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Cryptic diversity and biogeography of South American salamanders

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FRANCISCA HERVAS SOTOMAYOR

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Santiago R. Ron, Ph.D

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A mi madre, hermana y abuela

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Cryptic diversity and biogeography of South American salamanders

Francisca Hervas[a], Kathryn R. Elmer[b], David B. Wake[c], Santiago R. Ron[a]

[a]Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Avenida 12 de Octubre y Roca, Apartado 17-01-2184, Quito, Ecuador

[b]Institute of Biodiversity, Animal Health & Comparative Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK

[c]Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, 3101 Valley Life Sciences Building, Berkeley, CA 94720-3160, USA

Abstract

Tropical regions harbor a majority of Earth's biodiversity. In most taxonomic groups there is a gradient of increasing diversity from polar to tropical latitudes. In a few groups, however, the gradient is partly inverted and species richness peaks in temperate regions. One of those groups are salamanders, a group with higher diversity in North and Middle America than in tropical South America. This inverse gradient could result from a time for diversification effect because they arrived more recently to South America. However, an additional contributing factor could be that salamander diversity is underestimated in South America. In this study we used mitochondrial and nuclear DNA sequences to obtain a more comprehensive phylogeny of South American salamanders, to estimate their time of colonization of this continent and assess their species diversity. Using published and new genetic data, we generated a time-calibrated phylogeny and reconstructed ancestral bioregions and altitudes to infer the colonization history of *Bolitoglossa* in South America. The phylogenetic analyses recognized 19 candidate species, of which 13 are confirmed candidate species. This represents an increase of at least 38% in the species content of South American salamanders demonstrating that species richness is severely underestimated. Our results show that the ancestor of *Bolitoglossa* first colonized middle altitudes of the Andes, between the Oligocene and the Miocene. Therefore, all extant species descend from an Andean ancestor, which subsequently colonized higher and lower elevations. Our results suggest that salamander history has been closely linked to the Andean uplift and agrees with previous studies showing that most of the South American biodiversity diversified prior to the Quaternary. Our results show that the Andes have been a species pump for lowland regions. This highlights the importance of the protection of montane regions to maintain the processes that promote species diversification of this group of organisms. We show that formally described species with wide geographical distributions are in fact species complexes on which each species has small geographic ranges. Therefore, the extinction probability of South American salamanders it is likely to be underestimated under the IUCN's Red List.

Keywords: *Bolitoglossa*, biogeography, South America, cryptic diversity, Andes.

1. Introduction

South America is recognized for harboring high levels of biodiversity. It contains five of the biodiversity 'hot spots' of the world (Myers et al., 2000; Jenkins et al., 2013). Despite its richness, the inventory of South American biodiversity is still incomplete and a large number of clades remain undescribed (Barletta et al., 2010; Fouquet et al., 2007a, 2007b; Giam et al., 2012; Pitman and Jorgensen, 2002; Torres-Carvajal et al., 2016). However, during the last decades, an increasing number of new tropical species have been described across a variety of taxa. This trend is partly a result of the increasing use of DNA sequence data in systematic reviews (e.g., Antonelli and Sanmartín, 2011; Bergmann and Russell, 2007; Fernandes et al., 2014; Fouquet et al., 2007b; Helgen et al., 2013; Piggott et al., 2011; Rheindt et al., 2013), but some regions, as the Amazonia, still harbor undescribed diversity, and are biologically and topographically complex (Elmer and Cannatella, 2008). Moreover, much less comprehension exists in regard to the forces that cause and maintain biodiversity in the region, even when this question (particularly for the Amazonia) has intrigued scientists and naturalists for centuries (Bates, 1863; von Humboldt, 1808; Wallace, 1853). Many hypotheses have been proposed to explain the high biodiversity in the region (for a discussion see Leite and Rogers, 2013), but these processes remain poorly understood. However, recent developments in biogeography, molecular phylogeography, and population genetics provide new insights to understand speciation processes in South America (e.g., Aleixo, 2004; Antonelli et al., 2010, 2009; Bell and Donoghue, 2005; Ceccarelli et al., 2016; Chaves et al., 2011; Chek et al., 2001; Collins and Dubach, 2000; da Silva and Patton, 1993; Fiaschi and Pirani, 2009; Funk et al., 2007; Glor et al., 2001; Lovette, 2004; Moritz et al., 2000; Zamudio and Greene, 1997).

For amphibians, South America is the most species-rich region in the world (Duellman, 1999). Moreover, the number of described species is rising at a fast rate as result of discoveries of new species and the recognition of cryptic diversity within previously known species complexes (Fouquet et al., 2007b; Vences et al., 2005a). This high diversity, however, is primarily a result of the high number of frog species, approximately 2631 (AmphibiaWeb, 2016). In contrast, salamanders are represented by only 36 species from a single family and two genera: *Bolitoglossa* and *Oedipina* (34 and 2 species, respectively) (Raffaëlli, 2013). Contrary to most organisms, salamanders do not fit the latitudinal diversity gradient as temperate and subtropical regions of North and Middle America have a greater diversity than tropical South America (Parra-Olea et al., 2004; Raffaëlli, 2013). Therefore, the study of the diversification of salamanders provides a unique opportunity to explore the processes that generate species richness in a group showing an inverse latitudinal diversity gradient.

Two hypotheses have been proposed to explain the inverse diversity gradient in New World salamanders. The first posits that low diversity in the southern region is a consequence of relatively recent colonization, possibly through the Isthmus of Panama. Under this scenario, South American salamanders have not had enough time to achieve high diversity (Kozak and Wiens, 2012; Parra-Olea et al., 2004; Wake, 2005). This hypothesis is compromised by recent estimates showing that *Bolitoglossa* colonized South America at least during the Early Miocene (Elmer et al., 2013; Pyron, 2014). Therefore, time for diversification may have been sufficient to result in a greater diversity than the one observed.

A second hypothesis proposes that the low diversity in South American salamanders could be an artifact of cryptic diversity (Elmer et al., 2013). The actual number of species could be higher so the difference in richness between Middle and South America may be lower than that implied by current estimates.

In this study, we test both hypotheses and for this we use published and new molecular sequences to: (1) provide a new estimate of the species richness of South American *Bolitoglossa*, (2) infer the phylogenetic relationships of *Bolitoglossa* with an expanded sample of species, and (3) determinate the colonization history of salamanders in South America, linking it with the geological events in the continent. Additionally we address the conservation implications of our results.

2. Materials and methods

2.1. Taxon sampling and laboratory protocols

We generated novel mitochondrial and nuclear sequences for 60 specimens of Peru, Colombia, and Ecuador (Fig.1), of which 6 were obtained from Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Peru. The remaining specimens are deposited at Museo de Zoología (QCAZ), Pontificia Universidad Católica del Ecuador, Quito, Ecuador. We amplified the mitochondrial genes 16S of rRNA (*16S*; from 60 individuals)*,* cytochrome b (*Cytb*; 55 individuals), and the nuclear gene recombination activating gene-1 (*Rag1*; 36 individuals).

We extracted DNA from frozen tissues (liver or muscle) stored in ethanol with a modified guanidinium isothiocyanate protocol (M. Fujita, unpublished). Sequences were amplified through the Polymerase Chain Reaction (PCR), performed as follows: a final reaction volume of 25 µl containing 1×PCR Buffer (–Mg), 3 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 µM of each primer, 0.1 U/µl of Platinum® *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA) and 1 µl of extracted DNA. All amplifications included a negative control. The mitochondrial gene *Cytb* (790 bp) was amplified using the primers by Moritz et al. (1992) and the thermocycler parameters described in Parra-Olea et al. (2004). For amplifying the *16S* sequence (≈813 bp), we used primers described by Evans et al. (2003), and reactions consisting of 35 cycles with a denaturing temperature of 94ºC (30 seconds), annealing at 53ºC (30 seconds), and extension at 72ºC (1 minute). The nuclear gene *Rag1* (805 bp) was amplified using the primers designed by Elmer et al. (2013), and the thermocycler parameters as follows: 40–45 cycles with a denaturing temperature of 95ºC (30 seconds), annealing at 55–57ºC (30 seconds) and extension at 72ºC (1 minute). PCR products were visualized by electrophoresis on 1% agarose gels, using SYBER® Safe dye (Invitrogen, Carlsbad, CA) on a Molecular Imager® Gel Doc™

XR+ Imaging System (Bio Rad, Hercules, CA) and purified with ExoSAP-IT (Affymetrix, Cleveland, OH). PCR products were sequenced in both directions by Macrogen Inc. New sequences are deposited in GenBank (Table 1)

2.2. Sequence alignment and phylogenetic analyses

In addition to the new sequences, we included all GenBank sequences of South American *Bolitoglossa* for a total of 131 specimens, and the species of the subgenus *Eladinea* (only when containing the sequences of the three genes). We also included representative species from the rest of the subgenera of *Bolitoglossa*. For estimating the phylogenetic relationships we included *Parvimolge townsendi, Pseudoeurycea rex* and *Ixalotriton niger,* as outgroup (Table 1 and S1). For the estimation of the divergence times we included representatives of all the subfamilies of Plethodontidae. For information about voucher numbers, localities and GenBank accession numbers, see Table 1 and S1 (Also see Fig. 1, for the sampling localities).

The sequences were assembled and aligned in Geneious v7.1.7 (Drummond et al., 2010) under default settings for the MAFFT plugin (Katoh and Standley, 2013). Finally, alignment errors were manually corrected in Mesquite v3.04 (Maddison and Maddison, 2015). We used PartitionFinder (Lanfear et al., 2012) to obtain the partition scheme and best-fit nucleotide substitution models using the Bayesian Information Criterion (BIC).

Phylogenetic relationships were inferred with maximum likelihood (ML) and Bayesian inference using Garli v2.0 (Zwickl, 2006) and MrBayes v3.2.2 (Ronquist et al., 2012), respectively. The ML analyses were run individually for mitochondrial and nuclear sequences and also for all genes combined. We ran 10 independent searches starting from random trees, and 10 searches starting from stepwise trees. All the analyses were run under the default settings of Garli v2.0 (Zwickl, 2006), and stopped when all searches resulted in similar likelihood values. Nodal support was assessed with 200 pseudoreplicates of non-parametric bootstrapping, using the same settings of the stepwise full search with a single replicate per search. The 50% majority rule consensus for the bootstrap trees was obtained with Mesquite v3.04 (Maddison and Maddison, 2015). For the Bayesian analyses, we conducted 4 independent runs, each with 5 MCMC chains that ran for 4×10^7 generations, sampling every 1000 generations. The resulting outputs were analyzed in Tracer v1.4 (Rambaut and Drummond, 2007) to evaluate convergence and effective sample sizes (ESS). Finally we discarded 10% of the trees, from each run, as burn-in and used the remaining trees to obtain a 50% majority rule consensus tree and the posterior probabilities for the nodes.

2. 3. Species delimitation

We calculated the uncorrected genetic distances between clades and haplotypes with MEGA v7 (Kumar et al., 2016) for *16S* and *Cytb*. Additionally, we performed a qualitative and quantitative morphological exploration of the specimens from Ecuador and Colombia (Tables S2-3). Principal Components Analysis (PCA) was used to assess the degree of morphometric differentiation among adult individuals. To remove the effect of covariation in size, the PCA were applied to the residuals from the linear regressions between the morphometric variables and SVL. Based on the morphological differences, we specified the genetic threshold for separating candidate species (3% for *16S* and 8% for *Cytb*). With the genetic and morphological data we classified the lineages in confirmed candidate species (CCS) and unconfirmed candidate species (UCS), following the categories defined by Vieites et al. (2009). CCS corresponds to clades that clearly differ morphologically and presented a genetic distance equal or higher than the thresholds specified, with respect to other clades. UCS corresponds to clades that do not differ morphologically or remain unstudied because we did not have access to the specimens, but had a genetic distance equal or higher than the thresholds specified, with respect to other clades.

2.4. Divergence times and ancestral areas

We estimated divergence times using the Bayesian inference as implemented in BEAST v1.8.0 (Drummond et al., 2012). For the reconstruction of the ancestral distributions, we conducted a reconstruction of ancestral biogeographic regions in BEAST v1.8.0 (Drummond et al., 2012) and an elevation reconstruction using R (R CoreTeam, 2013) with the phylotools package (Revell, 2012). We used the nuclear and mitochondrial sequences used for the phylogenetic analyses and the matrix partitions and nucleotide substitutions models previously chosen by PartitionFinder (Lanfear et al., 2012). We added representative species of the subfamilies of Plethodontidae (Table 1 and S1). We obtained three chronograms using three different priors for the crown date of Plethondontidae: 58 MYA (lowest time estimate from Zhang and Wake, 2009), 109 MYA (highest time estimate from Zhang and Wake, 2009) and 129 MYA (Mueller, 2006). Additionally, we obtained a chronogram using as calibration point the crown date of all South American *Bolitoglossa*, 18.2 MYA (Pyron, 2014). All time priors were normally distributed, with and standard deviation of ± 1. Finally, we also estimated a chronogram using the mutation rate of *Cytb* and *16S* for salamanders estimated by Mueller, 2006 (Table 2). Each search consisted of four runs for 6×10^7 generations, sampling every 10000 generations. We used a lognormal relaxed clock (uncorrelated) and a Yule speciation prior across the tree.

For the reconstruction of the ancestral distributions, we defined the regions based on the classifications by Duellman (1999), with modifications for the elevation limit of the Andes. We recognized four regions: Middle America, Choco (< 300 masl), Amazonia (< 600 masl), and Andes. The reconstruction was carried out using the Bayesian stochastic search variable (BSSVS) under a symmetric substitution model and a strict clock. All BEAST analyses were run in the CIPRES Science Gateway processors (Miller et al., 2010). The results were analyzed in Tracer v1.4 (Rambaut and Drummond, 2007) to evaluate convergence and ESS for all parameters. Files were combined in LogCombiner v1.8.0 (Drummond et al., 2012), after discarding the 10% of the trees. We used TreeAnnotator v1.8.0 (Drummond et al., 2012) to estimate the maximum clade credibility tree.

For the elevation reconstruction, performed in R (R CoreTeam, 2013), we used the time-calibrated phylogeny previously estimated in BEAST v1.8.0 (Drummond et al., 2012). Most of the described species of South American *Bolitoglossa* correspond to species complexes, making it difficult to determine the geographic band of distribution for those species. Thus, we used all the sequences of all available specimens of each species; therefore elevation data correspond to the individual value of each specimen. Elevation data were obtained from AmphibiaWeb, 2016, QCAZ collection database, and the Museum of Vertebrate Zoology at the University of California Berkeley collection (available at mvz.berkeley.edu). When altitude data were not available, we obtained an elevation estimate from the geographic coordinates plotted on an elevation layer (30-m ASTER Satellite, ASTGTM v.2), obtained from METI and NASA (EROS Center, 2001), in ArcGIS 10.0 (ESRI). When neither the altitude nor the geographic coordinates were available, we used the average of the elevation band described for the species, obtained from Amphibian Species of the World (Frost, 2016), AmphibiaWeb, 2016 and Raffaëlli (2013).

3. Results

3.1. Phylogenetic and divergence time analyses

The best random and stepwise ML trees had the same likelihood values (log likelihood = -28711.1756). We show the best stepwise tree in Figure 2. The topology of the Bayesian tree is consistent with the ML topology, except in the nodes with weak support (boostrap value <70 and posterior probability <0.9). This is the case for the basal nodes in the phylogeny. However, other clades show strong support values and the same topology in all the analyses. These phylogenies are derived from a matrix of combined mitochondrial and nuclear DNA that comprised the following 6 partitions and models: (i) *16S* [GTR + I + G]; (ii) *Cytb*, 1st codon position [SYM + I + G]; (iii) *Cytb*, 2nd codon position [HKY + I + G]; (iv) *Cytb*, 3rd codon position [GTR + G]; (v) *Rag1*, 1st and 2nd codon position [K80 + I + G]; and (vi) *Rag1*, 3rd codon position [SYM + G]. The best mitochondrial and nuclear trees have congruent topologies (Fig. S1-2). However, there are some differences in respect to the relationships between basal clades, which correspond to weakly supported nodes in the concatenated tree. Despite these differences, all the trees showed the same topology for the distal relationships. The combined mitochondrial and nuclear ML tree is shown in Figure 2.

Monophyly of *Bolitoglossa* from South America is strongly supported (Fig 2). However the phylogenetic location of some of the described species remain unknown, given the weak support of certain nodes. This is the case for *B. caldwellae,* which is phylogenetically distant to other species from Brazil. Additionally, the relationship between *Bolitoglossa* from South America and *B. medemi* was not resolved, as evidenced by the low phylogenetic resolution of this node (Fig. 2).

