *et al.*, 2000), most works addressing species diversification and biogeographical patterns and processes in the rain forests of tropical America are limited to specific geographical areas and/or single or few species. Taxonomically and geographically broad studies that include an explicit historical approach (i.e. genealogical hypotheses combined with biogeographical scenarios) are scarce (e.g. Antonelli *et al.*, 2009; Ribas *et al.*, 2009; Santos *et al.*, 2009; Drummond *et al.*, 2012).

Frogs constitute excellent subjects for the study of biogeography and species diversification across large groups because, in general, species show limited dispersal capabilities and strong habitat dependence (e.g. Zeisset & Beebee, 2008). At the same time, some anuran clades are species rich and distributed across large geographical areas. Hence, by studying amphibian evolutionary history, we can gain greater insight into the history of their habitats and of the other inhabitants of these areas (Crawford & Smith, 2005; van der Meijden *et al.*, 2007). Furthermore, amphibians from the American tropics are of exceptional relevance because of their diversity (*c.* 50% of the planet's anurans inhabit the American tropics) and because the Neotropics is the area where they are most threatened (Stuart *et al.*, 2004). Despite this, only a limited number of works have addressed their evolutionary radiations within a phylogenetic framework and at a large geographical scale (Crawford & Smith, 2005; Wiens *et al.*, 2006; Santos *et al.*, 2009; Gonzalez-Voyer *et al.*, 2011).

We focus our study on glassfrogs (family Centrolenidae) and their sister taxon (family Allophrynidae). This clade (Allocentroleniae) is endemic to the Neotropical rain forests and constitutes one of the most interesting anuran groups in the region because of their morphological and ecological characteristics, as well as their phylogenetic and biogeographical complexity (Castroviejo-Fisher *et al.*, 2007; Guayasamin *et al.*, 2009). Species of both families are nocturnal, epiphyllous and arboreal. All species of glassfrogs have a partially or completely transparent venter, and deposit their eggs out of the water on vegetation (leaves, mosses or branches) overhanging streams or on rocks close to the water (Guayasamin *et al.*, 2009). They constitute an evolutionary radiation of *c.* 150 currently recognized nominal species organized in 12 genera (Guayasamin *et al.*, 2009) and distributed across the main tropical American rain forests from sea level to 3500 m a.s.l. Their dependence on rain forests, combined with their presence across tropical America, makes them an ideal group for the study of species diversification in Neotropical rain forests.

To evaluate the tempo, mode and area relationships of the diversification of Allocentroleniae in the Neotropics, we reconstructed phylogenetic relationships using an extensive taxon (109 species) and gene (seven nuclear and three mitochondrial genes) sampling to address the following questions: (1) What is the history of the glassfrog biotic exchange between South America and Central America? (2) What was the temporal and biogeographical scenario within which glassfrogs diversified? Here we assess the relative importance of Oligocene and Miocene geological changes – i.e. uplifts of the proto-Andes, marine incursions, and establishment of the transcontinental Amazon drainage system – versus Pliocene and Quaternary changes – i.e. major and rapid uplifts of the Andes, closure of the Isthmus of Panama, establishment of the Orinoco and Amazon rivers, and climatic changes. (3) Have the Andes acted as a species pump for other areas? (4) Does the radiation of Andean taxa follow a south-to-north pattern of speciation? (5) What is the origin of the vicariant rain forest fauna of the Atlantic Forest? (6) Does glassfrog diversification correspond to an evolutionary museum or cradle? The 'evolutionary museum' hypothesis predicts that most diversification events are relatively old (≥ 5.3 Ma), occurring before the Pliocene, and that accumulation of species has not experienced significant changes, accumulating at a steady rate. The 'evolutionary cradle' hypothesis predicts species diversification to be concentrated in one or more periods of time with significant diversification shifts (accelerations and slow-downs).

MATERIALS AND METHODS

Taxon sampling

The family Allophrynidae currently comprises two described and one undescribed species (Castroviejo-Fisher *et al.*, 2012; J. Faivovich, Museo Argentino de Ciencias Naturales, pers. comm.). The alpha diversity of the family Centrolenidae is far from stable (Castroviejo-Fisher *et al.*, 2011) and for this work we have compiled all available information (up to 1 December 2012) to estimate the species diversity of the group (166 species; see Appendix S1 in Supporting Information).

Therefore, Allocentroleniae is considered here to have 169 extant species.

We obtained molecular data for 109 species, including 102 formally named and seven undescribed centrolenid species (Appendix S1). The ingroup sampling represents 65% of the known alpha diversity of Allocentroleniae and includes representatives from all currently recognized genera and all major ecoregions and elevational ranges in which these anurans occur (Appendix S1). We included five species as outgroups (Appendix S1) and rooted the phylogeny with *Xenopus leavis* (Haas, 2003; Frost *et al.*, 2006).

DNA sequence collection

Tissue samples were obtained from specimens listed in Appendix S1. Additional sequences were downloaded from GenBank (NCBI; Appendix S1). The genes chosen for this study are the mitochondrial 12S rRNA (*12S*), 16S rRNA (*16S*) and NADH dehydrogenase subunit 1 (*ND1*), and portions of the nuclear brain-derived neurotrophic factor (*BDNF*), proto-oncogene cellular myelocytomatosis (*C-MYC*), chemokine receptor 4 (*CXCR4*), sodium-calcium exchanger 1 (*SLC8A1*), proopiomelanocortin A (*POMC*), recombination activating gene 1 (*RAG1*), and solute carrier family 8 member 3 (*SLC8A3*). In total, we generated 406 new sequences, adding 22 species and four genes to the last phylogenetic review of the family (Guayasamin *et al.*, 2008). For primers and laboratory protocols, see Appendix S1.

Phylogenetic analyses

We aligned protein-coding genes in MAFFT 7 (Katoh & Toh, 2008) under the E-INS-i option; in a few cases, we changed the placement of gaps to ensure the reading frame was maintained. We used maximum parsimony (MP) as implemented in POY 4.1.2 (Varón *et al.*, 2010) to analyse the complete concatenated dataset. We considered protein-coding genes as static alignments, while for ribosomal genes (*12S* and *16S*) we used direct optimization (Wheeler, 1996). We used POY with equal weights for all transformations (substitutions and insertion/deletion events) for seven consecutive 8-h searches using 32 processors. The resulting trees were submitted to a final round of swapping using iterative pass optimization (Wheeler, 2003a). Also using POY, we inferred an implied alignment (Wheeler, 2003b) for the ribosomal fragments. Node support for MP was assessed by 1000 jackknife resamplings with removal probability of 0.36 ($\approx e^{-1}$) (Farris *et al.*, 1996) calculated in TNT (Goloboff *et al.*, 2008), considering gaps as a fifth character and using 10 random addition sequences (RAS) + tree bisection–reconnection (TBR) branch swapping per replicate.