We estimated the divergence times using different calibration points and the molecular rate for mitochondrial sequences. The comparison between analyses is shown in Table 2. We chose the estimation based on the calibration of the crown of Plethondotidae, dated by Mueller (2006), to present our results of the ancestral area reconstruction and chronogram (Figs. 3 and S3). With exception of the chronogram based on the molecular rate, all chronograms indicate that *Bolitoglossa* first colonized South American between the Oligocene and the Miocene (Table 2). The earliest date for the colonization of South America is 30.7 MYA (95% HPD 27.9–33.7 MYA), while the latest date is 14.3 MYA (95% HPD 12.9–15.9 MYA) (Table 2 and Fig. S3). The chronogram based on the molecular rate indicates that the earliest colonization of South America took place in the Eocene, much earlier than estimated by chronograms based on fossil calibrations.

3.2. Cryptic diversity

The morphological data suggests differences between most of the clades (Tables S2-3) and allowed us to identify 13 confirmed candidate species (CCS) and 6 unconfirmed candidate species (UCS). The PCA show an overlap among species in the morphometric space, especially for the clades CCS9-13 and UCS6 (Fig. S4). Four components with eigenvalues > 1.0 were extracted from the PCA for the group A, including CSS1-8, *B. sima*, *B.* cf. *equatoriana*, UCS1 and 3 (Table S4). The components accounted for 72.7% of the total variation. For the clade including CCS9-13 and UCS6, four components with eigenvalues > 1.0 were extracted. These components accounted for 68.2% of the total variation (Table S4).

Our analyses revealed high levels of cryptic diversity among South American salamanders, including 19 candidate species: 1 from Colombia (CCS6), 3 from Peru (UCS2, 4−5), 15 from Ecuador (CCS1−5, 7−13, UCS1, 6−7) and 1 lineage shared between the last two countries (UCS3). The candidate species from Colombia and Peru are Amazonian, but the ones from Ecuador are distributed in the Chocoan, Andean and Amazonian regions (Fig. 1). The description of these new clades could mean a raise between 38% (only considering CCS) and 56% (considering UCS and CCS) of the *Bolitoglosssa* species of South America.

All 19 clades correspond to ancient lineages that diverged during the Neogene and therefore display great levels of genetic divergences, as shown by the genetic distances between the closest species. The genetic distances range from 8.7% to 17.4% for *Cyb* and from 3.4% to 7.2% for *16S* (Table 3-5). As for the mean genetic distances within the 19 clades, the variation range from 0% to 4.4% for *Cytb* and 0% to 1.8% for *16S*. The only exception was the Ecuadorian species from the Choco, which we morphologically confirmed as *Bolitoglossa sima.* The genetic distance within this clade is high for the *16S* range (highest value = 3.1%, Table 3). However we did not separate this clade in two, because this value corresponds to only one specimen. In this regard, the specimen of *B.* cf. *sima* from Colombia has a mean genetic distance of 9.7% for the *Cytb*, when compared to the clade from Ecuador, suggesting that the Colombian specimen may be another species. The genetic distance for the *16S* has a lower value (2%) than the threshold we used to separate the clades (3%), but the size of the Colombian sequence is smaller (540 bp) than the ones from Ecuador (861 bp); what may be the cause of the low genetic difference.

Most of the candidate species occur in the Amazonian Basin. We could not ascribe unequivocally any of those candidate species to the available species binomials

B. peruviana, B. equatoriana, and *B. altamazonica* because we lacked samples from the type localities. However, we tentatively consider clade UCS2 as *B.* cf. *peruviana* because of the proximity of this sample to the type locality of *B. peruviana*. Similarly, we consider that clade "*B.* cf. *equatoriana*" is likely to represent *B. equatoriana sensu stricto* because it is similar to the morphology from the original description of the species (Fig. 2).

3.3. Biogeography

Results of the ancestral area reconstruction for regions are shown in Figure 3. Most of the clades distribute in a single altitude band; but some of them contain populations located at different elevations (*B. sima*, UCS2, 7, and CCS7, 9,11–12). Therefore the distribution area of some lineages includes more than one region (Table S1). The chronogram suggests that, during the Oligocene or Miocene, *Bolitoglossa* colonized South American from Middle America (Fig. 3, Table 2). The reconstruction of the ancestral distributions indicates that the ancestor of all South American species inhabited the Andes. There have been subsequent colonization events to the Choco and Amazon region, as well as episodes of re-colonization to Middle America and the Andes. But these ancestral clades from the Andes did not only colonize lower altitudes, but the higher ones too (Fig. 4). These species remained in the Andes and started to migrate to higher elevations as early as the Late Oligocene to the Early Miocene.

The ancestor of the Chocoan lineages and *B. biseriata* most likely occurred in the Andes. This ancestor re-colonized Middle America from the Andes during the Miocene (Table 2, node C from Fig. 3.), before the closure of the Panamanian land bridge. The colonization of the Chocoan region took place in two events between the Middle Miocene and the Early Pleistocene. The Amazonian lineages descend from seven independent events of colonization from the Andes; the earliest event occurred between the Late Oligocene and the Middle Miocene and corresponds to the Amazonian colonization of *B. caldwellae*. The next colonization event occurred during the Miocene and gave rise to CCS8 and *B.* cf. *equatoriana*. Afterwards, between the late Oligocene and the Miocene, the ancestor of the clade that includes CCS9-13 and UCS6 colonized the Amazonia. The remaining colonization events took place between the Miocene and the Pliocene. The Amazonian lineages have re-colonized the Andes between the Middle Miocene and Early Pleistocene. These colonizations have not reached altitudes higher than 900 masl (Fig. 4).

The reconstruction of the ancestral elevations is shown in Figure 4. This reconstruction shows that *Bolitoglossa* colonized South America probably at altitudes of approximately 900 masl. The reconstruction show that most of the colonization history of *Bolitoglossa* has been dynamic given that many species have colonized lower elevations but other have arrived to higher altitudes than the ancestral one, reaching elevations higher than 2000 masl. This is specially the case for the species of the Northern Andes, for which most of the ascension has occurred during the Miocene.

4. Discussion

4.1. Genetic distances and cryptic diversity

Our results show that South American *Bolitoglossa* have high levels of genetic divergence for *16S* and *Cytb*. Therefore these salamander species show a similar level of genetic fragmentation that the one observed in the species from Middle America (García-París et al., 2000; Hanken and Wake, 1982; Parra-Olea and Wake, 2001; Rovito et al., 2012). In fact, this level of genetic divergence has been reported before as a common pattern for amphibians around the world (Elmer et al., 2007; Fouquet et al., 2007a, 2007b; Kieswetter and Schneider, 2013; Vences et al., 2005a, 2005b). Moreover, it has been proposed that in tropical species, the genetic diversity is higher than in temperate ones (Chek et al., 2003; Martin and McKay, 2004; Miraldo et al., 2016; Pereira, 2016). Previous studies in mammals and amphibians have reported a latitudinal gradient for genetic diversity that peaks in the Equatorial band (mean=of 1−3%), and decreases towards the polar latitudes (mean=0.1−1%) (Miraldo et al., 2016). Thus, South American salamanders may be exhibiting the common genetic diversity pattern that is displayed by other species from the tropics.

It has been proposed that the main speciation process driving divergence in tropical amphibians is geographic speciation (Elmer et al., 2007; Fouquet et al., 2007a; Slatkin, 1993), because of the life characteristics of amphibians along with the complex landscape of South America, and the genetic divergence between species. In this regard, the geographically distant linages of South American salamanders show higher genetic distances, thus they seem to show a relationship between their genetic structure geographic distributions. Therefore the speciation process in these lineages could be the same reported for other tropical salamanders and anurans: geographic speciation.

Our results indicate that the number of salamander species in South America is severely underestimated. The number of candidate species that we found represents an increase of at least 38% in the richness of South American *Bolitoglossa.* The increase is even larger when sampling is more exhaustive. In Ecuador, the number of species increases between 172% and 215% (considering the CCS and all the species, respectively). Although an underestimation of South American salamander diversity have been suggested before (Elmer et al., 2013; Hanken and Wake, 1982; Parra-Olea et al., 2004), we show that the magnitude is much higher than previously thought. These findings are comparable with previous estimates of undescribed diversity in tropical frogs (e.g. Funk et al., 2012 reported 150-350% of undescribed species richness in Amazonian frogs, for other examples see Elmer et al., 2007; Fouquet et al., 2012, 2007a; Funk et al., 2012; Kieswetter and Schneider, 2013). Thus, Neotropical salamanders are showing levels of undescribed diversity comparable to that of other amphibians. This level of diversity in these lineages may be a consequence of an earlier colonization of South America than previously thought (see the biogeography discussion).

Our results demonstrate that salamander diversity is underestimated in three bioregions of South America: Amazonia, Andes and Choco. Even though Elmer et al. (2013) already proved that *Bolitoglossa* from the region showed high levels of cryptic diversity, reporting eight potentially new species for Ecuador, here we show that denser sampling in the same region yields a significant increase of undescribed species. We report 19 new candidate species from South America, of which eight coincide with clades reported by Elmer et al. (2013). For example, our CCS2 was originally reported by Elmer et al. (2013) as a new species from the northern Andes of Ecuador ("*B*. sp. Chilma"). Also the former study identified CCS4 and CCS5 as "*B*. sp. Condor" and "*B. sp.* ECSanFran", respectively. Additionally, Clade K includes the species reported by Elmer et al. (2013) as the *B. peruviana* complex. Lineages CCS9, CCS10 and CCS13 correspond to the species named as "Lower Napo/Lower Aguarico", "Upper Aguarico" and "Upper Napo", respectively. We found additional candidate species to those reported by Elmer et al. (2013). We separated the species "*B*. cf. *peruviana* altamazonica" from Elmer et al. (2013) in two CCS based on genetic distances (8.8% for *Cytb* and 3.2% for *16S*). This disagreement could be caused by the our larger sample size, more than twice the amount of sequences that Elmer et al. (2013) used for the same clade. However, the previous study did show that the mean genetic distance within this species (6.2% for *Cytb*) was higher than the genetic distances within the rest of the species (0% to 3.3% for *Cytb*). Additionally, we also report an extra clade (CCS12) for this complex from the lower Amazonia.

Elmer et al. (2013) results suggested that the number of *Bolitoglossa* species from western Amazonia was twice the number of currently described species. Our results show an even higher increase. This illustrates the importance of having exhaustive sampling to achieve a better approximation to the real species richness of Amazonian biodiversity. This is especially true for organisms with restricted distribution ranges such as tropical salamanders (Wake and Lynch, 1976; Wake et al., 1992). The underestimation of diversity in the tropics has been shown in other studies (Barletta et al., 2010; Fouquet et al., 2007b; Funk et al., 2012; Giam et al., 2012; Pitman and Jorgensen, 2002) .

Salamander diversity in South America has always been considered depauperate (Wiens et al., 2007). Our results, however, suggest that species richness is much higher than previously thought. Most of our samples were collected in Ecuador and represent a small area within the South American distribution of *Bolitoglossa*. Comparing to larger areas in Brazil, Colombia, Peru, and Venezuela, our sampling was poor. If the species density found in Ecuador is comparable to that of other South American countries we anticipate a substantial increase in the number of species.

4.2. Biogeography

Most of our estimates (the ones based on fossil calibration) indicate that colonization of South America by *Bolitoglossa* took place between 30.7 MYA and 14.3 MYA, that is as early as the Oligocene and as late as the Middle Miocene (Table 2). Previous estimates have dated this event in the Early Miocene (Pyron, 2014). But our results coincide with the dates reported by Elmer et al. (2013) that dated this colonization between the Oligocene and the Miocene. Yet, the range of ages suggested by Elmer et al. (2013) correspond to the crown age of South American *Bolitoglossa*, that is the first divergence event within this clade. However, the age of the colonization is the stem age of the South American clade. Here, we show a wider range of estimates for the age of colonization of South American *Bolitoglossa.* So together the results from Elmer et al (2016), Pyron (2014) and ours propose a range of time when *Bolitoglossa* probably colonized South America, that is as between the Oligocene and the Middle Miocene. However, one of our estimates (the one based in the molecular rate) shows an even earlier colonization, during the Eocene, expanding the former range. Even though the date of the South American colonization remains inaccurate, it is clear that this event occurred much earlier than the accepted date for the closure of the Isthmus of Panama (3−4 MYA). However, it has been suggested a previous closure age for the Isthmus (ca. 23–25 MYA) (Coates et al., 2004; Duque-Caro, 1990; Farris et al., 2011; Mann and Kolarsky, 1995; Montes et al., 2012), but this affirmation is still debated. Nevertheless, the latter scenario could give an explanation for the early establishment of *Bolitoglossa* in South America, as suggested by Elmer et al. (2013). Still, other colonization mechanisms could have been involved such as short-distance oceanic dispersal between adjacent continents, as seen in frogs (e.g., Bossuyt and Milinkovitch, 2001; Heinicke et al., 2007).

Our findings indicate that the establishment of *Bolitoglossa* in South America took place in middle altitudes of the Andes (approx. 900 mals). Wiens et al. (2007) reported a similar scenario for the colonization of Middle American bolitoglossines. They also found that colonization of mid-altitudes was followed by colonization of lower and higher elevations. Given that Middle America was colonized by a temperate species, it could have been easier for that species to colonize montane areas in the tropics. Thus, the pattern of colonization along elevation gradients may be influenced by ancestral environmental tolerances (Smith et al., 2005; Wiens et al., 2007). The ancestral tolerance of South American *Bolitoglossa* could also explain patterns of species richness along elevation gradients. Species richness of Central American *Bolitoglossa* (Subgenus *Eladinea*) peaks at intermediate elevations (Raffaëlli, 2013). For South American species, it is most likely that the *Bolitoglossa* ancestor that colonized the Andes was distributed at the highest elevations available at the time, given that this colonization occurred during the Oligocene-Miocene. The Northern Andes reached an altitude slightly over 1000 masl during the Middle Miocene. The most intense uplift started between the Middle Miocene (approx. 12 MYA) and the Early Pliocene (approx. 4.5 MYA), when the Cordillera was not more than 40% of its modern elevation (Gregory-Wodzicki, 2000; Hoorn et al., 2010).

Our results support previous studies showing that most contemporary species of vertebrates in the Amazon basin originated during the Neogene (23.7–1.8 MYA) (Antonelli et al., 2010; Turchetto-Zolet et al., 2013). Many events have been associated with the high diversity in the Neotropics, including the uplift of the Andes, marine incursions and climate oscilations (Antonelli et al., 2010). Our results indicate that all the lineages from the Amazonian and the Chocoan region are descendants of an Andean ancestor, therefore the Andes have acted as a source for lowland diversity. A similar pattern was reported by Santos et al. (2009) for the dendrobatid frogs. They showed that the diversity of dendrobatids in the Amazon and the Choco–Middle America region was the consequence of a series of dispersal events from the Andes during the Miocene. Salamanders from South America show a similar pattern demonstrating that the Andes have acted as a species pump for lowland diversity (Fjeldså, 1994; Sedano and Burns, 2010). There is also evidence from birds of the genus *Tangara*, proposing that the Northern Andes have been the source of the tanagers species from other regions including Middle America (Burns and Naoki, 2004; Sedano and Burns, 2010).

The first events of colonization to the Amazonia occurred soon after the original Andean colonization. Therefore diversification of *Bolitoglossa* in the Amazon region is not much recent than that of Andean species. Most present-day species of *Bolitoglossa* from the Amazonia diversified during the Miocene, agreeing with a time of huge tectonic events in South America, such as the Andean uplift (Garzione et al., 2008; Gregory-Wodzicki, 2000; Hoorn et al., 2010). The formation of the Andes had an enormous impact on South American history, since it modified the landscape, climate and the river system (Antonelli et al., 2010; Hoorn et al., 2010). Consequently, the Andean orogeny influenced the Amazonian biota by shaping the climatic conditions, as proposed for many taxa (Antonelli et al., 2010, 2009; Castroviejo-Fisher et al., 2014; Ceccarelli et al., 2016; Fernandes et al., 2014; Turchetto-Zolet et al., 2013). Additionally, our results show events of secondary colonization of the Andes from the Amazonia since the late Miocene until the Quaternary. The upper elevations of the Andes have been also colonized by lineages from lower elevations of the Amazonia. Most of these colonization episodes, particularly of the northern lineages that have reached altitudes higher than 2000 masl, occurred during the peak of Andean uplift, that is between the Miocene and the Pliocene (Gregory-Wodzicki, 2000; Hoorn et al., 1995; Mora et al., 2010). Therefore these latest colonization events may have been facilitated by the rapid Andean orogeny, which may have caused great levels of isolation, as shown for many taxa (Antonelli et al., 2010; Turchetto-Zolet et al., 2013).

We propose that the Andes have played as the source of all living *Bolitoglossa* species in South America, and that the diversification of the lineages was strongly tied to Andean orogeny. Therefore the diversification of *Bolitoglossa* has been influenced by the geologic events that occurred since the Oligocene in South America. The *Bolitoglossa* species from South America are ancient linages that went through speciation during the last periods of the Paleogene and the Neogene, being the latter a period with high rates of diversification in other Neotropical amphibians (Chek et al., 2001; Kieswetter and Schneider, 2013; Lougheed et al., 2006). Thus, our results are consistent with previous studies that have suggested that most of the South American fauna and flora diversified prior to the Quaternary (Antonelli et al., 2010; Chaves et al., 2011; Collins and Dubach, 2000; Cortes-Ortiz et al., 2003; Grau et al., 2005; Guarnizo et al., 2009; Luebert et al., 2011; Moritz et al., 2000; Quintero et al., 2013; Ribas et al., 2007).

4.3. Conservation insights

Our results have important conservation significance, primarily because of the large number of cryptic species that are currently identified as a few formally described species with wide geographic distributions. This is especially true for the western Amazonian species. Currently only three species are recognized, *B. peruviana, B. altamazonica*, and *B. equatoriana.* The IUCN Red List categorizes *B. peruviana* as "Least Concern" because it is assumed that it has large populations across its extensive distribution range (Almendáriz et al., 2004; Azevedo-Ramos et al., 2010; Coloma et al., 2004). However, our results and those of Elmer et al. (2013) have shown that "*B. peruviana*" is a species complex on which each species has small distribution ranges, a feature that characterizes bolitoglossines in the Neotropics (Wake and Lynch, 1976; Wake et al., 1992). Consequently, the extinction risk of each species should be higher than current IUCN Red List estimates. Moreover, the Neotropics harbor ~50% of the world amphibians, a group that has suffered severe population declines and extinctions (Stuart et al., 2004). Therefore, numerous unknown species may be threatened with extinction in poorly studied groups like South American salamanders. Finally, our findings highlight the importance of the protection of montane regions to maintain the processes that promote species diversification.

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Figures

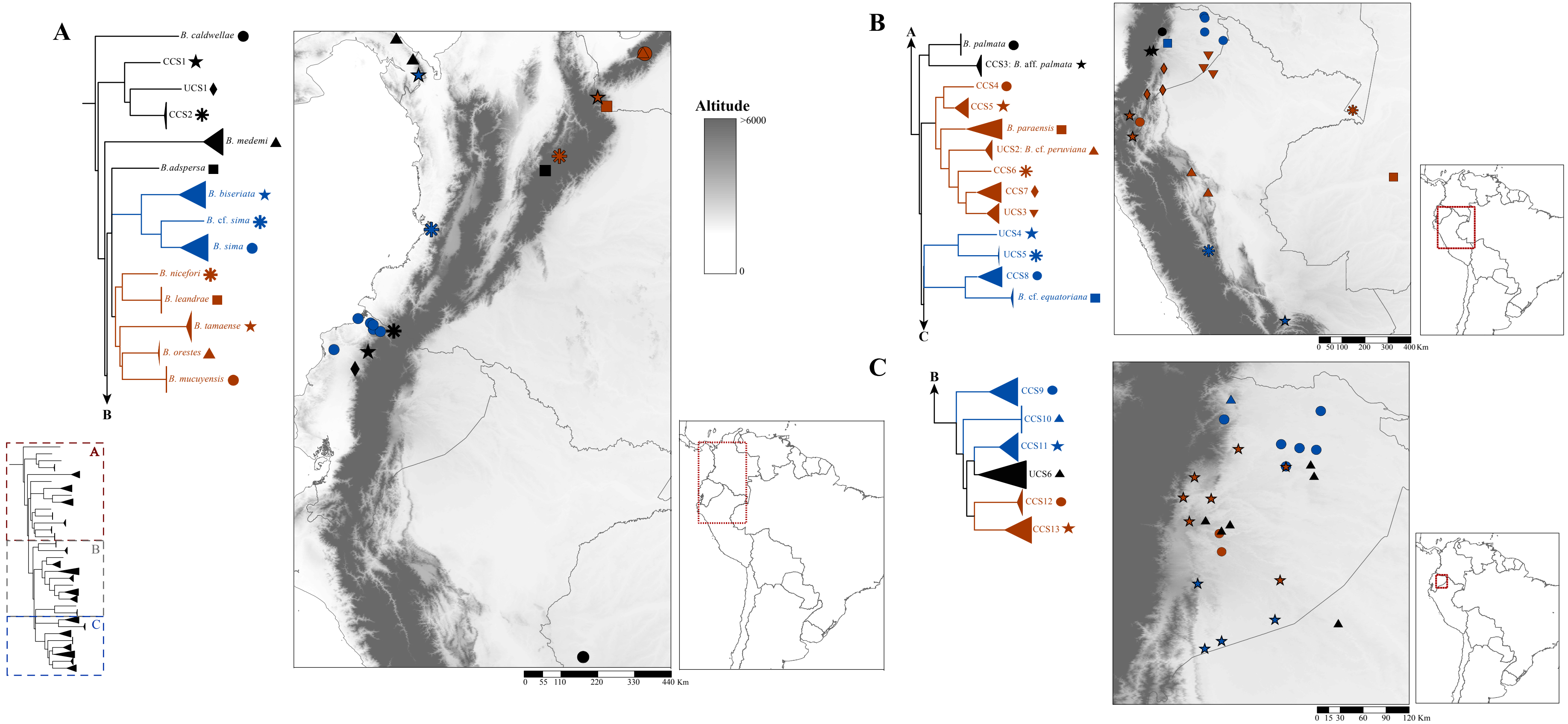
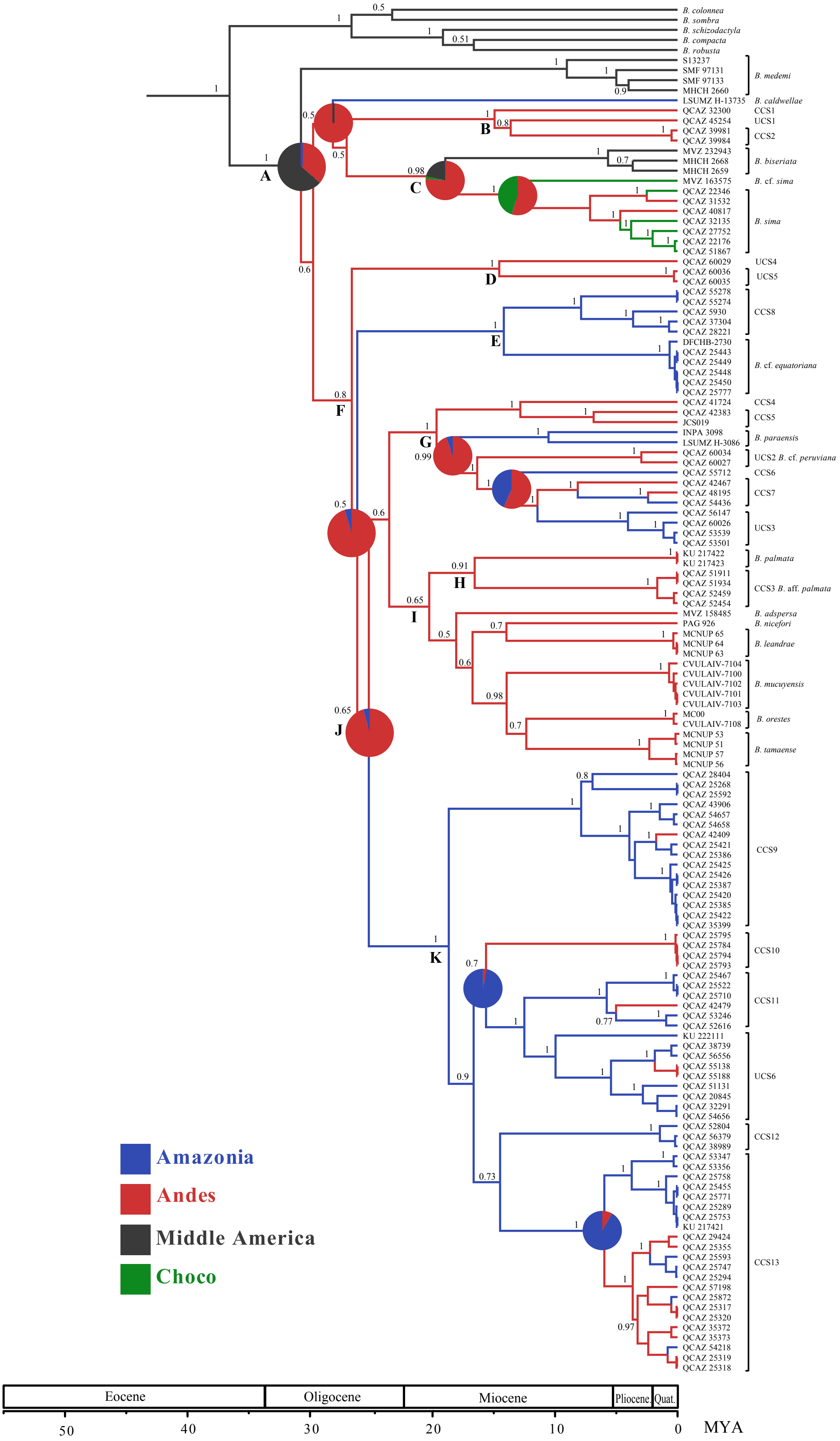


Figure 1. Simplified phylogeny of *Bolitoglossa* from South America and maps showing the sampling localities of the sequences used in this study. The detailed phylogeny is presented in Fig. 2. Localities details, voucher and GenBank numbers are listed in Tables 1 and S1.


MVZ 225875 *B. sombra* CR
B. colonnea PA
B. schizodactyla PA
UCR 20532 *B. compacta* CR
MVZ 190830 *B. robusta* CR
LSUMZ H-13735 *B. caldwellae* BR
QCAZ 32300 Los Cedros Imbabura EC
CCS1
QCAZ 45254 Santo Domingo EC
UCS1
QCAZ 39981 Chilma bajo Carchi EC
QCAZ 39984 Chilma bajo Carchi EC
CCS2
S13237 PA
MHCH 2660 PA
SMF 97131 PA
SMF 97133 PA
B. medemi
MVZ 158485 *B. adspersa* CO
MVZ 232943 PA
MHCH 2668 PA
MHCH 2659 PA
B. biseriata
MVZ 163575 *B.* cf. *sima* CO
QCAZ 22346 La Tola Esmeraldas EC
QCAZ 31532 Bilsa Esmeraldas EC
QCAZ 40817 El Achotero ImbaburaEC
QCAZ 32135 Imbabura EC
QCAZ 27752 Alto Tambo Esmeraldas EC
QCAZ 22176 Durango Esmeraldas EC
QCAZ 51867 Durango Esmeraldas EC
B. sima
PAG 926 *B. nicefori* CO
MCNUP 63 CO
MCNUP 65 CO
MCNUP 64 CO
B. leandrae
MCNUP 51 CO
MCNUP 53 CO
MCNUP 56 CO
MCNUP 57 CO
B. tamaense
CVULAIV-7108 VE
MC00 VE
B. orestes
CVULAIV-7101 VE
CVULAIV-7100 VE
CVULAIV-7102 VE
CVULAIV-7103 VE
CVULAIV-7104 VE
B. mucuyensis
KU 217422 Guacamayos EC
KU 217423 Guacamayos EC
B. palmata
QCAZ 51934 Río Verde Tugurahua EC
QCAZ 51911 Río Verde Tugurahua EC
QCAZ 52454 Río Verde Tugurahua EC
QCAZ 52459 Río Verde Tugurahua EC
CCS3: *B.* aff. *palmata*
QCAZ 41724 Zurmi Zamora Chinchipe EC
CCS4
QCAZ 42383 Chito Zamora Chinchipe EC
JCS019 Zamora Chinchipe EC
CCS5
LSUMZ H-3086 *B. paraensis* BR
INPA 3098 *B. paraensis* BR
QCAZ 60034 Chambirillo Picota PE
QCAZ 60027 Pinto Recodo Lamas PE
UCS2: *B.* cf. *peruviana*
QCAZ 55712 Puerto Nariño Amazonas CO
CCS6
QCAZ 42467 Wisui Morona Santiago EC
QCAZ 54436 LaVirgen Morona Santiago EC
QCAZ 48195 Limón Morona Santiago EC
CCS7
QCAZ 56147 Lorocachi Pastaza EC
QCAZ 60026 Andoas Loreto PE
QCAZ 53539 Juyuintza Pastaza EC
QCAZ 53501 Juyuintza Pastaza EC
UCS3
QCAZ 60029 Poyentimari La Convención PE
UCS4
QCAZ 60036 3 de Mayo Leoncio Prado PE
QCAZ 60035 3 de Mayo Leoncio Prado PE
UCS5
QCAZ 55278 Tambococha Orellana EC
QCAZ 55274 Tambococha Orellana EC
QCAZ 28221 Cuyabeno Sucumbíos EC
QCAZ 5930 Cuyabeno Sucimbíos EC
QCAZ 37304 Tiputini Orellana EC
CCS8
QCAZ 25449 Jatun Sacha Napo EC
QCAZ 25450 Jatun Sacha Napo EC
QCAZ 25448 Jatun Sacha Napo EC
QCAZ 25777 Jatun Sacha Napo EC
QCAZ 25443 Jatun Sacha Napo EC
DFCHB-2730 Jatun Sacha Napo EC
B. cf. *equatoriana*
QCAZ 28404 Cuyabeno Sucumbíos EC
QCAZ 25268 Auca Orellana EC
QCAZ 25592 Auca OrellanaEC
QCAZ 43906 El Descanso Orellana EC
QCAZ 54657 Parque Nacional Yasuní Orellana EC
QCAZ 54658 Parque Nacional Yasuní Orellana EC
QCAZ 42409 Santa Rosa Napo EC
QCAZ 25421 La Selva Lodge Orellana EC
QCAZ 25386 La Selva Lodge Orellana EC
QCAZ 25425 La Selva Lodge Orellana EC
QCAZ 35399 La Selva Lodge Orellana EC
QCAZ 25426 La Selva Lodge Orellana EC
QCAZ 25422 La Selva Lodge Orellana EC
QCAZ 25420 La Selva Lodge Orellana EC
QCAZ 25385 La Selva Lodge Orellana EC
QCAZ 25387 La Selva Lodge Orellana EC
CCS9
QCAZ 25795 Aguarico Sucumbíos EC
QCAZ 25784 Aguarico Sucumbíos EC
QCAZ 25794 Aguarico Sucumbíos EC
QCAZ 25793 Aguarico Sucumbíos EC
CCS10
QCAZ 25467 Kapawi Lodge Pastaza EC
QCAZ 25710 Kapawi Lodge Pastaza EC
QCAZ 25522 Kapawi Lodge Pastaza EC
QCAZ 42479 Wisui Morona Santiago EC
QCAZ 53246 Tukupi Morona Santiago EC
QCAZ 52616 Puerto Morona Morona Santiago EC
CCS11
KU 222111 Teniente López Loreto PE
QCAZ5 1131 Parque Nacional Yasuní Orellana EC
QCAZ 20845 Estación Ciéntifica Yasuní Orellana EC
QCAZ 32291 Estación Ciéntifica Yasuní Orellana EC
QCAZ 54656 Parque Nacional Yasuní Orellana EC
QCAZ 56556 Tarangaro Pastaza EC
QCAZ 38739 Villano Pastaza EC
QCAZ 55138 Oglán Pastaza EC
QCAZ 55188 Oglán Pastaza EC
UCS6
QCAZ 52804 Sarayacu Pastaza EC
QCAZ 56379 Kurintza Pastaza EC
QCAZ 38989 Kurintza Pastaza EC
CCS12
QCAZ 53356 Payamino Orellana EC
QCAZ 53347 Payamino Orellana EC
QCAZ 25455 Jatun Sacha Napo EC
QCAZ 25771 Jatun Sacha Napo EC
QCAZ 25289 J atun Sacha Napo EC
KU 217421 Jatun Sacha Napo EC
QCAZ 25758 Jatun Sacha Napo EC
QCAZ 25753 Jatun Sacha Napo EC
QCAZ 25294 Jatun Sacha Napo EC
QCAZ 25747 Jatun Sacha Napo EC
QCAZ 25593 Auca Orellan EC
QCAZ 29424 Mariscal Sucre Pastaza EC
QCAZ 25355 Santa Clara Pastaza EC
QCAZ 57198 Cotundo Napo EC
QCAZ 25320 Cando Napo EC
QCAZ 25317 Cando Napo EC
QCAZ 25872 Cando Napo EC
QCAZ 35373 Cando Napo EC
QCAZ 54218 Kurintza Pastaza EC
QCAZ 35372 Cando Napo EC
QCAZ 25319 Cando Napo EC
QCAZ 25318 Cando Napo EC
CCS13
0.04

Figure 2. Phylogeny of South American *Bolitoglossa*. Maximum likelihood tree, an analysis obtained using two mitochondrial (*16S* and *Cytb*) and one nuclear gene (*Rag1*). Numbers above branches correspond to ML bootstrap support, values <50 are not shown. Numbers below branches correspond to Bayesian posterior probabilities, values <0.5 are not shown. The voucher number and the locality are shown in each terminal. Clades are delimitated by square brackets. Names in red correspond to species for which we produced new sequences. Abbreviations of the localities: CR−Costa Rica; PA−Panama; BR−Brazil, EC−Ecuador; CO−Colombia; VE−Venezuela; PE−Peru.