A matrix containing the alignments of the ribosomal and protein-coding genes was analysed using the program JMODELTEST 0.1.1 (Posada, 2008) to select the model of nucleotide evolution that best fits the data for each marker based on the Akaike information criterion (AIC; Akaike,

1974). Maximum likelihood (ML) analyses were performed in GARLI 2.0 (Zwickl, 2006) with 10 independent partitions, corresponding to each marker, under the models selected by JMODELTEST and 120 independent searches. Node support was estimated with 1000 nonparametric bootstrap pseudoreplicates (Felsenstein, 1985; see Appendix S1 for details).

Divergence time estimates

We performed Bayesian Markov chain Monte Carlo analyses, as implemented in the program BEAST 1.6.2 (Drummond & Rambaut, 2007), to analyse the same matrix used for ML tree inferences. We defined 10 unlinked partitions and clock models, corresponding to the different genes, but linked trees. We used the uncorrelated relaxed lognormal clock (Drummond *et al.*, 2006), with a Yule tree prior of speciation. For more details see Appendix S1.

We used divergence time constraints, applying uniform distributions to the prior probabilities of the dates, to the following nodes. First, to the split between Anomocoela and Neobatrachia we assigned a minimum date of 144 Ma based on a fossil of a Pelobatidae *incertae sedis* from the Late Jurassic (Evans & Milner, 1993), and a maximum date of 200 Ma (Roelants *et al.*, 2007). Second, to the split of *Hyla meridionalis* and *H. arenicolor* a minimum date of 15.97 Ma based on the oldest fossil of *Hyla* sp. in Europe from the Burdigalian in the early Miocene (Rage & Rocek, 2003), and a maximum of 50 Ma based on previous divergence time estimates of Hylinae (Wiens *et al.*, 2006; Roelants *et al.*, 2007). There are no known centrolenid fossils, but we identified two pairs of sister lowland taxa isolated by the Andes (*Teratohyla amelie*/*T. pulverata* and *T. spinosa*/*T. midas*–*T. adenocheira*) and set a minimum date for their split at 2.7 Ma, corresponding to the most recent uplift of the Eastern Cordillera (Gregory-Wodzicki, 2000; Hooghiemstra *et al.*, 2006). Two pairs of montane sister rain forest species were found to be isolated by the depression of the Uanare River in Venezuela and to each we set a minimum divergence time of 5.0 Ma, time of the final uplift of the Venezuelan Cordillera de la Costa (Mattson, 1984): *Vitreorana castroviejoi*/*V. antisthenesi* and *Celsiella revocata*/*C. vozmedianoi*. Additionally, for the crown age of glassfrogs, a minimum date of 15.0 Ma and a maximum of 30.0 Ma was implemented based on the estimates of Roelants *et al.* (2007). As explained in Appendix S1 special care was taken regarding our ingroup calibrations because they are not based on fossils and can be problematic (Upchurch, 2008; Kodandaramaia, 2011).

Biogeography

To study the dynamics of elevational distribution, we coded species as 'lowland' taxa when they lived from sea level to 900 m a.s.l., 'highland' from 800 to 3500 m a.s.l., and 'continuous' from lowland-to-highland when they occupied both elevational bands (see Appendix S1 for more details). Ancestral states were reconstructed using ML with the Mk1 model

(Lewis, 2001) in MESQUITE 2.74 (Maddison & Maddison, 2010). For each node, we favoured the ancestral character state reconstruction with a proportional likelihood ≥ 0.65, otherwise the ancestral state was considered as ambiguous.

For the characterization of amphibian biogeographical areas in the Neotropics, we followed Duellman (1999) and defined nine areas (Fig. 1, Appendix S1): Central America, Chocó, Cordillera de la Costa, Amazon, Guiana Shield, Northern Andes, Central Andes, Atlantic Forest and Sierra Nevada de Santa Marta. To identify dispersal, vicariance and extinction events between the biogeographical areas, we used statistical dispersal–vicariance analysis (S-DIVA; Yu *et al.*, 2010) as implemented in RASP v2.0b, following the approach of Nylander *et al.* (2008). Biogeographical reconstructions were simultaneously inferred from the strict consensus of the shortest trees from MP, the best topology of ML, and

the maximum clade credibility tree of Bayesian analysis, with the maximum upper bound to tree length of the optimal reconstruction = 32767. The results were mapped on the Bayesian chronogram. To evaluate the role of an area as a source or a sink we quantified the percentage of dispersals in and out of each area in relation to the total number of dispersals between areas according to the shortest reconstructions from RASP.