A
B
C
D
E
F
G
H
I
J
K
B. colonnea
B. sombra
B. schizodactyla
B. compacta
B. robusta
S13237
SMF 97131
SMF 97133
MHCH 2660
B. medemi
LSUMZ H-13735
B. caldwellae
QCAZ 32300
CCS1
QCAZ 45254
UCS1
QCAZ 39981
QCAZ 39984
CCS2
MVZ 232943
MHCH 2668
MHCH 2659
B. biseriata
MVZ 163575
B. cf. sima
QCAZ 22346
QCAZ 31532
QCAZ 40817
QCAZ 32135
QCAZ 27752
QCAZ 22176
QCAZ 51867
B. sima
QCAZ 60029
UCS4
QCAZ 60036
QCAZ 60035
UCS5
QCAZ 55278
QCAZ 55274
QCAZ 5930
QCAZ 37304
QCAZ 28221
CCS8
DFCHB-2730
QCAZ 25443
QCAZ 25449
QCAZ 25448
QCAZ 25450
QCAZ 25777
B. cf. equatoriana
QCAZ 41724
CCS4
QCAZ 42383
JCS019
CCS5
INPA 3098
LSUMZ H-3086
B. paraensis
QCAZ 60034
QCAZ 60027
UCS2 B. cf. peruviana
QCAZ 55712
CCS6
QCAZ 42467
QCAZ 48195
QCAZ 54436
CCS7
QCAZ 56147
QCAZ 60026
QCAZ 53539
QCAZ 53501
UCS3
KU 217422
KU 217423
B. palmata
QCAZ 51911
QCAZ 51934
QCAZ 52459
QCAZ 52454
CCS3 B. aff. palmata
MVZ 158485
B. adspersa
PAG 926
B. nicefori
MCNUP 65
MCNUP 64
MCNUP 63
B. leandrae
CVULAIV-7104
CVULAIV-7100
CVULAIV-7102
CVULAIV-7101
CVULAIV-7103
B. mucuyensis
MC00
CVULAIV-7108
B. orestes
MCNUP 53
MCNUP 51
MCNUP 57
MCNUP 56
B. tamaense
QCAZ 28404
QCAZ 25268
QCAZ 25592
QCAZ 43906
QCAZ 54657
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QCAZ 25425
QCAZ 25426
QCAZ 25387
QCAZ 25420
QCAZ 25385
QCAZ 25422
QCAZ 35399
CCS9
QCAZ 25795
QCAZ 25784
QCAZ 25794
QCAZ 25793
CCS10
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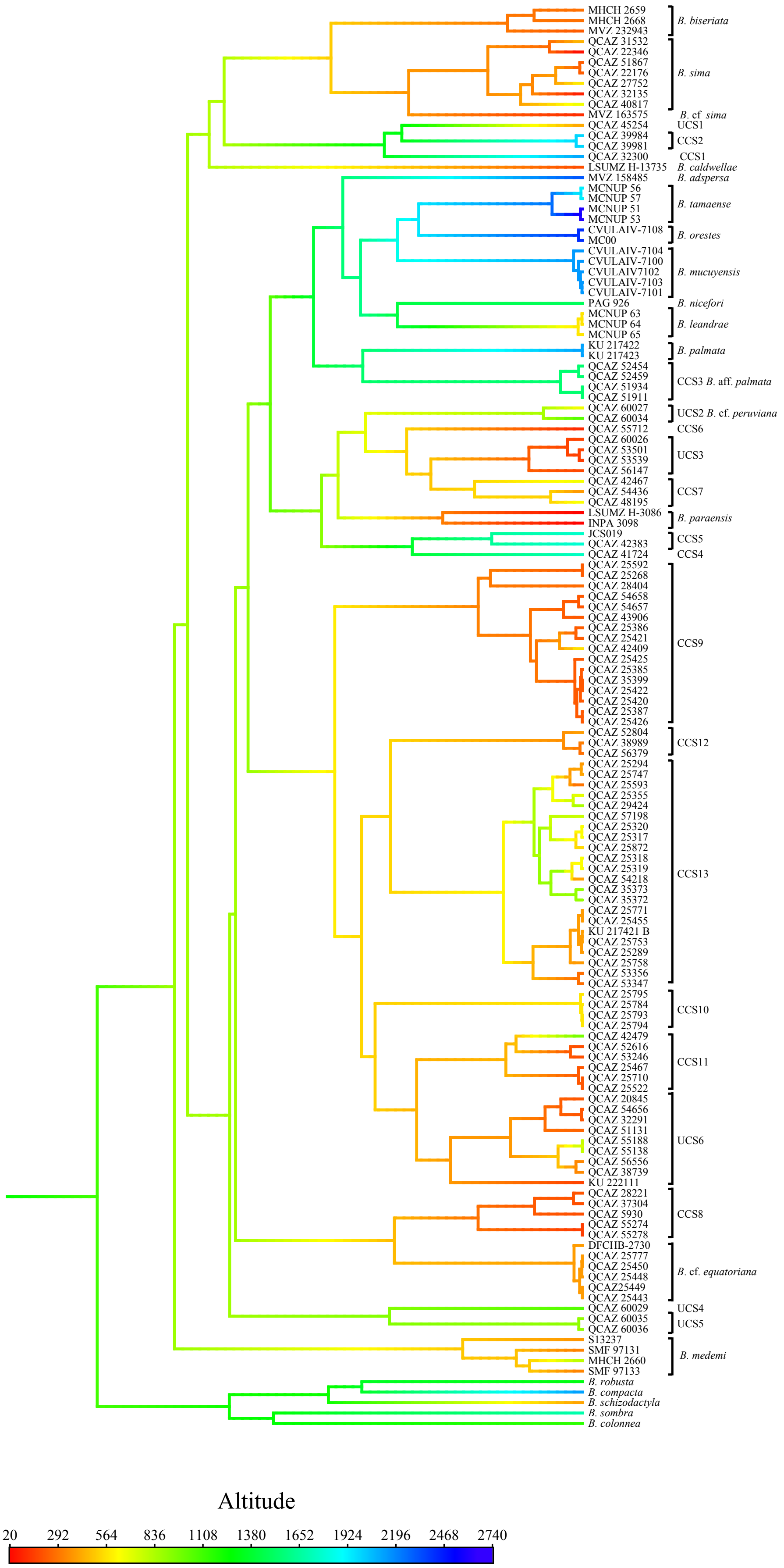
QCAZ 52616
CCS11
KU 222111
QCAZ 38739
QCAZ 56556
QCAZ 55138
QCAZ 55188
QCAZ 51131
QCAZ 20845
QCAZ 32291
QCAZ 54656
UCS6
QCAZ 52804
QCAZ 56379
QCAZ 38989
CCS12
QCAZ 53347
QCAZ 53356
QCAZ 25758
QCAZ 25455
QCAZ 25771
QCAZ 25289
QCAZ 25753
KU 217421
QCAZ 29424
QCAZ 25355
QCAZ 25593
QCAZ 25747
QCAZ 25294
QCAZ 57198
QCAZ 25872
QCAZ 25317
QCAZ 25320
QCAZ 35372
QCAZ 35373
QCAZ 54218
QCAZ 25319
QCAZ 25318
CCS13
🟦 Amazonia
🟥 Andes
⬛ Middle America
🟩 Choco
Eocene
Oligocene
Miocene
Pliocene
Quat.
50
40
30
20
10
0
MYA

Figure 3. Reconstruction of ancestral bioregions and chronogram for South American *Bolitoglossa*. Bayesian estimation based on *16S*, *Cytb* and *Rag1*. Numbers close to the nodes correspond to Bayesian posterior probabilities values. Branches are colored according to the most probable region, which are based on the classifications by Duellman (1999), with modifications for the elevation limit of the Andes: Choco (< 300 masl) and Amazonia (< 600 masl). The pie charts near to the nodes represent the probability of each region. The analysis was performed in BEAST by calibrating the crown group Plethodontidae at 129 MYA (according to Mueller, 2006). Letters A−K correspond to nodes for which we present the estimated ages in Table 2.


MHCH 2659
MHCH 2668
MVZ 232943
B. biseriata
QCAZ 31532
QCAZ 22346
QCAZ 51867
QCAZ 22176
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QCAZ 32135
QCAZ 40817
B. sima
MVZ 163575
B. cf *sima*
QCAZ 45254
UCS1
QCAZ 39984
QCAZ 39981
CCS2
QCAZ 32300
CCS1
LSUMZ H-13735
B. caldwellae
MVZ 158485
B. adspersa
MCNUP 56
MCNUP 57
MCNUP 51
MCNUP 53
B. tamaense
CVULAIV-7108
MC00
B. orestes
CVULAIV-7104
CVULAIV-7100
CVULAIV7102
CVULAIV-7103
CVULAIV-7101
B. mucuyensis
PAG 926
B. nicefori
MCNUP 63
MCNUP 64
MCNUP 65
B. leandrae
KU 217422
KU 217423
B. palmata
QCAZ 52454
QCAZ 52459
QCAZ 51934
QCAZ 51911
CCS3 *B.* aff. *palmata*
QCAZ 60027
QCAZ 60034
UCS2 *B.* cf. *peruviana*
QCAZ 55712
CCS6
QCAZ 60026
QCAZ 53501
QCAZ 53539
QCAZ 56147
UCS3
QCAZ 42467
QCAZ 54436
QCAZ 48195
CCS7
LSUMZ H-3086
INPA 3098
B. paraensis
JCS019
QCAZ 42383
CCS5
QCAZ 41724
CCS4
QCAZ 25592
QCAZ 25268
QCAZ 28404
QCAZ 54658
QCAZ 54657
QCAZ 43906
QCAZ 25386
QCAZ 25421
QCAZ 42409
QCAZ 25425
QCAZ 25385
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QCAZ 25420
QCAZ 25387
QCAZ 25426
CCS9
QCAZ 52804
QCAZ 38989
QCAZ 56379
CCS12
QCAZ 25294
QCAZ 25747
QCAZ 25593
QCAZ 25355
QCAZ 29424
QCAZ 57198
QCAZ 25320
QCAZ 25317
QCAZ 25872
QCAZ 25318
QCAZ 25319
QCAZ 54218
QCAZ 35373
QCAZ 35372
QCAZ 25771
QCAZ 25455
KU 217421 B
QCAZ 25753
QCAZ 25289
QCAZ 25758
QCAZ 53356
QCAZ 53347
CCS13
QCAZ 25795
QCAZ 25784
QCAZ 25793
QCAZ 25794
CCS10
QCAZ 42479
QCAZ 52616
QCAZ 53246
QCAZ 25467
QCAZ 25710
QCAZ 25522
CCS11
QCAZ 20845
QCAZ 54656
QCAZ 32291
QCAZ 51131
QCAZ 55188
QCAZ 55138
QCAZ 56556
QCAZ 38739
KU 222111
UCS6
QCAZ 28221
QCAZ 37304
QCAZ 5930
QCAZ 55274
QCAZ 55278
CCS8
DFCHB-2730
QCAZ 25777
QCAZ 25450
QCAZ 25448
QCAZ25449
QCAZ 25443
B. cf. *equatoriana*
QCAZ 60029
UCS4
QCAZ 60035
QCAZ 60036
UCS5
S13237
SMF 97131
MHCH 2660
SMF 97133
B. medemi
B. robusta
B. compacta
B. schizodactyla
B. sombra
B. colonnea
Altitude
20
292
564
836
1108
1380
1652
1924
2196
2468
2740

Figure 4. Reconstruction of ancestral altitudes for South American *Bolitoglossa*. Reconstruction made in R, using the phylotools package and based on the Bayesian phylogeny obtained from the sequences of *16S*, *Cytb* and *Rag1*. The color of the branches corresponds to the most probable altitude. The guide on the bottom indicates the color scale according to the altitudes.

Tables

Table 1. Voucher and accession number information. Institutional abbreviations: CARIE—Colección de Referencia de Anfibios y Reptiles del Instituto de Ecología, AC (Mexico), CORBIDI−División de Herpetología, Centro de Ornitología y Biodiversidad (Peru), CVULA−Colección de Vertebrados de la Universidad de Los Andes (Colombia), DFCH−Diego Francisco Cisneros-Heredia at Universidad San Francisco de Quito (Ecuador), GP−Gabriela Parra field number, IBH−Instituto de Biología, Universidad Nacional Autónoma de México, herpetology collection (México) INPA−Instituto Nacional de Pesquisas da Amazônia (Brazil), JCS−field collection Juan Carlos Sánchez, KU−University of Kansas Biodiversity Institute-Herpetology Collection (USA), LSUMZ−Louisiana State University Museum of Natural Science (USA), MCNUP−Museo de Ciencias Naturales de la Universidad de Pamplona (Spain) MVZ−Museum of Vertebrate Zoology, University of California, Berkeley (USA) QCAZ− Museo de Zoología, Pontificia Universidad Católica del Ecuador (Ecuador) S−uncatalogued specimens in MVZ, SMF−Senckenberg Museum collection (Germany), UCR−Universidad de Costa Rica (Costa Rica), USNM−United States National Museum collection (USA).

Species	**Voucher**	**GenBank accession number**		
		16S	***cytb***	***Rag1***
B. adspersa	MVZ 158485	-	AF212984[a]	-
B. biseriata	MVZ 232943 (S13236)	AY526118[b]	AY526161[b]	KC614436[c]
B. biseriata	MHCH 2668	KM527334[d]	-	-
B. biseriata	MHCH 2659	KM527330[d]	-	-
B. caldwellae	LSUMZ H-13735	AY526129[b]	AY526168[b]	-
B. cf. *sima*	MVZ 163575	AY526134[b]	AY526172[b]	-
B. leandrae	MCNUP 63	KC257102[e]	-	-
B. leandrae	MCNUP 64	KC257103[e]	-	-
B. leandrae	MCNUP 65	KC257104[e]	-	-
B. medemi	S13237	AY526123[b]	AY526163[b]	KC614437[c]
B. medemi	SMF 97133	KM527328[d]	-	-
B. medemi	SMF 97131	KM527327[d]	-	-
B. medemi	MHCH 2660	KM527325[d]	-	-
B. mucuyensis	CVULAIV-7100	JN635335[f]	JQ665282[g]	-
B. mucuyensis	CVULAIV-7101	JN635336[f]	-	-
B. mucuyensis	CVULAIV-7102	JN635337[f]	-	-
B. mucuyensis	CVULAIV-7103	JN635338[f]	-	-
B. mucuyensis	CVULAIV-7104	JN635339[f]	-	-
B. nicefori	PAG 926	KC257105[e]	-	-