Diversification

To analyse the temporal pattern of speciation and extinction, we employed a likelihood-based method that simultaneously accommodates undersampling of extant taxa, rate variation over time, and potential periods of declining diversity (Morlon *et al.*, 2011). We compared eight scenarios of
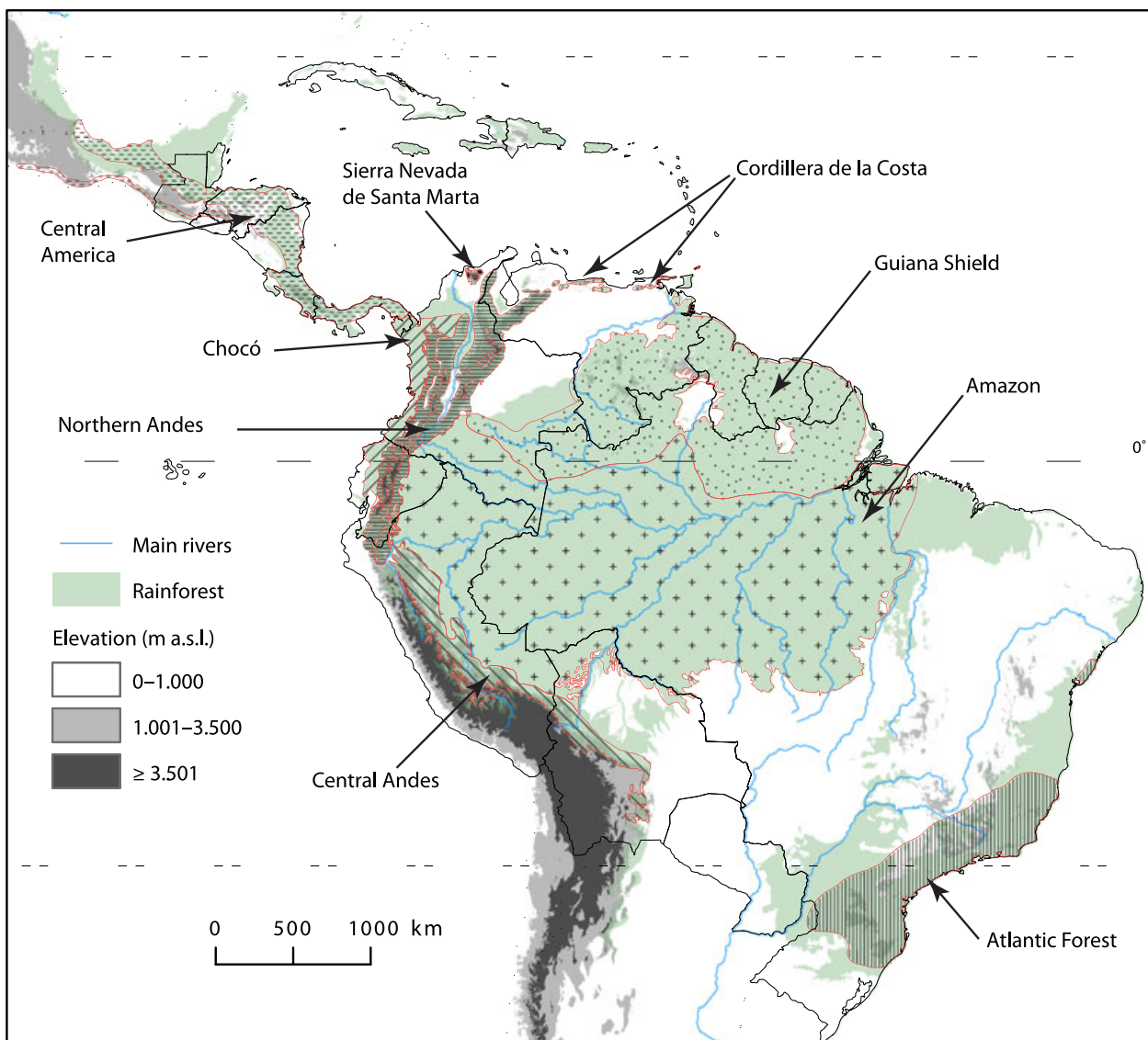

Figure 1 Map of the Neotropical region showing the geographical distribution of Allocentroleniae divided into the nine biogeographical areas considered in this study. Note that the distribution of Allocentroleniae broadly overlaps with the presence of rain forest (in green).

diversification covering most currently discussed hypotheses concerning diversification in the tropics that span from the simple Yule model with null extinction, through time-varying speciation and/or extinction rates, to diversity-saturated models (e.g. Rabosky, 2009a,b; Morlon *et al.*, 2010). All analyses were performed in R (R Core Development Team, 2011), with scripts provided by H. Morlon (Center for Applied Mathematics, Palaiseau Cedex). We also used TURBOMEDUSA (http://www.webpages.uidaho.edu/~lukeh/software/software.html) to identify shifts in the rate of speciation or extinction along the phylogeny. TURBOMEDUSA combines phylogenetic and taxonomical information, thus controlling for incomplete taxon sampling in the phylogeny. The tree was pruned to 47 tips, representing our best knowledge on the relationships of these frogs (Appendix S1). The method fits a birth–death model, estimating the likelihood of obtaining a given combination of phylogenetic relationships, species richness data, and particular values of speciation and extinction. This null birth–death model is then compared to models of increasing complexity with instantaneous shifts in the rate of diversification at a single breakpoint on one branch of the tree where the descendant clade from this branch has its own set of speciation and extinction rates, whereas the remainder of the tree has different rates (Alfaro *et al.*, 2009). We defined a maximal limit of 10 break points for our analyses.

RESULTS

Phylogenetics

As expected from previous studies (Guayasamin *et al.*, 2008, and references therein), Allocentroleniae (Centrolenidae + Alloprhynidae) was retrieved as monophyletic and received high support in all analyses (Appendix S2). Both families were also inferred as reciprocally monophyletic with maximum support. Within the subfamilies Centroleninae and Hyalinobatrachinae, the relationships among genera are fully congruent with those obtained by Guayasamin *et al.* (2008). Some 'shallow' relationships among particular species are different, probably because of our increased taxon and character sampling.

The MP searches yielded 10 most parsimonious topologies (tree length = 16278); the strict consensus of these trees is shown in Appendix S2. All differences among the shortest trees were related to two shallow clades: one containing *Nymphargus ocellatus* + *N. pluvialis* + *N. posadae* and the other containing *Rulyrana flavopunctata* + *R. mcdiarmidi* + *R. saxiscandens* + *R. tagarana*. Jackknife values for most clades

(76.7% of the 103 clades present in the strict consensus) were relatively high (≥ 75%) and only 13.6% of the clades in the strict consensus received low jackknife values (< 50%).

The models selected for each partition of the alignment (total number of characters = 6531) implemented in the parametric methods are shown in Appendix S2. The ML searches resulted in one single optimal tree (ln-likelihood = −77302.85), which is shown in Appendix S2. Bootstrap values for most clades (76% of the 108 clades of the ingroup taxa) were relatively high (≥ 75%) and only 6% of the clades received bootstrap values < 50%. The Bayesian maximum clade credibility chronogram with 95% highest posterior distributions (HPD) of dates is shown in Fig. 2 (see also Appendix S2). After applying the correction of Randle & Pickett (2010), 77% of the clades present in the ingroup received significant support, which in this case are those with clade posterior probabilities equal to 1.