Species	**Voucher**	**GenBank accession number**		
		16S	***cytb***	***Rag1***
B. orestes	No voucher (Isolate MC00)	JN635351[f]	JQ665281[h]	-
B. orestes	CVULAIV-7108	JN635340[f]	JQ665280[g]	-
B. palmata	KU 217422	AY526125[b]	AY526164[b]	-
B. palmata	KU 217423	AY526126[b]	AY526165[b]	-
B. paraensis	INPA 3098	AY526127[b]	AY526166[b]	-
B. paraensis	LSUMZ H-3086	AY526128[b]	AY526167[b]	-
B. sima	QCAZ 22176	To be added	To be added	To be added
B. sima	QCAZ 22346	To be added	To be added	To be added
B. sima	QCAZ 27752	To be added	To be added	To be added
B. sima	QCAZ 31532	To be added	To be added	To be added
B. sima	QCAZ 32135	To be added	To be added	To be added
B. sima	QCAZ 40817	To be added	To be added	To be added
B. sima	QCAZ 51867	To be added	To be added	To be added
B. tamaense	MCNUP 51	KC257100[e]	-	-
B. tamaense	MCNUP 53	KC257101[e]	-	-
B. tamaense	MCNUP 56	KC257098[e]	-	-
B. tamaense	MCNUP 57	KC257099[e]	-	-
CCS1	QCAZ 32300	To be added	To be added	To be added
CCS2	QCAZ 39981	-	KC614431[c]	KC614456[c]
CCS2	QCAZ 39984	To be added	To be added	To be added
CCS3 *B.* aff. *palmata*	QCAZ 51911	To be added	To be added	To be added
CCS3 *B.* aff. *palmata*	QCAZ 51934	To be added	To be added	To be added
CCS3 *B.* aff. *palmata*	QCAZ 52454	To be added	To be added	To be added
CCS3 *B.* aff. *palmata*	QCAZ 52459	To be added	To be added	To be added
CCS4	QCAZ 41724	To be added	To be added	To be added
CCS5	JCS 019	-	KC699921[c]	KC699927[c]
CCS5	QCAZ 42383	To be added	To be added	To be added
CCS6	QCAZ 55712	To be added	To be added	To be added
CCS7	QCAZ 42467	To be added	To be added	To be added
CCS7	QCAZ 48195	To be added	To be added	To be added
CCS7	QCAZ 54436	To be added	To be added	-
CCS8	QCAZ 5930 (LSUMZ H-12838)	AY526130[b]	AY526169[b]	-
CCS8	QCAZ 28221	-	KC614428[c]	-
CCS8	QCAZ 37304	-	DQ353845[c]	KC614452[c]
CCS8	QCAZ 55274	To be added	To be added	-
CCS8	QCAZ 55278	To be added	To be added	-
CCS9	QCAZ 25268	To be added	DQ353830[c]	KC614447[c]
CCS9	QCAZ 25385	-	DQ353835[c]	KC614449[c]

Species	**Voucher**	**GenBank accession number**		
		16S	***cytb***	***Rag1***
CCS9	QCAZ 25386	-	DQ353833[c]	-
CCS9	QCAZ 25387	-	DQ353836[c]	KC614450[c]
CCS9	QCAZ 25420	-	DQ353838[c]	-
CCS9	QCAZ 25421	-	DQ353832[c]	-
CCS9	QCAZ 25422	-	DQ353834[c]	-
CCS9	QCAZ 25425	-	DQ353839[c]	-
CCS9	QCAZ 25426	-	DQ353837[c]	-
CCS9	QCAZ 25592	-	DQ353831[c]	KC614448[c]
CCS9	QCAZ 28404	To be added	KC614429[c]	KC614454[c]
CCS9	QCAZ 35399	To be added	To be added	-
CCS9	QCAZ 42409	To be added	To be added	To be added
CCS9	QCAZ 43906	To be added	To be added	-
CCS9	QCAZ 54657	To be added	To be added	-
CCS9	QCAZ 54658	To be added	To be added	To be added
CCS10	QCAZ 25784	To be added	DQ353813[c]	-
CCS10	QCAZ 25793	-	DQ353814[c]	-
CCS10	QCAZ 25794	-	DQ353815[c]	KC614443[c]
CCS10	QCAZ 25795	-	DQ353812[c]	
CCS11	QCAZ 25467	-	DQ353811[c]	-
CCS11	QCAZ 25522	To be added	DQ353809[c]	KC614442[c]
CCS11	QCAZ 42479	To be added	To be added	To be added
CCS11	QCAZ 52616	To be added	To be added	To be added
CCS11	QCAZ 53246	To be added	To be added	-
CCS11	QCAZ 25710	-	DQ353810[c]	-
CCS12	QCAZ 38989	To be added	To be added	To be added
CCS12	QCAZ 52804	To be added	To be added	-
CCS12	QCAZ 56379	To be added	To be added	-
CCS13	KU 217421	AY526131[b]	AY526170[b]	-
CCS13	QCAZ 25289	-	DQ353826[c]	-
CCS13	QCAZ 25294	-	DQ353816[c]	-
CCS13	QCAZ 25317	-	DQ353822[c]	-
CCS13	QCAZ 25318	-	DQ353824[c]	-
CCS13	QCAZ 25319	-	DQ353823[c]	-
CCS13	QCAZ 25320	-	DQ353821[c]	KC614445[c]
CCS13	QCAZ 25355	-	DQ353818[c]	-
CCS13	QCAZ 25455	-	DQ353829[c]	-
CCS13	QCAZ 25593	-	DQ353819[c]	KC614444[c]
CCS13	QCAZ 25747	-	DQ353817[c]	-
CCS13	QCAZ 25753	To be added	DQ353827[c]	KC614446[c]
CCS13	QCAZ 25758	To be added	DQ353825[c]	-
CCS13	QCAZ 25771	-	DQ353828[c]	-
CCS13	QCAZ 25872	-	DQ353820[c]	-
CCS13	QCAZ 29424	To be added	To be added	To be added
CCS13	QCAZ 35372	To be added	To be added	To be added

Species	**Voucher**	**GenBank accession number**		
		16S	***cytb***	***Rag1***
CCS13	QCAZ 35373	To be added	To be added	To be added
CCS13	QCAZ 53347	To be added	To be added	To be added
CCS13	QCAZ 53356	To be added	To be added	-
CCS13	QCAZ 54218	-	To be added	-
CCS13	QCAZ 57198	To be added	To be added	-
UCS1	QCAZ 45254	To be added	To be added	To be added
UCS2 *B.* cf. *peruviana*	QCAZ 60027 (CORBIDI 6332)	To be added	To be added	To be added
UCS2 *B.* cf. *peruviana*	QCAZ 60034 (CORBIDI 9206)	To be added	To be added	-
UCS3	QCAZ 53501	To be added	To be added	To be added
UCS3	QCAZ 53539	To be added	To be added	-
UCS3	QCAZ 56147	To be added	To be added	-
UCS3	QCAZ 60026 (CORBIDI 4666)	To be added	To be added	To be added
UCS4	QCAZ 60029 (CORBIDI 10709)	To be added	To be added	To be added
UCS5	QCAZ 60035 (CORBIDI 15488)	To be added	To be added	To be added
UCS5	QCAZ 60036 (CORBIDI 15489)	To be added	To be added	To be added
B. cf. *equatoriana*	DFCH-2730	-	DQ353846[c]	-
B. cf. *equatoriana*	QCAZ 25443	-	DQ353841[c]	-
B. cf. *equatoriana*	QCAZ 25448	-	DQ353842[c]	KC614451[c]
B. cf. *equatoriana*	QCAZ 25449	-	DQ353843[c]	-
B. cf. *equatoriana*	QCAZ 25450	-	DQ353844[c]	-
B. cf. *equatoriana*	QCAZ 25777	-	DQ353840[c]	-
UCS6	KU 222111	AY526117[b]	AY526160[b]	-
UCS6	QCAZ 20845	-	KC614427[c]	KC614453[c]
UCS6	QCAZ 32291	-	KC614430[c]	KC614455[c]
UCS6	QCAZ 38739	To be added	To be added	-
UCS6	QCAZ 51131	To be added	To be added	To be added
UCS6	QCAZ 54656	To be added	To be added	-
UCS6	QCAZ 55138	To be added	To be added	-
UCS6	QCAZ 55188	To be added	To be added	To be added
UCS6	QCAZ 56556	To be added	To be added	-
B. alberchi	MVZ 264191	KP735249[i]	KP735278[i]	KP735306[i]
B. alvaradoi	MVZ 215735	AY526157[b]	AY526194[b]	

Species	**Voucher**	**GenBank accession number**		
		16S	***cytb***	***Rag1***
B. bramei	UCR 20483	JQ899159[j]	JQ899189[j]	
B. carri	USNM 523277	AY526139[b]	AY526176[b]	KC614458[c]
B. cerroensis	MVZ S 12921	AF199233[k]	AF199195[k]	
B. colonnea	No voucher	AY526119[b]	AY526162[b]	
B. compacta	UCR 20532	JQ899163	JQ899193	
B. dofleini	MVZ 263450	KP886842[i]	KP900047[i]	KP900133[i]
B. dunni	USNM 523280	AY526145[b]	AY526182[b]	KC614438[c]
B. engelhardti	MVZ 251495	GU725448[l]	GU725461[l]	
B. epimela	MVZ 181260	AY526120[b]	AF212097[m]	
B. flavimembris	MVZ 177786	GU725449[l]	GU725462[l]	KP900132[i]
B. franklini	MVZ 185991	AY526147[b]	AY526184[b]	KC614439[c]
B. hartwegi	MVZ 263458	KC288004[n]	KC288103[n]	KP900131[i]
B. helmrichi	MVZ 257804	GU725450[l]	GU725463[l]	
B. lincolni	MVZ 143564	AY526148[b]	AY526185[b]	KC614440[c]
B. longissima	USNM 523285	AY526149[b]	AY526186[b]	KC614441[c]
B. nympha	MVZ 257812	KC287924[n]	KC288068[n]	KP900130[i]
B. oaxacensis	IBH 13374	AF416690[o]	AF416681[o]	
B. occidentalis	MVZ 194254	AY526115[b]	AY526158[b]	KC614435[c]
B. odonnelli	MVZ 229068	AF218476[a]	AF212977[a]	KC699922[c]
B. platydactyla	GP 108	AF218487[a]	AF212981[a]	
B. riletti	MVZ 194328	AF416696[o]	AF416682[o]	
B. robusta	MVZ 190830	EU448109[p]	EU448110[p]	
B. schizodactyla	No voucher	AY526133[b]	AY526171[b]	
B. sombra	MVZ 225875	AY728235[q]	AY728235[q]	EU275810[r]
B. striatula	MVZ 181280	AF218488[a]	AF212982[a]	
B. subpalmata	MVZ 229172	AF416697[m]	AF212094[o]	
Outgroup				
Batrachoseps attenuatus	MVZ 230761	AY728228[q]	AY728228[q]	KP900175[i]
Desmognathus fuscus	MVZ 224931	AY728227[q]	AY728227[q]	EU275781[r]
Eurycea bislineata	MVZ 225074	AY728217[q]	AY728217[q]	EU275784[r]
Hemidactylium scutatum	MVZ 225077	AY728231[q]	AY728231[q]	AY691711[s]
Hydromantes brunus	MVZ 230641	AY728234[q]	AY728234[q]	HM797658[t]
Ixalotriton niger	IBH 29715	KP886874[i]	KP900077[i]	KP900163[i]
Oedipina taylori	MVZ 267200	KP886887[i]	KP900080[i]	KP900167[i]
Parvimolge townsendi	CARIE 1174	KP886876[i]	KP900078[i]	KP900165[i]
Plethodon cinereus	MVZ 225101	AY728232[q]	AY728232[q]	JF449378[u]

Species	**Voucher**	**GenBank accession number**		
		16S	***cytb***	***Rag1***
Pseudoeurycea galeanae	IBH 24595	KP886847[i]	KP900051[i]	KP900137[i]
Pseudoeurycea rex	MVZ 263590	KP886852[i]	KP900056[i]	KP900142[i]

[a] García-París et al. (2000b)
[b] Parra-Olea et al. (2004)
[c] Elmer et al. (2013)
[d] Batista et al. (2014)
[e] Acevedo et al. (2013)
[f] Fermin et al. (2012)
[g] García-Gutiérrez et al. (2013)
[h] Unpublished
[i] Rovito et al. (2015)
[j] Boza-Oviedo et al. (2012)
[k] García-París and Wake (2000)
[l] Rovito et al. (2010)
[m] García-París et al. (2000a)
[n] Rovito et al. (2012)
[o] Parra-Olea et al. (2002)
[p] García-París et al. (2008)
[q] Mueller et al. (2004)
[r] Vieites et al. (2007)
[s] Wiens et al. (2006)
[t] Rovito (2010)
[u] Vieites et al. (2011)

Table 2. Alternative divergence time estimates based on different calibrations.

The estimation based on the calibration by Mueller (2006), shaded in gray, was used in the results shown in Figs. 3 and S1. The letters in the first column correspond to the nodes labeled in Fig. 3. For each node we present the age estimated along with the 95% high posterior density interval (HPD) between parentheses.

Node	Bayesian (95% HPD) (MYA)				
	Mueller, 2006	**Zhang and Wake, 2009 (Highest)**	**Zhang and Wake, 2009 (Lowest)**	**Pyron, 2014**	**Molecular rate**
Plethodontidae	129 (109–152)*	109 (85–133)*	58 (16–104)*		213.8 (194.2–233.9)
Bolitoglossa	55.7 (51.3–60.2)	48.9 (44.7–53.2)	26.2 (23.8–28.5)		94.4 (86.5–102.6)
Eladinea	43.4 (39.3–47.6)	37.8 (34.1–41.8)	20.2 (18.2–22.5)		72.1 (52.8–87.5)
A: SA *Bolitoglossa*	30.7 (27.9–33.7)	26.7 (24.1–29.4)	14.3 (12.9–15.9)	18.2*	50.6 (40.1–60.4)
B	14.9 (12.5–17.3)	12.9 (10.9–15.2)	6.9 (4.1–7.7)	10.9 (7.4–14.7)	25.1 (21.3–29.3)
C	18.9 (15.8–22.2)	16.5 (13.8–19.4)	8.8 (7.4–10.4)	13.3 (10.1–16.5)	31.9 (27–37.4)
D	14.6 (11.9–17.4)	12.6 (10.3–15.1)	6.8 (5.5–8.1)	8.7 (4.8–12.5)	24.8 (20.5–29.5)
E	14.2 (11.6–17)	12.3 (10.1–14.8)	6.7 (5.4–7.9)	8.5 (5.6–11.5)	22.8 (18.4–27.1)
F	26.6 (24.3–28.9)	23.1 (20.9–25.3)	12.4 (11.2–13.6)	17.5 (15.7–19.8)	44.2 (40.6–47.9)
G	19.7 (17.7–21.8)	17.1 (15.3–18.9)	9.1 (8.1–10.2)	14 (11.7–16.4)	32.5 (29.4–35.9)
H	16.6 (13.4–19.8)	14.4 (11.6–17.2)	7.7 (6.3–9.3)	10.3 (6.5–14.2)	26.7 (21.6–31.8)
I	20.3 (17.5–23.3)	17.6 (15.1–20.2)	9.4 (8.1–10.9)	13.7 (10.7–16.7)	32.2 (27.8–36.8)
J	25.2 (23.1–27.5)	21.8 (20–24.1)	11.7 (10.6–12.9)	17.3 (15.2–19.3)	41.7 (38.2–45.2)
K	18.7 (16.6–20.9)	16.2 (14.3–18.2)	8.7 (7.6–9.7)	12.7 (10.3–15.2)	31.6 (28.1–35.2)

*calibration points

Table 3. Uncorrected genetic distances of the clades from the Andes and Choco. Mean ± SD is given along with the range in parenthesis (only when n>1). The genetic distance of *Cytb* is given below the diagonal and the genetic distance of *16S* is given above the diagonal. The diagonal shows (in bold) the genetic distance within clades. Not applicable (na) is shown for the clades when n=1. Asterisks indicate identical pairwise comparisons obtained from more than one specimen per clade.