The optimal and maximum clade credibility trees inferred from the different methods are almost identical (Appendix S2). However, relationships among genera of the tribe Cochranellini were resolved differently in nonparametric and parametric methods. Nevertheless, all the conflicts among the three phylogenetic methods involve weakly supported clades in, at least, one of the conflicting methods.
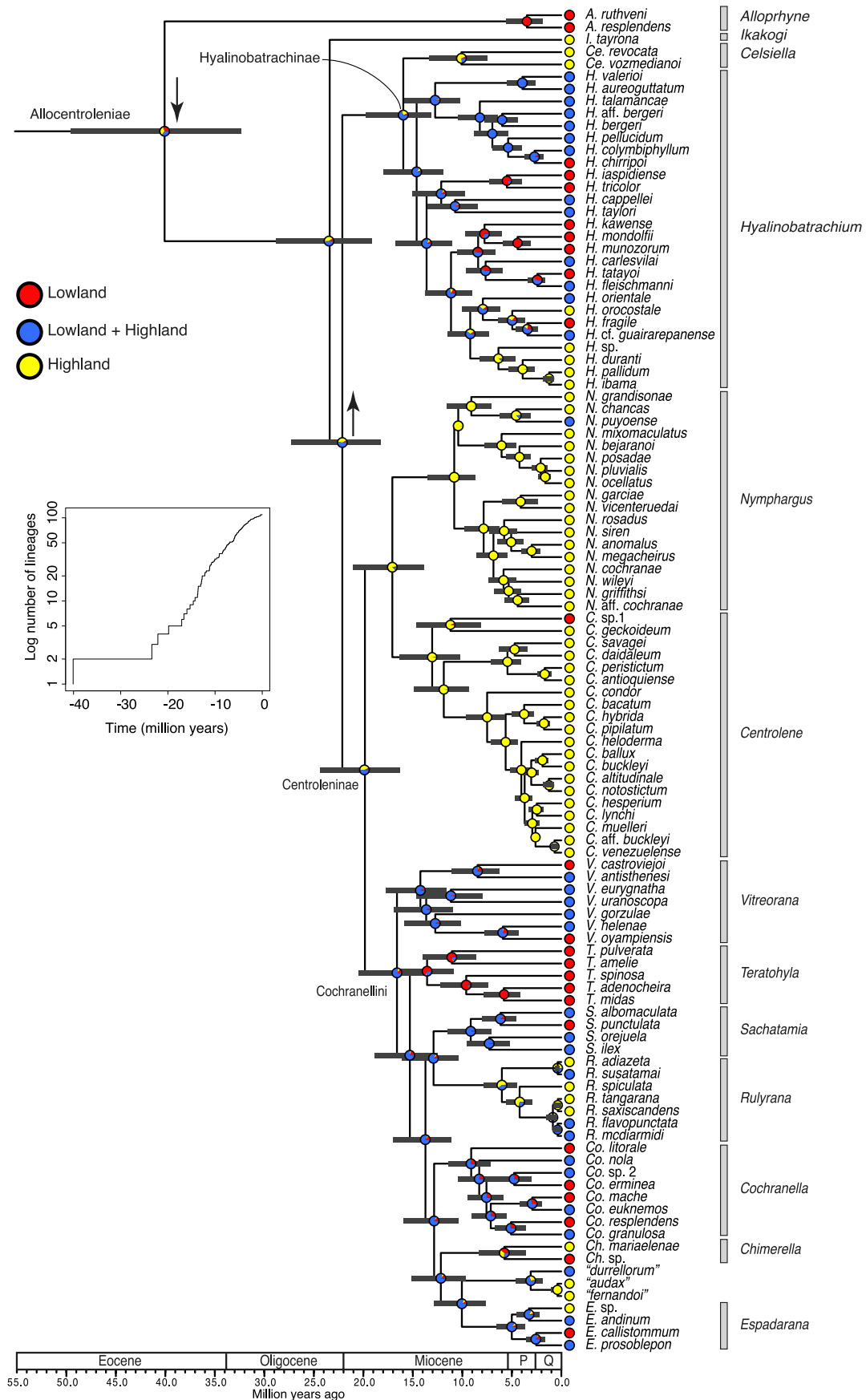
Divergence times

Our results (Fig. 2, Appendix S2) indicate that the split between Allophrynidae and Centrolenidae occurred about 40.8 Ma (95% HPD: 32.37–49.59 Ma), in the late–middle Eocene. The family Centrolenidae started to diversify in the Miocene, about 23.4 Ma (95% HPD: 19.6–28.82 Ma). All currently recognized genera within Centrolenidae appeared during the Miocene, later than 9 Ma (Appendix S2). The majority of current Allocentroleniae species originated during the Pliocene and Miocene (Fig. 3, Appendix S2).

Biogeography

A graphic summary of the ML reconstruction (rate = 0.025; −log*L* = 93.01) is shown in Fig. 2. The elevational distribution of four basal nodes (the three first splits within Allocentroleniae and the node defining Centroleninae) was ambiguous. However, the ancestral state estimate suggests that the most recent common ancestor (MRCA) of Hyalinobatrachidae, as well as that of Cochranellini, occupied both lowlands and highlands (proportional likelihood = 0.69 and 0.79, respectively). In both clades, ancestors with broad elevational distributions in several instances (a minimum of

Figure 2 Ancestral elevational distributions for Allocentroleniae reconstructed by maximum likelihood analysis and mapped on the Bayesian maximum clade credibility chronogram with 95% highest posterior distributions of dates indicated by grey bars. Circles on the tips of the tree indicate the elevational distribution of extant species sampled in the phylogeny. Circles on nodes show the maximum likelihood reconstruction of elevational distributions; the proportion of a colour in any given circle on a node is proportional to the likelihood of the reconstruction. Arrows indicate significant increases (pointing up) or decreases (pointing down) in background diversification rates. A lineage-through-time plot of Allocentroleniae is included. P = Pleistocene; Q = Quaternary.
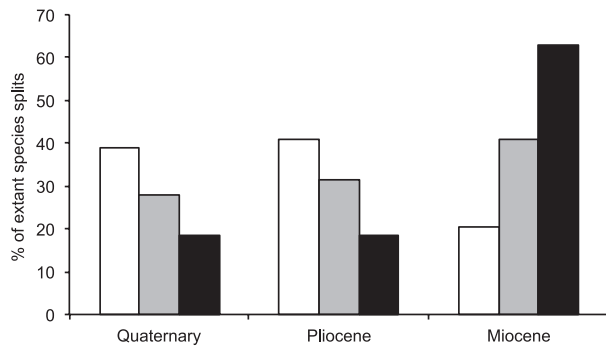
Figure 3 Percentage of the age of extant species of Allocentroleniae distributed in the Quaternary, Pliocene and Miocene based on the average (grey) estimate of divergence times and the lower (white) and upper (black) values of the 95% highest posterior distributions.