CLADE	***B.* cf. *sima***	***B. palmata***	**CCS1**	**UCS1**	**CCS2**	***B. sima***	**CCS3** ***B.* aff. *palmata***
B.* cf. *sima	**na** **na**	0.047*	0.047	0.047	0.053	0.022 ± 0.005 (0.020 - 0.025)	0.053*
B. palmata	0.122*	**0.000** **0.000**	0.051*	0.049*	0.053*	0.046 ± 0.008 (0.041 - 0.051)	0.036 ± 0.008 (0.035 - 0.037)
CCS1	0.151	0.117*	**na** **na**	0.053	0.049	0.069 ± 0.008 (0.061 - 0.075)	0.067*
UCS1	0.147	0.124*	0.087	**na** **na**	0.051	0.067 ± 0.007 (0.058 - 0.071)	0.076*
CCS2	0.126*	0.115 ± 0.012 (0.115 - 0.116)	0.09 ± 0.010 (0.087 - 0.094)	0.103 ± 0.011 (0.098 - 0.107)	**na** **0.003**	0.055 ± 0.007 (0.052 - 0.058)	0.071*
B. sima	0.097 ± 0.016 (0.076 - 0.112)	0.119 ± 0.012 (0.114 - 0.122)	0.124 ± 0.010 (0.118 - 0.128)	0.134 ± 0.010 (0.127 - 0.140)	0.142 ± 0.011 (0.127 - 0.142)	**0.018 ± 0.003 (0.000 - 0.031)** **0.029 ±0.004 (0.001 - 0.047)**	0.074 ± 0.008 (0.070 - 0.079)
CCS3 ***B.* aff. *palmata***	0.137*	0.107 ± 0.012 (0.104 - 0.110)	0.131 ± 0.012 (0.129 - 0.132)	0.139 ± 0.012 (0.138 - 0.140)	0.14 ± 0.012 (0.137 - 0.144)	0.133 ± 0.011 (0.131 - 0.137)	**0.005 ± 0.002 (0.000 - 0.007)** **0.004 ± 0.002 (0.000 - 0.006)**

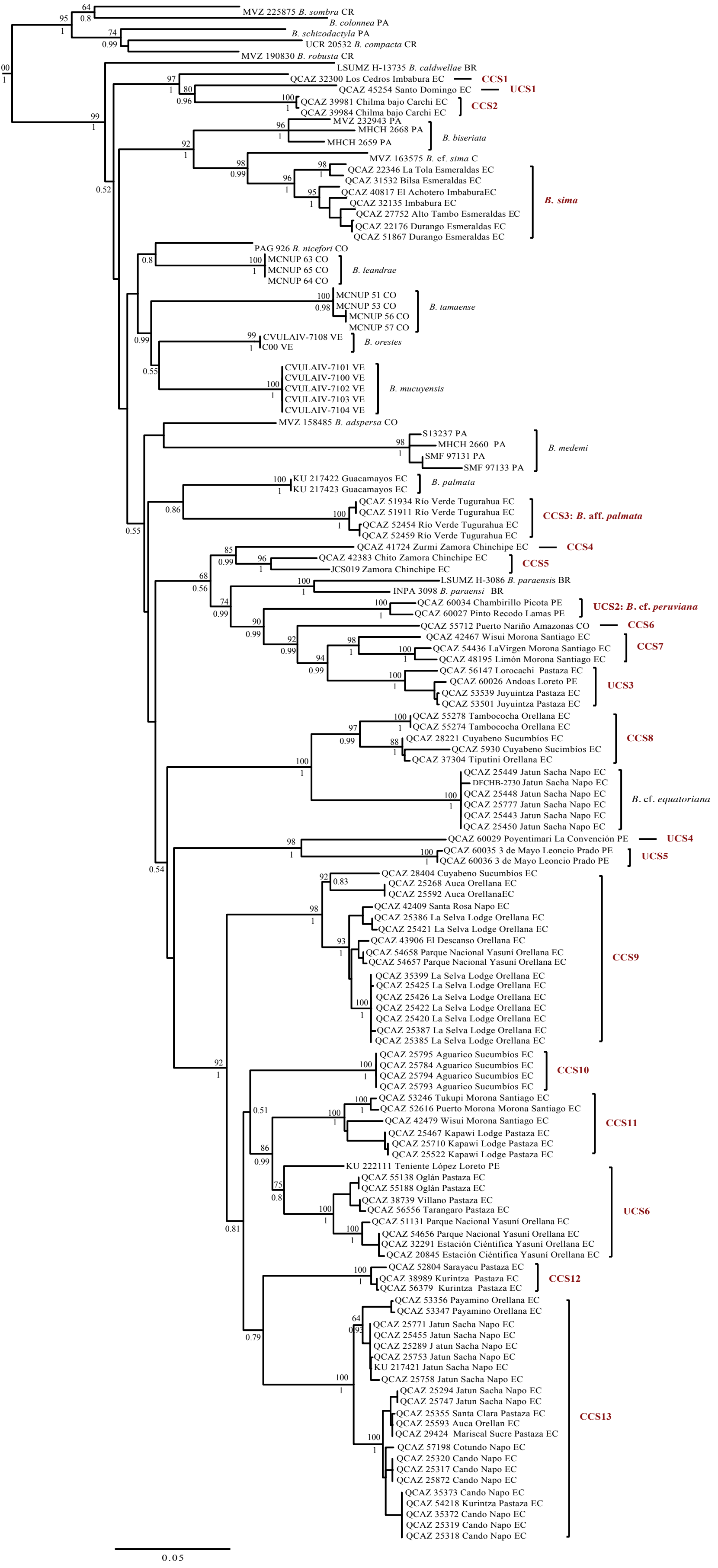
Table 4. Uncorrected genetic distances of the clades CCS4-7 and UCS2-5, from the Amazonia. Mean ± SD is given along with the range in parenthesis (only when n>1). The genetic distance of *Cytb* is given below the diagonal and the genetic distance of *16S* is given above the diagonal. The diagonal shows (in bold) the genetic distance within clades. Not applicable (na) is shown for the clades when n=1. Asterisks indicate identical pairwise comparisons obtained from more than one specimen per clade.

CLADE	**CCS4**	**CCS5**	**UCS2** **B. cf. *peruviana***	**CCS6**	**CCS7**	**UCS3**	**UCS4**	**UCS5**
CCS4	**Na** **Na**	0.034	0.051 ± 0.007 (0.050 - 0.052)	0.063	0.052 ± 0.007 (0.051 - 0.053)	0.051 ± 0.007 (0.048 - 0.055)	0.067	0.068 ± 0.008 (0.067 - 0.070)
CCS5	0.083 ± 0.009 (0.075 - 0.091)	**na** **0.055**	0.043 ± 0.006 (0.042 - 0.044)	0.051	0.044 ± 0.009 (0.044 - 0.045)	0.042 ± 0.007 (0.040 - 0.045)	0.058	0.056 ± 0.008 (0.053 - 0.060)
UCS2 ***B*. cf. *peruviana***	0.102 ± 0.010 (0.101 - 0.104)	0.122 ± 0.011 (0.115 - 0.131)	**0.017** **0.020**	0.058 ± 0.007 (0.055 - 0.061)	0.058 ± 0.006 (0.049 - 0.062)	0.054 ± 0.007 (0.049 - 0.062)	0.072 ± 0.008 (0.070 - 0.074)	0.065 ± 0.008 (0.061 - 0.069)
CCS6	0.109	0.112 ± 0.010 (0.105 - 0.118)	0.094 ± 0.010 (0.091 - 0.097)	**na** **na**	0.052 ± 0.007 (0.048 - 0.050)	0.049 ± 0.007 (0.048 - 0.050)	0.083	0.077 ± 0.009 (0.077 - 0.078)
CCS7	0.115 ± 0.010 (0.105 - 0.126)	0.112 ± 0.010 (0.101 - 0.121)	0.106 ± 0.009 (0.103 - 0.110)	0.082 ± 0.008 (0.079 - 0.085)	**0.018 ± 0.0045 (0.006 - 0.025)** **0.044 ± 0.006 (0.022 - 0.058)**	0.035 ± 0.005 (0.030 - 0.043)	0.062 ± 0.008 (0.060 - 0.066)	0.068 ± 0.008 (0.065 - 0.071)
UCS3	0.123 ± 0.011 (0.118 - 0.126)	0.12 ± 0.010 (0.105 - 0.133)	0.112 ± 0.009 (0.108 - 0.117)	0.094 ± 0.010 (0.087 - 0.098)	0.087 ± 0.009 (0.085 -0.092)	**0.012 ± 0.003 (0.000 - 0.023)** **0.017 ± 0.003 (0.003 - 0.028)**	0.067 ± 0.008 (0.066 - 0.067)	0.068 ± 0.008 (0.064 - 0.070)
UCS4	0.129	0.143 ± 0.012 (0.134 - 0.152)	0.15*	0.141	0.144 ± 0.011 (0.141 - 0.150)	0.151 ± 0.012 (0.151 - 0.154)	**na** **na**	0.053 ± 0.008 (0.053 - 0.053)
UCS5	0.124 ± 0.012 (0.123 - 0.125)	0.134 ± 0.011 (0.119 - 0.148)	0.137 ± 0.012 (0.136 - 0.138)	0.136 ± 0.011 (0.135 - 0.138)	0.146 ± 0.011 (0.139 - 0.157)	0.151 ± 0.011 (0.139 - 0.157)	0.091 ± 0.010 (0.090 - 0.091)	**0.001** **0.003**

Table 5. Uncorrected genetic distances of the clades CCS8-13, UCS6 and *B.* cf *equatoriana,* from the Amazonia. Mean ± SD is given along with the range in parenthesis (only when n>1). The genetic distance of *Cytb* is given below the diagonal and the genetic distance of *16S* is given above the diagonal. The diagonal shows (in bold) the genetic distance within clades. Not applicable (na) is shown for the clades when n=1. Asterisks indicate identical pairwise comparisons obtained from more than one specimen per clade.

CLADE	**CCS8**	***B.* cf. *equatoriana***	**CCS9**	**CCS10**	**CCS11**	**USC6**	**CCS12**	**CCS13**
CCS8	**0.012 ± 0.004 (0.000 - 0.017)** **0.040 ± 0.005 (0.000 - 0.065)**	-	0.065 ± 0.008 (0.043 - 0.078)	0.075 ± 0.008 (0.064 - 0.082)	0.065 ± 0.008 (0.048 - 0.077)	0.062 ± 0.007 (0.049 - 0.073)	0.059 ± 0.008 (0.041 - 0.072)	0.069 ± 0.008 (0.052 - 0.081)
B.* cf. *equatoriana	0.102 ± 0.010 (0.097 - 0.110)	**na** **0.002 ± 0.001 (0.000 - 0.006)**	-	-	-	-	-	-
CCS9	0.12 ± 0.010 (0.099 - 0.141)	0.137 ± 0.012 (0.124 - 0.156)	**0.014 ± 0.003 (0.000 - 0.026)** **0.026 ± 0.003 (0.000 - 0.061)**	0.048 ± 0.007 (0.047 - 0.050)	0.041 ± 0.006 (0.030 - 0.048)	0.043 ± 0.006 (0.036 - 0.048)	0.039 ± 0.007 (0.036 - 0.041)	0.045 ± 0.006 (0.039 - 0.051)
CCS10	0.133 ± 0.011 (0.129 - 0.137)	0.141 ± 0.012 (0.139 - 0.143)	0.101 ± 0.010 (0.087 - 0.113)	**na** **0***	0.045 ± 0.007 (0.043 - 0.046)	0.042 ± 0.006 (0.036 - 0.044)	0.044 ± 0.007 (0.042 - 0.047)	0.052 ± 0.008 (0.050 - 0.054)
CCS11	0.145 ± 0.010 (0.124 - 0.158)	0.160 ± 0.013 (0.141 - 0.170)	0.100 ± 0.008 (0.084 - 0.119)	0.088 ± 0.009 (0.087 - 0.106)	**0.009 ± 0.003 (0.001 - 0.013)** **0.031 ± 0.004 (0.000 - 0.045)**	0.032 ± 0.005 (0.030 - 0.036)	0.036 ± 0.006 (0.031 - 0.040)	0.035 ± 0.006 (0.027 - 0.042)
UCS6	0.136 ± 0.011 (0.125 - 0.148)	0.147 ± 0.012 (0.133 - 0.155)	0.095 ± 0.009 (0.075 - 0.129)	0.084 ± 0.009 (0.077 - 0.099)	0.088 ± 0.008 (0.077 - 0.130)	**0.006 ± 0.002 (0.000 - 0.011)** **0.026 ± 0.004 (0.000 - 0.045)**	0.034 ± 0.006 (0.032 - 0.039)	0.038 ± 0.006 (0.026 - 0.046)
CCS12	0.131 ± 0.011 (0.18 - 0.136)	0.15 ± 0.013 (0.147 - 0.53)	0.107 ± 0.010 (0.096 - 0.120)	0.097 ± 0.011 (0.095 - 0.098)	0.114 ± 0.010 (0.099 - 0.124)	0.104 ± 0.009 (0.099 - 0.109)	**0.004 ± 0.002 (0.000 - 0.006)** **0.008 ± 0.003 (0.003 - 0.012)**	0.038 ± 0.006 (0.033 - 0.043)
CCS13	0.140 ± 0.011 (0.132 - 0.148)	0.138 ± 0.012 (0.133 - 0.148)	0.108 ± 0.010 (0.092 - 0.129)	0.092 ± 0.009 (0.087 - 0.098)	0.105 ± 0.009 (0.089 - 0.130)	0.098 ± 0.009 (0.089 - 0.111)	0.101 ± 0.010 (0.090 - 0.110)	**0.008 ± 0.002 (0.000 - 0.014)** **0.025 ± 0.003 (0.000 - 0.056)**

Supplementary material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B. colonnea PA
B. schizodactyla PA
UCR 20532 *B. compacta* CR
MVZ 190830 *B. robusta* CR
LSUMZ H-13735 *B. caldwellae* BR
QCAZ 32300 Los Cedros Imbabura EC
CCS1
QCAZ 45254 Santo Domingo EC
UCS1
QCAZ 39981 Chilma bajo Carchi EC
QCAZ 39984 Chilma bajo Carchi EC
CCS2
MVZ 232943 PA
MHCH 2668 PA
MHCH 2659 PA
B. biseriata
MVZ 163575 *B.* cf. *sima* C
QCAZ 22346 La Tola Esmeraldas EC
QCAZ 31532 Bilsa Esmeraldas EC
QCAZ 40817 El Achotero ImbaburaEC
QCAZ 32135 Imbabura EC
QCAZ 27752 Alto Tambo Esmeraldas EC
QCAZ 22176 Durango Esmeraldas EC
QCAZ 51867 Durango Esmeraldas EC
B. sima
PAG 926 *B. nicefori* CO
MCNUP 63 CO
MCNUP 65 CO
MCNUP 64 CO
B. leandrae
MCNUP 51 CO
MCNUP 53 CO
MCNUP 56 CO
MCNUP 57 CO
B. tamaense
CVULAIV-7108 VE
C00 VE
B. orestes
CVULAIV-7101 VE
CVULAIV-7100 VE
CVULAIV-7102 VE
CVULAIV-7103 VE
CVULAIV-7104 VE
B. mucuyensis
MVZ 158485 *B. adspersa* CO
S13237 PA
MHCH 2660 PA
SMF 97131 PA
SMF 97133 PA
B. medemi
KU 217422 Guacamayos EC
KU 217423 Guacamayos EC
B. palmata
QCAZ 51934 Río Verde Tugurahua EC
QCAZ 51911 Río Verde Tugurahua EC
QCAZ 52454 Río Verde Tugurahua EC
QCAZ 52459 Río Verde Tugurahua EC
CCS3: *B.* aff. *palmata*
QCAZ 41724 Zurmi Zamora Chinchipe EC
CCS4
QCAZ 42383 Chito Zamora Chinchipe EC
JCS019 Zamora Chinchipe EC
CCS5
LSUMZ H-3086 *B. paraensis* BR
INPA 3098 *B. paraensi* BR
QCAZ 60034 Chambirillo Picota PE
QCAZ 60027 Pinto Recodo Lamas PE
UCS2: *B.* cf. *peruviana*
QCAZ 55712 Puerto Nariño Amazonas CO
CCS6
QCAZ 42467 Wisui Morona Santiago EC
QCAZ 54436 LaVirgen Morona Santiago EC
QCAZ 48195 Limón Morona Santiago EC
CCS7
QCAZ 56147 Lorocachi Pastaza EC
QCAZ 60026 Andoas Loreto PE
QCAZ 53539 Juyuintza Pastaza EC
QCAZ 53501 Juyuintza Pastaza EC
UCS3
QCAZ 55278 Tambococha Orellana EC
QCAZ 55274 Tambococha Orellana EC
QCAZ 28221 Cuyabeno Sucumbios EC
QCAZ 5930 Cuyabeno Sucimbios EC
QCAZ 37304 Tiputini Orellana EC
CCS8
QCAZ 25449 Jatun Sacha Napo EC
DFCHB-2730 Jatun Sacha Napo EC
QCAZ 25448 Jatun Sacha Napo EC
QCAZ 25777 Jatun Sacha Napo EC
QCAZ 25443 Jatun Sacha Napo EC
QCAZ 25450 Jatun Sacha Napo EC
B. cf. *equatoriana*
QCAZ 60029 Poyentimari La Convención PE
UCS4
QCAZ 60035 3 de Mayo Leoncio Prado PE
QCAZ 60036 3 de Mayo Leoncio Prado PE
UCS5
QCAZ 28404 Cuyabeno Sucumbios EC
QCAZ 25268 Auca Orellana EC
QCAZ 25592 Auca OrellanaEC
QCAZ 42409 Santa Rosa Napo EC
QCAZ 25386 La Selva Lodge Orellana EC
QCAZ 25421 La Selva Lodge Orellana EC
QCAZ 43906 El Descanso Orellana EC
QCAZ 54658 Parque Nacional Yasuní Orellana EC
QCAZ 54657 Parque Nacional Yasuní Orellana EC
QCAZ 35399 La Selva Lodge Orellana EC
QCAZ 25425 La Selva Lodge Orellana EC
QCAZ 25426 La Selva Lodge Orellana EC
QCAZ 25422 La Selva Lodge Orellana EC
QCAZ 25420 La Selva Lodge Orellana EC
QCAZ 25387 La Selva Lodge Orellana EC
QCAZ 25385 La Selva Lodge Orellana EC
CCS9
QCAZ 25795 Aguarico Sucumbios EC
QCAZ 25784 Aguarico Sucumbios EC
QCAZ 25794 Aguarico Sucumbios EC
QCAZ 25793 Aguarico Sucumbios EC
CCS10
QCAZ 53246 Tukupi Morona Santiago EC
QCAZ 52616 Puerto Morona Morona Santiago EC
QCAZ 42479 Wisui Morona Santiago EC
QCAZ 25467 Kapawi Lodge Pastaza EC
QCAZ 25710 Kapawi Lodge Pastaza EC
QCAZ 25522 Kapawi Lodge Pastaza EC
CCS11
KU 222111 Teniente López Loreto PE
QCAZ 55138 Oglán Pastaza EC
QCAZ 55188 Oglán Pastaza EC
QCAZ 38739 Villano Pastaza EC
QCAZ 56556 Tarangaro Pastaza EC
QCAZ 51131 Parque Nacional Yasuní Orellana EC
QCAZ 54656 Parque Nacional Yasuní Orellana EC
QCAZ 32291 Estación Científica Yasuní Orellana EC
QCAZ 20845 Estación Científica Yasuní Orellana EC
UCS6
QCAZ 52804 Sarayacu Pastaza EC
QCAZ 38989 Kurintza Pastaza EC
QCAZ 56379 Kurintza Pastaza EC
CCS12
QCAZ 53356 Payamino Orellana EC
QCAZ 53347 Payamino Orellana EC
QCAZ 25771 Jatun Sacha Napo EC
QCAZ 25455 Jatun Sacha Napo EC
QCAZ 25289 J atun Sacha Napo EC
QCAZ 25753 Jatun Sacha Napo EC
KU 217421 Jatun Sacha Napo EC
QCAZ 25758 Jatun Sacha Napo EC
QCAZ 25294 Jatun Sacha Napo EC
QCAZ 25747 Jatun Sacha Napo EC
QCAZ 25355 Santa Clara Pastaza EC
QCAZ 25593 Auca Orellan EC
QCAZ 29424 Mariscal Sucre Pastaza EC
QCAZ 57198 Cotundo Napo EC
QCAZ 25320 Cando Napo EC
QCAZ 25317 Cando Napo EC
QCAZ 25872 Cando Napo EC
QCAZ 35373 Cando Napo EC
QCAZ 54218 Kurintza Pastaza EC
QCAZ 35372 Cando Napo EC
QCAZ 25319 Cando Napo EC
QCAZ 25318 Cando Napo EC
CCS13
0.05