17 changes considering both clades together) gave rise to lowland or highland specialists (Fig. 2). On the other hand, the MRCA of *Centrolene* + *Nymphargus* is inferred as highland restricted (proportional likelihood = 0.84), a character that is maintained through the radiation of these two genera except for two terminal changes, one in each genus (Fig. 2).

Our results unambiguously indicate that Allocentroleniae originated in South America (Fig. 4, Appendix S2). The presence of glassfrogs in Central America was explained in our analysis as resulting from range expansions of extant species from South America to Central America. In total, 11 independent dispersals into Central America from South America were inferred.

The reconstruction of the biogeographical origin of glassfrogs within South America (Fig. 4) was more problematic – i.e. there were numerous equally parsimonious reconstructions. Eight nodes (the three most basal, the first split within Cochranellini, and four basal nodes within *Hyalinobatrachium*) included more than four equally parsimonious reconstructions. The most common recent ancestor of Allocentroleniae was inferred to have a broad distribution encompassing four biogeographical areas. The only area present in all of the 10 equally parsimonious reconstructions is the Amazon. However, because of the high number of alternative biogeographical scenarios for these eight nodes, their ancestral area reconstructions are not discussed further. Of the remaining 100 nodes, 82 were unambiguously reconstructed, including many vicariance and dispersal events, 10 involved two alternative reconstructions, five nodes show three alternative reconstructions, and three nodes show four equally parsimonious reconstructions.

Our results indicated that the 181 dispersal events inferred among biogeographical areas were not evenly distributed (Table 1). Four of the nine areas (Amazonas, Atlantic Forest, Central America and Cordillera de la Costa) can be considered as sinks (more dispersals into the area than out of the area), while only two areas (Central and Northern Andes) qualify as sources. The Guiana Shield and Chocó had

roughly the same proportion of emigrant and immigrant species. The Andes (Northern and Central) and Chocó alone were the source for 81% of the dispersals. The Northern Andes was the most important source for dispersals, accounting for 49% of them. Interestingly, the Northern Andes were also the most important sink area (26% of dispersals), followed by the Chocó (18%) and the Amazon (15%). Of the 88 dispersals from the Northern Andes, 34% were into the Central Andes, which was the main sink for dispersals out of the Northern Andes. On the other hand, 30% of the potential dispersals out of the Central Andes were into the Northern Andes.

Rate of diversification

Five of the eight different diversification models we evaluated were rejected by the exact likelihood approach (Table 2, Appendix S2). The three models that could not be rejected implied expanding diversity. The simplest one was the Yule model, which implies a constant rate of speciation and no extinction. The other two models assumed changes in speciation and extinction rates through time, with the variation in the rates being either exponential or linear.

When using turbomedusa, we found support for two shifts from the background diversification rate ($r = \lambda - \mu = 0.1445$; $\varepsilon = \mu/\lambda = 5.7396 \times 10^{-7}$): one dramatic decrease in diversification rate at the base of the tree ($r = -0.9998$; $\varepsilon = 85820.9574$), approximately 40 Ma, and one moderate increase (Fig. 2) in the radiation of Centrolenidae excluding *Ikakogi tayrona* ($r = 0.15401$; $\varepsilon = 0.008689$).

DISCUSSION

Biogeography

Although the ancestral area reconstruction was ambiguous for several nodes (Fig. 4), it also reconstructed congruent events among the multiple optimal solutions. Coupling these events with the temporal framework inferred from the dating analysis, the dynamics of elevational distributions (Fig. 2), and the palaeogeographical model of Hoorn & Wesselingh (2010) provided insights into the biogeographical scenario of the diversification of Allocentroleniae.

Elevational distribution

The rate of changes in the elevational range distribution is small (rate = 0.025). For example, most species of *Centrolene*, *Nymphargus*, *Teratohyla*, *Vitreorana* and several subclades within *Hyalinobatrachium* retain their ancestral elevational state (Fig. 2) in spite of their old origin (e.g. 16.29–24.39 Ma MRCA of *Nymphargus* + *Centrolene* and 12.5–18.85 Ma MRCA of *Teratohyla*). Such reduced variation in elevational distributions is compatible with the phylogenetic niche conservatism hypothesis: taxa tend to retain ancestral ecological constraints and, therefore, closely related
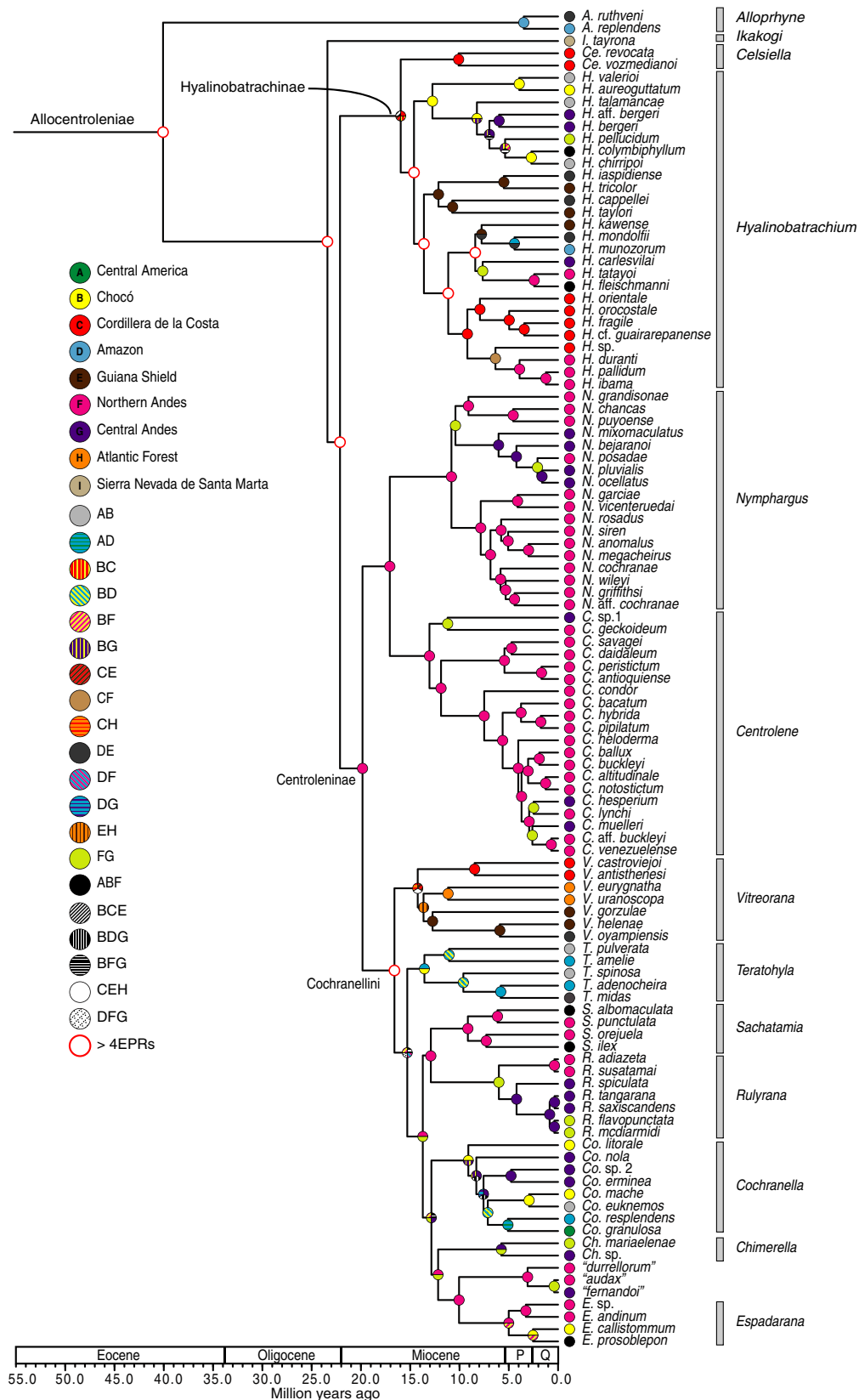
Figure 4 Ancestral area reconstructions for Allocentroleniae inferred by S-DIVA analysis and mapped on the Bayesian maximum clade credibility chronogram. Circles on the tips of the tree indicate the geographical distribution of extant species sampled in the phylogeny. Circles on nodes show the most parsimonious reconstructions of ancestral areas. Nodes with more than four equally parsimonious reconstructions are referred to as '> 4EPRs'. P = Pleistocene; Q = Quaternary.

Table 1 Percentage of total dispersals inferred for Allocentroleniae, indicating when areas acted as sources or sinks.

Area	Total dispersal (%)	
	Source	Sink
Chocó	18.2	17.7
Cordillera de la Costa	3.9	10.5
Amazonas	3.3	14.9
Guiana Shield	3.9	3.9
Northern Andes	48.6	26.0
Central Andes	13.8	4.4
Atlantic Forest	0.6	8.8
Central America	0.0	7.8
Ambiguous*	7.7	6.0

*Ambiguous refers to those dispersals involving composite areas in each of the categories (i.e. source and sink).

Table 2 Log-likelihood and AIC_c values of the eight diversification models fitted to the branching times derived from the Bayesian maximum clade credibility chronogram of Allocentroleniae (models which are not rejected by the data are shown in bold).

Model	LogL	AIC_c
Expanding diversity; constant speciation and extinction	−331.04	665.56
Expanding diversity; exponential variation in speciation and constant extinction	−330.74	667.60
Expanding diversity; linear variation in speciation and constant extinction	−377.17	760.46
Expanding diversity; linear variation in speciation and extinction	**−327.17**	**662.52**
Expanding diversity; constant speciation and exponential variation in extinction	−331.02	668.16
Expanding diversity; exponential variation in speciation and extinction	**−327.49**	**663.17**
Expanding diversity; constant speciation and no extinction (Yule)	**−331.04**	**664.10**
Expanding diversity; exponential variation in speciation and no extinction	−330.75	665.56

AIC_c, Akaike information criterion corrected for small sample sizes.

species inhabit areas that are environmental equivalents (Ricklefs & Latham, 1992; Peterson et al., 1999).

It is also noteworthy that only clades that contain elevational generalist lineages (*Hyalinobatrachium* and *Cochranellini*) have been able to colonize and radiate into different biogeographical areas. Clear examples of clades restricted to highlands are *Celsiella*, *Centrolene* and *Nymphargus*, which in spite of their relatively old origins (≥ 13.11 Ma) and high species richness (roughly 50% of the species diversity), are only present in one biogeographical area, while other equally old clades (*Hyalinobatrachium* and *Cochranellini*) are present across virtually all rain forests of the Neotropics (Fig. 4). Thus, the success of some clades in spreading across all the rain forests of the New World tropics might have been, at least to some extent, related to the presence of elevational generalists in certain clades.

Temporal and biogeographical scenario of glassfrog diversification

Although the ancestral area reconstruction of the MRCA of Allocentroleniae is ambiguous, the Amazon was the only area consistently present in all alternative reconstructions. Additionally, the sister group of Allocentroleniae, Leptodactylidae (Frost et al., 2006; Guayasamin et al., 2008), is more diverse in the lowland rain forests of the Amazon and the Guiana Shield. Thus, it seems likely that the MRCA of Allocentroleniae was present in the lowland and upland rain forests of northern South America. The split between Allophrynidae and Centrolenidae was inferred to be 40.08 Ma (95% HPD: 32.37–49.59 Ma) and is coincidental in time with marine incursions (e.g. Pozo Embayment; Roddaz et al., 2010) that could have played a role as a potential barrier isolating the proto-Andes from the lowland and upland rain forests of north-eastern South America. The marine incursions of the late Eocene were followed by a period of uplift in the northern Central Cordillera c. 40 Ma, a biologically important event providing rising uplands where coastal swamps had been (Graham, 2009). This uplift of the proto-Andes might have also facilitated the isolation of Centrolenidae from Allophrynidae.