Figure S1. Mitochondrial phylogeny of South American *Bolitoglossa*. Maximum likelihood tree, an analysis obtained using two mitochondrial genes (*16S* and *Cytb*). Numbers above branches correspond to ML bootstrap support, values <50 are not shown. Numbers below branches correspond to Bayesian posterior probabilities, values <0.5 are not shown. The voucher number and the locality are shown in each terminal. Clades are delimitated by square brackets. Names in red correspond to species for which we produced new sequences. Abbreviations of the localities: CR−Costa Rica; PA−Panama; BR−Brazil, EC−Ecuador; CO−Colombia; VE−Venezuela; PE−Peru.

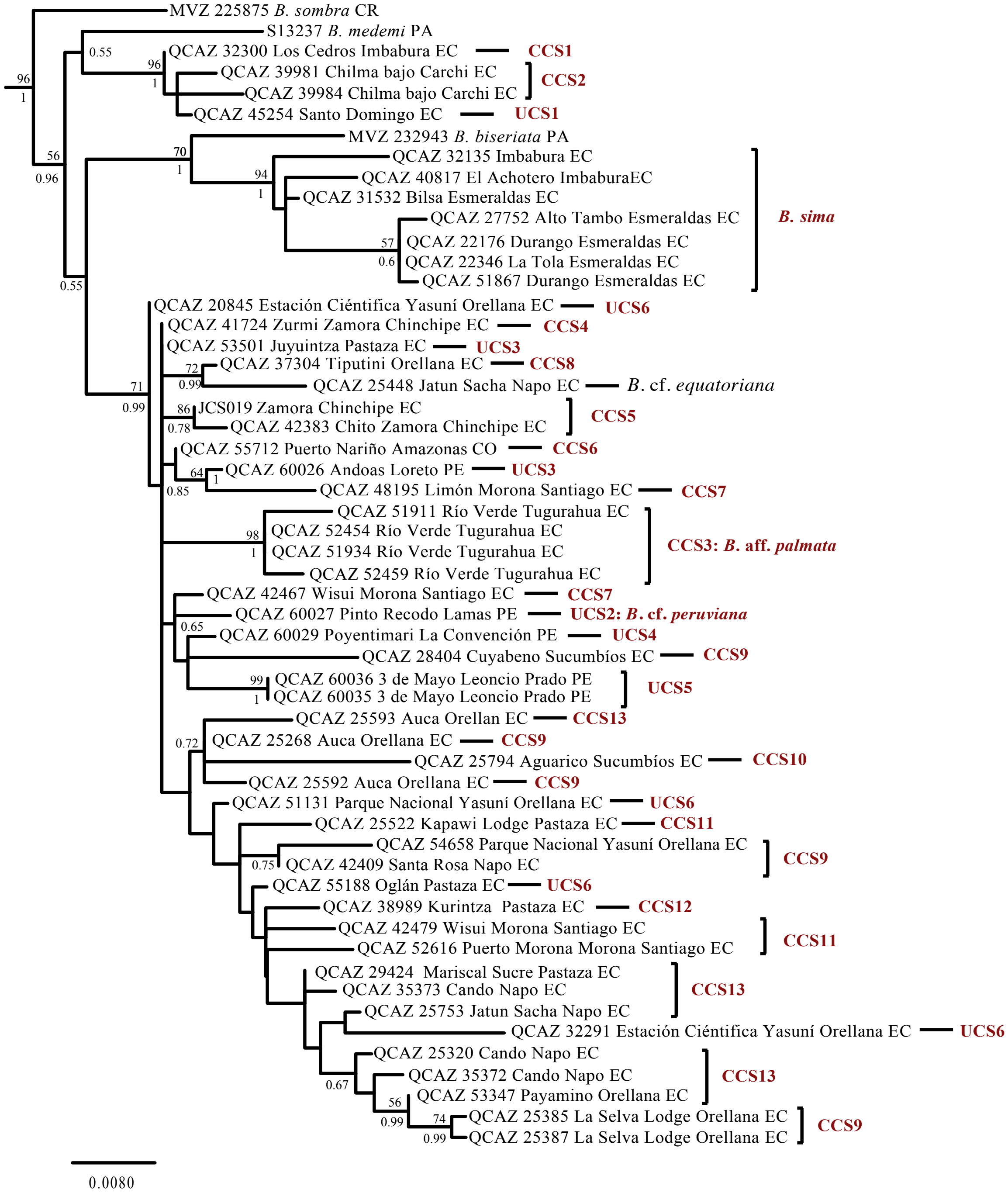
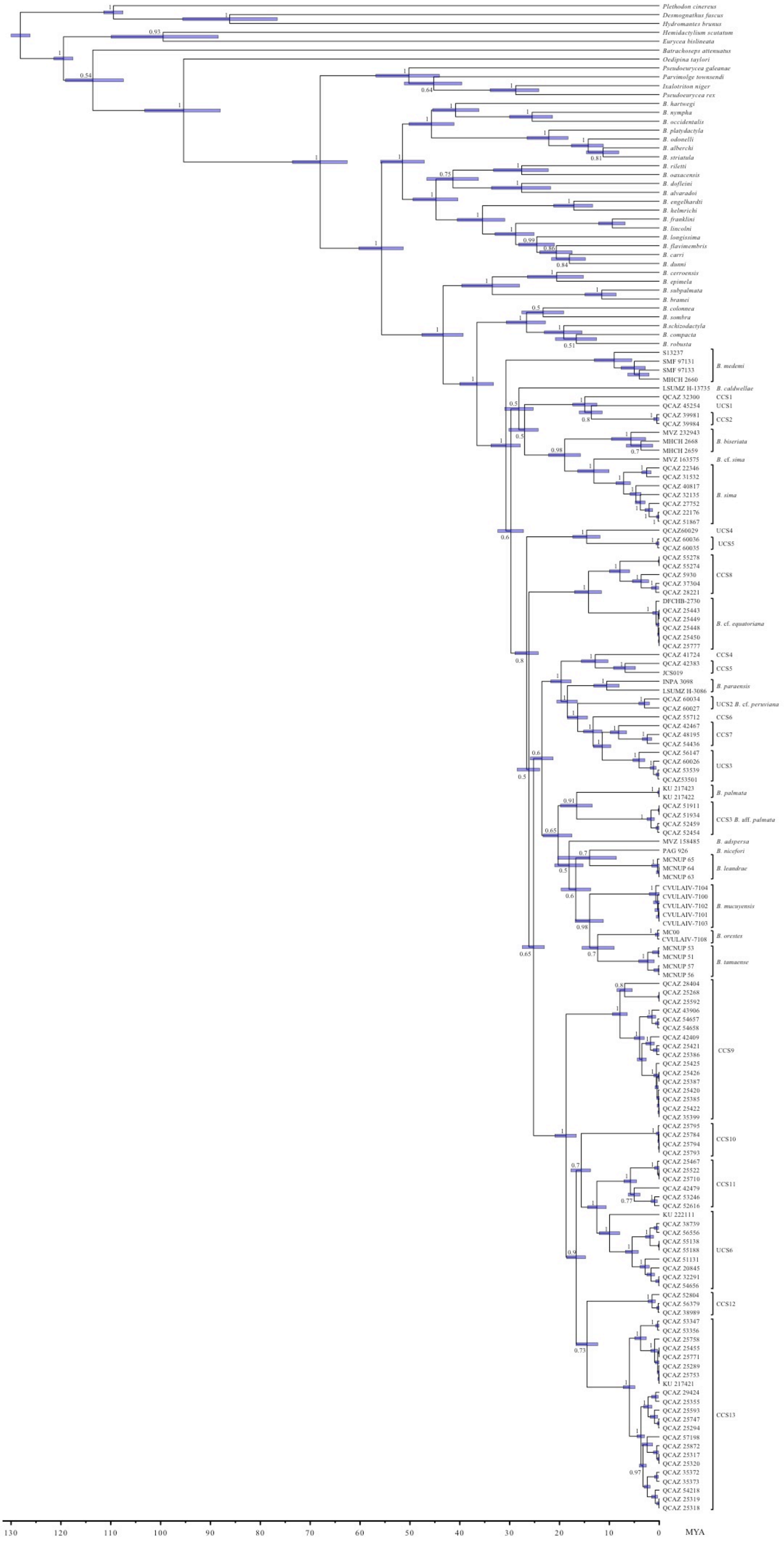

MVZ 225875 *B. sombra* CR
S13237 *B. medemi* PA
QCAZ 32300 Los Cedros Imbabura EC
CCS1
QCAZ 39981 Chilma bajo Carchi EC
QCAZ 39984 Chilma bajo Carchi EC
CCS2
QCAZ 45254 Santo Domingo EC
UCS1
96
1
0.55
96
1
MVZ 232943 *B. biseriata* PA
QCAZ 32135 Imbabura EC
QCAZ 40817 El Achotero ImbaburaEC
QCAZ 31532 Bilsa Esmeraldas EC
QCAZ 27752 Alto Tambo Esmeraldas EC
QCAZ 22176 Durango Esmeraldas EC
QCAZ 22346 La Tola Esmeraldas EC
QCAZ 51867 Durango Esmeraldas EC
B. sima
56
0.96
70
1
94
1
57
0.6
0.55
QCAZ 20845 Estación Científica Yasuní Orellana EC
UCS6
QCAZ 41724 Zurmi Zamora Chinchipe EC
CCS4
QCAZ 53501 Juyuintza Pastaza EC
UCS3
QCAZ 37304 Tiputini Orellana EC
CCS8
QCAZ 25448 Jatun Sacha Napo EC
B. cf. *equatoriana*
72
0.99
JCS019 Zamora Chinchipe EC
QCAZ 42383 Chito Zamora Chinchipe EC
CCS5
86
0.78
71
0.99
QCAZ 55712 Puerto Nariño Amazonas CO
CCS6
QCAZ 60026 Andoas Loreto PE
UCS3
QCAZ 48195 Limón Morona Santiago EC
CCS7
64
1
0.85
QCAZ 51911 Río Verde Tugurahua EC
QCAZ 52454 Río Verde Tugurahua EC
QCAZ 51934 Río Verde Tugurahua EC
QCAZ 52459 Río Verde Tugurahua EC
CCS3: *B.* aff. *palmata*
98
1
QCAZ 42467 Wisui Morona Santiago EC
CCS7
QCAZ 60027 Pinto Recodo Lamas PE
UCS2: *B.* cf. *peruviana*
QCAZ 60029 Poyentimari La Convención PE
UCS4
0.65
QCAZ 28404 Cuyabeno Sucumbíos EC
CCS9
QCAZ 60036 3 de Mayo Leoncio Prado PE
QCAZ 60035 3 de Mayo Leoncio Prado PE
UCS5
99
1
QCAZ 25593 Auca Orellan EC
CCS13
QCAZ 25268 Auca Orellana EC
CCS9
0.72
QCAZ 25794 Aguarico Sucumbíos EC
CCS10
QCAZ 25592 Auca Orellana EC
CCS9
QCAZ 51131 Parque Nacional Yasuní Orellana EC
UCS6
QCAZ 25522 Kapawi Lodge Pastaza EC
CCS11
QCAZ 54658 Parque Nacional Yasuní Orellana EC
QCAZ 42409 Santa Rosa Napo EC
CCS9
0.75
QCAZ 55188 Oglán Pastaza EC
UCS6
QCAZ 38989 Kurintza Pastaza EC
CCS12
QCAZ 42479 Wisui Morona Santiago EC
QCAZ 52616 Puerto Morona Morona Santiago EC
CCS11
QCAZ 29424 Mariscal Sucre Pastaza EC
QCAZ 35373 Cando Napo EC
QCAZ 25753 Jatun Sacha Napo EC
CCS13
QCAZ 32291 Estación Científica Yasuní Orellana EC
UCS6
QCAZ 25320 Cando Napo EC
QCAZ 35372 Cando Napo EC
QCAZ 53347 Payamino Orellana EC
CCS13
0.67
56
0.99
QCAZ 25385 La Selva Lodge Orellana EC
QCAZ 25387 La Selva Lodge Orellana EC
CCS9
74
0.99
0.0080

Figure S2. Nuclear phylogeny of South American *Bolitoglossa*. Maximum likelihood tree, an analysis obtained one nuclear gene (*Rag1*). Numbers above branches correspond to ML bootstrap support, values <50 are not shown. Numbers below branches correspond to Bayesian posterior probabilities, values <0.5 are not shown. The voucher number and the locality are shown in each terminal. Clades are delimitated by square brackets. Names in red correspond to species for which we produced new sequences. Abbreviations of the localities: CR−Costa Rica; PA−Panama; EC−Ecuador; CO−Colombia; VE−Venezuela; PE−Peru.