The ancestral area reconstruction of the MRCA of Centrolenidae is also ambiguous. Nonetheless, the first split within Centrolenidae, isolating *Ikakogi tayrona* from the clade containing all other glassfrogs, was inferred to have occurred 23.4 Ma (95% HPD: 19.16–28.82 Ma). During the Oligocene (c. 24–34 Ma), the Central and Eastern Cordilleras of the Andes experienced uplifts, probably facilitating the early colonization/vicariance of a proto-Sierra Nevada de Santa Marta (Hoorn & Wesselingh, 2010, Plate 14). The small size and isolation of the Sierra Nevada de Santa Marta from other rain forests offers an explanation for the occurrence of only one species in the genus (i.e. reduced chances for dispersal and speciation), although extinctions and undiscovered species cannot be ruled out. Centrolenidae further split into the MRCA of Hylinobatrachinae and Centroleninae around 22.12 Ma (95% HPD: 18.21–27.26 Ma). The MRCA of Centroleninae was inferred as a Northern Andes taxon (Fig. 4). The MRCA of Hyalinobatrachidae was inferred to be an elevational generalist (Fig. 2) present in the Cordillera de la Costa in all equally parsimonious ancestral area reconstructions (Fig. 4).

The MRCA of Centroleninae, inferred to be restricted to the Northern Andes, split into the MRCA of *Centrolene* + *Nymphargus* (restricted to the highlands of the Northern Andes) and the MRCA of Cochranellini, which was inferred to be present in both highlands and lowlands. The MRCA of *Nymphargus* diversified in the Northern Andes 8.69–16.34 Ma and dispersed twice into the Central Andes between 7.32 and 11.53 Ma, which is coincidental with the connection between the two areas due to the elevation of the Andes at the latitude of the Guayaquil Gulf (Hoorn & Wesselingh, 2010, Plate 15). A second group of dispersals between the Central and Northern Andes were inferred to

have occurred between 1.05 and 3.56 Ma both in *Centrolene* and *Nymphargus*. These dispersals followed some of the major, fastest, and concerted uplifts in the Andes and were coincidental with major climate changes during the Quaternary that most likely promoted range expansions and contractions (Hooghiemstra & Van der Hammen, 2004; Hooghiemstra *et al.*, 2006). The ancestral area reconstruction of the MRCA of Cochranellini is ambiguous.

Previous work has emphasized the possible link between the diversity of Neotropical taxa and the major changes of the Pliocene and Quaternary – i.e. major and rapid uplifts of the Andes, closure of the isthmus of Panama, establishment of the Orinoco and Amazon rivers, climatic changes (e.g. Haffer, 1969; Gentry, 1982; Haffer & Prance, 2001; Richardson *et al.*, 2001; Brumfield & Edwards, 2007; Ribas *et al.*, 2007; Chaves *et al.*, 2011; Weir & Price, 2011). We consider that for some locally restricted groups or new colonizers of South America this has possibly been the case (Hughes & Eastwood, 2006; Moore & Donoghue, 2007; Drummond *et al.*, 2012). However, our findings are concordant with other works that have studied the biogeography of different groups of plants and animals over a large geographical range, in that the diversification of Neotropical taxa is better understood as a continuous process acting over the last 50 Myr or so than a sporadic and recent event (McKenna & Farrell, 2006; Antonelli *et al.*, 2009; Santos *et al.*, 2009; Pennington *et al.*, 2010; Symmank *et al.*, 2011). The elevation of the proto-Andes and its associated changes (e.g. marine incursions) provided the geographical context for the diversification of the main lineages of glassfrogs (and other groups of animals and plants) and later uplifts may have led to a burst of species numbers in localized lineages, but the overall contribution of the main recent Andean uplifts and of Pleistocene glaciations to the formation of deep and divergent lineages within glassfrogs was very limited (Figs 3 & 4).

The Andes as a species pump

The hypothesis that the highlands of the Andes have acted as a species pump for lowland diversity and vice versa is based on the idea that *in situ* radiations in either highlands or lowlands eventually promoted multiple independent colonizations of adjacent areas at different elevations (Fjeldså, 1994; Fjeldså & Rahbek, 2006; Aleixo & Rossetti, 2007; Sedano & Burns, 2010). Our results show that although the vast majority of the inferred dispersals involve the Andes as a source area (62.4%), roughly a third of these dispersals took place between the Central and the Northern Andes so the overall contribution of these areas to other regions is relatively low. It is also remarkable that the Andean diversity contributed differently to *trans*-Andean (west of the Andes) and *cis*-Andean (east of the Andes) diversity. There is little evidence for influence of Andean taxa on the diversity of Amazonian glassfrogs; however, multiple relationships between Andean and Chocóan taxa are observed both in Cochranellini and *Hyalinobatrachium* since 15–20 Ma (Fig. 4). This is compatible with the presence of a large lacustrine environment, the Pebas System, which isolated the eastern versant of the Andes from the *cis*-Andean lowland rain forests.

A south-to-north speciation pattern

Our results allow us to revisit the south-to-north speciation hypothesis (SNSH) of diversification in the Andes. Doan (2003) predicted that because the Andean orogeny proceeded from south to north (Gregory-Wodzicki, 2000; Garzione *et al.*, 2008), we should expect a pattern of cladogenesis of Andean species following the rise of the Andes, with basal lineages occurring in the southern areas and derived ones towards lower latitudes, and with a predominance of ancestral area reconstructions for ancient nodes pointing to the southern Andes. Our results show that none of the Andean clades follow a south-to-north pattern of diversification. On the contrary, clades such as *Centrolene* and *Nymphargus* are inferred to have an ancestral area in the Northern Andes, from which they have colonized the Central Andes (Fig. 4). Only two dispersals from the Central to the Northern Andes were inferred, one in *Nymphargus* and one in *Rulyrana*, and they seem to be relatively recent, representing reversals from the Northern Andes ancestral distribution. Our results are similar to those of Torres-Carvajal (2007) and Goicoechea *et al.* (2012) for Andean lizards.

Great American Biotic Interchange

As suggested by Guayasamin *et al.* (2008), glassfrogs originated in South America and dispersed several times into Central America (at least 11 according to our analyses; Fig. 4, Appendix S2). In all cases but one, the presence of glassfrogs in Central America was explained as range expansions from species distributed in the Chocó and Andes (Fig. 4, Appendix S2). Thus, it is impossible to establish a precise date of the dispersal event (i.e. they are not associated with a node) with the data at hand, although the simplest explanation would be that they occurred after the formation of the Panama Gap isthmus *c.* 3.6 Ma (Coates & Obando, 1996).

Only Andean clades with continuous elevational distributions and that inhabit the lowlands of the Amazon Basin and/or the Chocó (*Cochranella*, *Teratohyla*, *Sachatamia*, *Hyalinobatrachium*) are also found in Central America (Fig. 4). In contrast, South American clades restricted to higher elevations (*Centrolene*, *Nymphargus*, *Rulyrana*) are not found in Central America. Therefore, lineages adapted to broad elevational distributions west of the Andes could explain the influence of Andean taxa in the diversification of the Chocó and Central America.

The origin of Atlantic Forest glassfrogs

The genus *Vitreorana* shows a very interesting biogeographical pattern, with all species restricted to the rain forests east of the Andes. Current species are grouped in three

clades, one restricted to the Cordillera de la Costa (*V. antisthenesi* + *V. castroviejoi*), a second restricted to the Guiana Shield and Amazon (*V. gorzulae* + *V. helenae* + *V. oyampiensis*), and a third restricted to the Atlantic Forest (*V. eurygnatha* + *V. uranoscopa*). The MRCA of *Vitreorana* was inferred to originate 16.6 Ma (95% HPD: 13.59–20.46 Ma), with a distribution covering the Cordillera de la Costa and the Guiana Shield or the Atlantic Forest or both. This inferred distribution would be compatible with the reconstructions of Díaz de Gamero (1996). He considered that the opening of the Pebas System would be situated in the Maracaibo Lake in Venezuela, allowing direct communication between the Guiana Shield and the Cordillera de la Costa, which was later interrupted by the current course of the Orinoco River in the late Miocene (*c.* 11–5 Ma). The first split within the genus isolated the species of the Cordillera de la Costa from those of the Precambrian Shields 14.24 Ma (95% HPD: 11.58–17.71 Ma). The lineage present in both the Guiana Shield and the Atlantic Forest split by vicariance 13.65 Ma (95% HPD: 10.92–16.91 Ma). It is possible that the Purus Arch acted as a corridor for *Vitreorana* between the Guiana Shield and the Atlantic Forest until approximately the end of the middle Miocene (11.2 Ma), when uplifts of the Central and Northern Andes caused the establishment of a transcontinental Amazon drainage system which could have acted as the vicariant barrier between the Guiana Shield–Amazon rain forests and the Atlantic Forest (see review in Hoorn *et al.*, 2010b). Several studies have confirmed this pattern of vicariance in a variety of taxa (Cracraft & Prum, 1988; Costa, 2003; Fouquet *et al.*, 2012) and support our results of a relatively old connection in the Miocene between the Atlantic Forest and Amazonia.

Diversification of rain forest fauna

We found a significant positive correlation between log-transformed species richness and clade age (Appendix S2), even when incorporating phylogenetic information. Therefore, taxon age is a possible explanation for the observed differences in species numbers across clades within Allocentroleniae. However, the result should be taken with caution because the residuals are heterocedastic (Freckleton, 2009) and the small sample size ($n = 13$ clades) could be a problem (Freckleton *et al.*, 2002). Nonetheless, ages in the compared clades differ by nearly one order of magnitude (varying from 3.09 to 23.4 Ma; Appendix S2) and simulations suggest that heterogeneity among clades in rate of diversification is not sufficient to eliminate a positive association between clade age and species richness (Rabosky, 2009a).

Extinction also seems to be playing an important role in explaining differences in species numbers across the compared clades. The old taxa *Allophryne* (32.37–49.59 Ma) and *Ikakogi* (19.16–28.82 Ma), with three and one species, respectively, are two of the most depauperate genera of Allocentroleniae. The analysis of TURBOMEDUSA supports this view of a high

extinction rate in the initial diversification of Allocentroleniae ($\mu = 0.99$).

Diversity-dependent cladogenesis has attracted considerable attention in recent years (McPeek, 2008; Rabosky, 2009a; and references therein). Under this scenario, diversification rates are high early in an adaptive radiation because more niches are available (Mayr, 1947; Schluter, 2001). Once the niche space has been filled, speciation declines and speciation–extinction dynamics lead clade diversity to stasis (McPeek, 2008; Rabosky, 2009a). However, we found no evidence for diversity-dependent cladogenesis in glassfrogs and the hypothesis provides an unlikely explanation for glassfrog diversity. The best-fitting models in our diversification analyses point towards a general trend of increasing diversity, either with a constant rate of speciation (Yule model) or with slight variations through time (linear or exponential) in speciation and extinction, contrary to what is predicted by density-dependent cladogenesis. A compelling visual support to our thesis is the lineage-through-time plot shown in Fig. 2. In spite of our incomplete taxon sampling, which is expected to flatten the increase in diversity towards the present, there is no clear sign of stabilization or saturation. Recent studies have also failed to support the diversity-dependent cladogenesis hypothesis in other tropical taxa (Couvreur *et al.*, 2011; Derryberry *et al.*, 2011).

Our analyses suggest that Allocentroleniae is an old group that started to diversify in the Eocene (32.37–49.59 Ma) and that, except for a potential episode of severe extinction in the early stages of the diversification of the clade and a mild increase in the diversification of Centrolenidae (excluding *Ikakogi*) around 18.21–27.26 Ma, the overall species diversity in glassfrogs is steadily increasing with time (Fig. 2, Table 2). Under a Yule model of diversification, the average rate of species diversification is 0.15 species per Myr (Appendix S2). This value is at least 10 times smaller than the highest reported diversification rates among animals and plants (Kocher, 2004; Hughes & Eastwood, 2006; Moyle *et al.*, 2009) and similar to the rates described for the most species-rich Neotropical frog clades, such as hylids or dendrobatids (Wiens *et al.*, 2006; Santos *et al.*, 2009). These studies, combined with the fact that very few species originated during the Quaternary (Fig. 3), a pattern previously reported for other amphibian groups (Rull, 2008), support the view of Neotropical anuran faunas as a 'museum' or an 'ancient cradle'.

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SUPPORTING INFORMATION

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

Appendix S1 Materials and methods – supplementary information.
Appendix S2 Supplementary results.

BIOSKETCH

As an evolutionary biologist and zoologist, **Santiago Castroviejo-Fisher**'s main interests are to discover, study, describe, name and understand the diversity of life. He has focused his research on amphibians and, to a lesser extent, reptiles of tropical America.

Author contributions: S.C-F., J.M.G. and C.V. designed the study; S.C-F. and J.M.G. conducted the fieldwork; S.C-F. and J.M.G. carried out the laboratory work; S.C-F. and A.G-V. analysed the data; S.C-F. wrote the first complete version of the manuscript; and J.M.G., A.G-V. and C.V. read and improved the manuscript.

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