Plethodon cinereus
Desmognathus fuscus
Hydromantes brunus
Hemidactylium scutatum
Eurycea bislineata
Batrachoseps attenuatus
Oedipina taylori
Pseudoeurycea galeanae
Parvimolge townsendi
Ixalotriton niger
Pseudoeurycea rex
B. hartwegi
B. nympha
B. occidentalis
B. platydactyla
B. odonelli
B. alberchi
B. striatula
B. riletti
B. oaxacensis
B. dofleini
B. alvaradoi
B. engelhardti
B. helmrichi
B. franklini
B. lincolni
B. longissima
B. flavimembris
B. carri
B. dunni
B. cerroensis
B. epimela
B. subpalmata
B. bramei
B. colonnea
B. sombra
B.schizodactyla
B. compacta
B. robusta
S13237
SMF 97131
SMF 97133
MHCH 2660
B. medemi
LSUMZ H-13735
B. caldwellae
QCAZ 32300
CCS1
QCAZ 45254
UCS1
QCAZ 39981
QCAZ 39984
CCS2
MVZ 232943
MHCH 2668
MHCH 2659
B. biseriata
MVZ 163575
B. cf. *sima*
QCAZ 22346
QCAZ 31532
QCAZ 40817
QCAZ 32135
QCAZ 27752
QCAZ 22176
QCAZ 51867
B. sima
QCAZ60029
UCS4
QCAZ 60036
QCAZ 60035
UCS5
QCAZ 55278
QCAZ 55274
QCAZ 5930
QCAZ 37304
QCAZ 28221
CCS8
DFCHB-2730
QCAZ 25443
QCAZ 25449
QCAZ 25448
QCAZ 25450
QCAZ 25777
B. cf. *equatoriana*
QCAZ 41724
CCS4
QCAZ 42383
JCS019
CCS5
INPA 3098
LSUMZ H-3086
B. paraensis
QCAZ 60034
QCAZ 60027
UCS2 *B.* cf. *peruviana*
QCAZ 55712
CCS6
QCAZ 42467
QCAZ 48195
QCAZ 54436
CCS7
QCAZ 56147
QCAZ 60026
QCAZ 53539
QCAZ53501
UCS3
KU 217423
KU 217422
B. palmata
QCAZ 51911
QCAZ 51934
QCAZ 52459
QCAZ 52454
CCS3 *B.* aff. *palmata*
MVZ 158485
B. adspersa
PAG 926
B. nicefori
MCNUP 65
MCNUP 64
MCNUP 63
B. leandrae
CVULAIV-7104
CVULAIV-7100
CVULAIV-7102
CVULAIV-7101
CVULAIV-7103
B. mucuyensis
MC00
CVULAIV-7108
B. orestes
MCNUP 53
MCNUP 51
MCNUP 57
MCNUP 56
B. tamaense
QCAZ 28404
QCAZ 25268
QCAZ 25592
QCAZ 43906
QCAZ 54657
QCAZ 54658
QCAZ 42409
QCAZ 25421
QCAZ 25386
QCAZ 25425
QCAZ 25426
QCAZ 25387
QCAZ 25420
QCAZ 25385
QCAZ 25422
QCAZ 35399
CCS9
QCAZ 25795
QCAZ 25784
QCAZ 25794
QCAZ 25793
CCS10
QCAZ 25467
QCAZ 25522
QCAZ 25710
QCAZ 42479
QCAZ 53246
QCAZ 52616
CCS11
KU 222111
QCAZ 38739
QCAZ 56556
QCAZ 55138
QCAZ 55188
QCAZ 51131
QCAZ 20845
QCAZ 32291
QCAZ 54656
UCS6
QCAZ 52804
QCAZ 56379
QCAZ 38989
CCS12
QCAZ 53347
QCAZ 53356
QCAZ 25758
QCAZ 25455
QCAZ 25771
QCAZ 25289
QCAZ 25753
KU 217421
QCAZ 29424
QCAZ 25355
QCAZ 25593
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QCAZ 25320
QCAZ 35372
QCAZ 35373
QCAZ 54218
QCAZ 25319
QCAZ 25318
CCS13
130
120
110
100
90
80
70
60
50
40
30
20
10
0
MYA

Figure S3. Bayesian chronogram with 95% highest posterior density for Plethodontidae. The analysis is based on *16S*, *Cytb* and *Rag1*. All the sequences used are shown. The numbers above each branch are posterior probabilities of the relationships. The bars are the height of the 95% highest posterior density (HPD). The analysis was performed in BEAST by calibrating the base of the crown group Plethodontidae at 129 MYA (according to Mueller, 2006).

Fig. S4. Principal component analysis of morphological variables. The analyses were made using 12 size-corrected variables for A (CCS1−8, UCS1,2, *B. sima*, and *B.* cf. *equatoriana*) and B (CCS9−13 and UCS6). See table S4 for character loadings on each component.

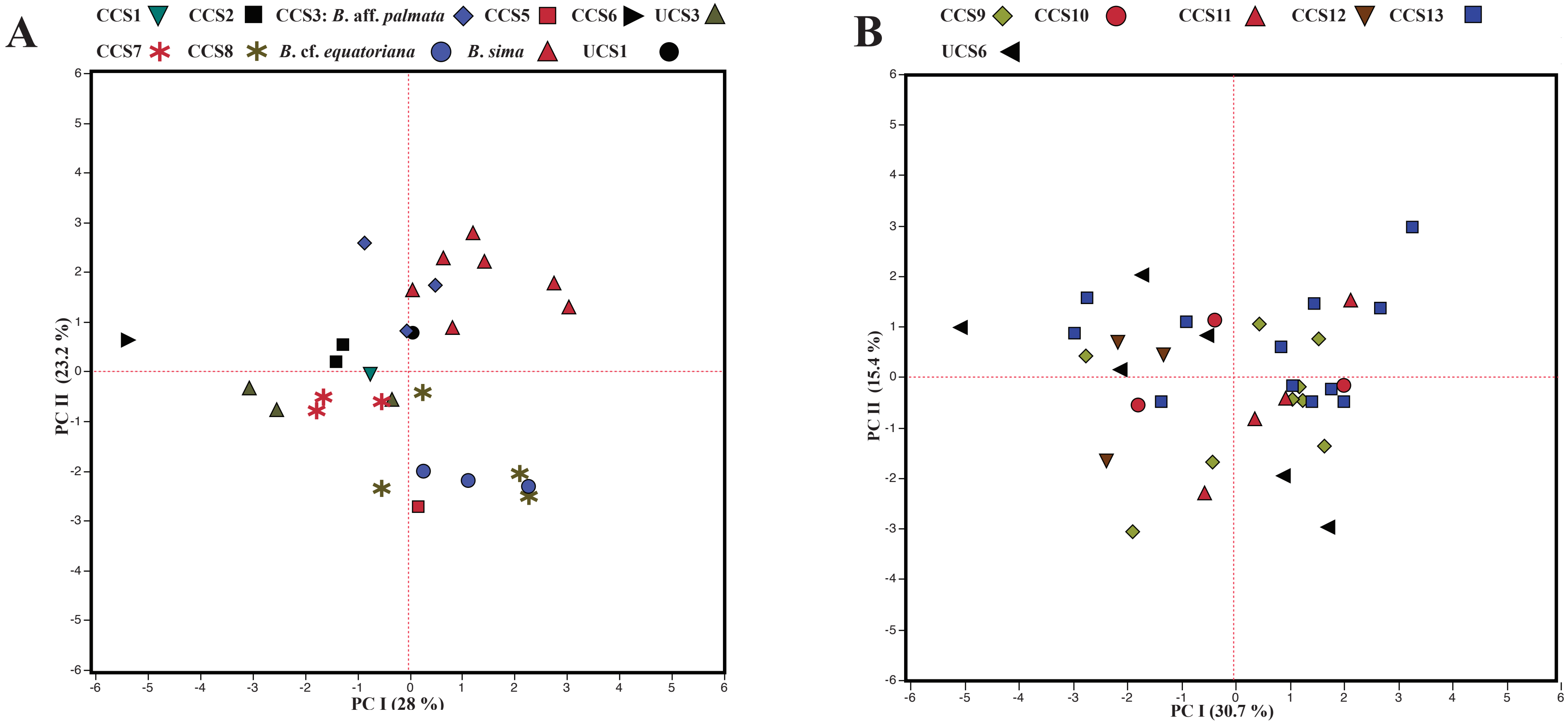

Table S1. Sample information: locality information, altitude and geographical coordinates. All institutional abbreviations are listed in Table 1.

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
B. adspersa	MVZ 158485	Colombia: Cundinamarca	2400	5,35	-73,83
B. biseriata	MVZ 232943 (S13236)	Panamá: Nusagandi: Kuna Yala	250		
B. biseriata	MHCH 2668	Panamá: Darién	306		
B. biseriata	MHCH 2659	Panamá: Darién	306		
B. caldwellae	LSUMZ H-13735	Brazil: Acre: 5 km N Porto Walter	215		
B. cf. *sima*	MVZ 163575	Colombia: Valle del Cauca	130	3,70	-77,00
B. leandrae	MCNUP 63	Colombia: Norte de Santander: San Antonio	600	7,15	-72,23
B. leandrae	MCNUP 64	Colombia: Norte de Santander: San Antonio	600	7,15	-72,23
B. leandrae	MCNUP 65	Colombia: Norte de Santander: San Antonio	600	7,15	-72,23
B. medemi	S13237	Panamá: Nusagandi: Kuna Yala	400		
B. medemi	SMF 97133	Panamá: Darién	344	9,06	–77.9796
B. medemi	SMF 97131	Panamá: Serranía de San Blas	340	9,06	–77.9797
B. medemi	MHCH 2660	Panamá: Darién	859	8,48	–77.5194
B. mucuyensis	CVULAIV-7100	Venezuela: Mérida: La Mucuy	2225		
B. mucuyensis	CVULAIV-7101	Venezuela: Mérida: La Mucuy	2225		
B. mucuyensis	CVULAIV-7102	Venezuela: Mérida: La Mucuy	2225		
B. mucuyensis	CVULAIV-7103	Venezuela: Mérida: La Mucuy	2225		
B. mucuyensis	CVULAIV-7104	Venezuela: Mérida: La Mucuy	2225		
B. nicefori	PAG 926	Colombia: Boyacá: Arcabuco	1550		
B. orestes	No voucher (Isolate MC00)	Venezuela: Mérida: Macho Capaz	2500		

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
B. orestes	CVULAIV-7108	Venezuela: Mérida: San Javier	2500		
B. palmata	KU 217422	Ecuador: Napo: Guacamayos at 31 km from Baeza	2200	-0,63	-77,81
B. palmata	KU 217423	Ecuador: Napo: Guacamayos at 31 km from Baeza	2200	-0,63	-77,81
B. paraensis	INPA 3098	Brazil: Amazonas: Rio Juruá	20		
B. paraensis	LSUMZ H-3086	Brazil: Amazonas: Rio Ituxi at the Madeireira Scheffer	20	-6,47	-68,77
B. sima	QCAZ 22176	Ecuador: Esmeraldas: Durango	253	1,04	-78,62
B. sima	QCAZ 22346	Ecuador: Esmeraldas: La Tola	31	1,21	-79,04
B. sima	QCAZ 27752	Ecuador: Esmeraldas: Alto Tambo (Río Balthazar)	645	0,91	-78,62
B. sima	QCAZ 31532	Ecuador: Esmeraldas: Reserva Ecológica Bilsa	500	0,34	-79,72
B. sima	QCAZ 32135	Ecuador: Imbabura: 13 km E Durango	85	1,09	-78,70
B. sima	QCAZ 40817	Ecuador: Imbabura: El Achotero (5 km E Lita)	729	0,85	-78,42
B. sima	QCAZ 51867	Ecuador: Esmeraldas: Durango	252	1,04	-78,62
B. tamaense	MCNUP 51	Colombia: Norte de Santander: La Asiria	2700	7,32	-72,37
B. tamaense	MCNUP 53	Colombia: Norte de Santander: La Asiria	2700	7,32	-72,37
B. tamaense	MCNUP 56	Colombia: Norte de Santander: Los Remansos	2000	7,33	-72,48
B. tamaense	MCNUP 57	Colombia: Norte de Santander: Los Remansos	2000	7,33	-72,48

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
CCS1	QCAZ 32300	Ecuador: Imbabura: Bosque Protector Los Cedros	2200	0,3	-78,77
CCS2	QCAZ 39981	Ecuador: Carchi: Chilma Bajo	2073	0,86	-78,05
CCS2	QCAZ 39984	Ecuador: Carchi: Chilma Bajo	2073	0,86	-78,05
CCS3 *B*. aff. *palmata*	QCAZ 51911	Ecuador: Tungurahua: (Río Verde)	1600	-1,40	-78,30
CCS3 *B*. aff. *palmata*	QCAZ 51934	Ecuador: Tungurahua: (Río Verde)	1600	-1,40	-78,30
CCS3 *B*. aff. *palmata*	QCAZ 52454	Ecuador: Tungurahua: (Río Verde)	1600	-1,38	-78,15
CCS3 *B*. aff. *palmata*	QCAZ 52459	Ecuador: Tungurahua: (Río Verde)	1600	-1,38	-78,15
CCS4	QCAZ 41724	Ecuador: Zamora Chinchipe: Zurmi (Las Orquídeas)	1820	-4,26	-78,68
CCS5	JCS 019	Ecuador: Zamora Chinchipe: Estación Científica San Francisco	1835	-3,97	-79,08
CCS5	QCAZ 42383	Ecuador: Zamora Chinchipe: Chito (Río Sangola)	1857	-4,82	-78,96
CCS6	QCAZ 55712	Colombia: Amazonas: Puerto Nariño	119	-3,78	-70,36
CCS7	QCAZ 42467	Ecuador: Morona Santiago: Wisui (Macas)	698	-2,11	-77,74
CCS7	QCAZ 48195	Ecuador: Morona Santiago: Limón	700	-3,14	-78,40
CCS7	QCAZ 54436	Ecuador: Morona Santiago: Puente La Virgen	396	-2,97	-77,79
CCS8	QCAZ 5930 (LSUMZ H-12838)	Ecuador: Sucumbíos: Cuyabeno Reserve	220	0,00	-76,18

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
CCS8	QCAZ 28221	Ecuador: Sucumbíos: Pto Bolivar (Cuyabeno)	240	-0,09	-76,14
CCS8	QCAZ 37304	Ecuador: Orellana: Tiputini Reserve	218	-0,64	-76,15
CCS8	QCAZ 55274	Ecuador: Orellana: Parque Nacional Yasuní (Tambococha)	177	-0,98	-75,43
CCS8	QCAZ 55278	Ecuador: Orellana: Parque Nacional Yasuní (Tambococha)	177	-0,98	-75,43
CCS9	QCAZ 25268	Ecuador: Orellana: Auca 14 Rd near Coca	255	-0,70	-76,73
CCS9	QCAZ 25385	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25386	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25387	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25420	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25421	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25422	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25425	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25426	Ecuador: Orellana: La Selva Lodge	245	-0,50	-76,37
CCS9	QCAZ 25592	Ecuador: Orellana: Auca 14 Rd near Coca	255	-0,70	-76,73
CCS9	QCAZ 28404	Ecuador: Sucumbíos Monte Tour (Cuyabeno)	290	-0,03	-76,32
CCS9	QCAZ 35399	Ecuador: Sucumbíos: Garzacocha (La Selva Lodge)	245	-0,50	-76,37
CCS9	QCAZ 42409	Ecuador: Napo: Santa Rosa (Río Coca Alto)	632	-0,13	-77,45

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
CCS9	QCAZ 43906	Ecuador: Orellana: El Descanso (Río Napo)	244	-0,43	-76,79
CCS9	QCAZ 54657	Ecuador: Orellana: Parque Nacional Yasuní (Sitio A05)	230	-0,45	-76,62
CCS9	QCAZ 54658	Ecuador: Orellana: Parque Nacional Yasuní (Sitio A45)	246	-0,48	-76,57
CCS10	QCAZ 25784	Ecuador: Sucumbíos: Aguarico	610	0,11	-77,37
CCS10	QCAZ 25793	Ecuador: Sucumbíos: Aguarico	610	0,11	-77,37
CCS10	QCAZ 25794	Ecuador: Sucumbíos: Aguarico	610	0,11	-77,37
CCS10	QCAZ 25795	Ecuador: Sucumbíos: Aguarico	610	0,11	-77,37
CCS11	QCAZ 25467	Ecuador: Pastaza: Kapawi Lodge	239	-2,54	-76,86
CCS11	QCAZ 25522	Ecuador: Pastaza: Kapawi Lodge	239	-2,54	-76,86
CCS11	QCAZ 42479	Ecuador: Morona Santiago: Wisui (Macas)	1045	-2,11	-77,76
CCS11	QCAZ 52616	Ecuador: Morona Santiago: Km 156 Puerto Morona Rd	208	-2,89	-77,68
CCS11	QCAZ 53246	Ecuador: Morona Santiago: Tukupi	218	-2,80	-77,48
CCS11	QCAZ 25710	Ecuador: Pastaza: Kapawi Lodge	239	-2,54	-76,86
CCS12	QCAZ 38989	Ecuador: Pastaza: Kurintza (Campo Villano, Bloque 10-Agip Oil)	362	-1,51	-77,51
CCS12	QCAZ 52804	Ecuador: Pastaza: Sarayacu	429	-1,73	-77,48
CCS12	QCAZ 56379	Ecuador: Pastaza: Kurintza (Campo Villano, Bloque 10-Agip Oil)	281	-1,51	-77,51
CCS13	KU 217421	Ecuador: Napo: Estación Biológica Jatun Sacha	420		

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
CCS13	QCAZ 25289	Ecuador: Napo: Estación Biológica Jatun Sacha (Inner Vision Lodge)	408	-1,10	-77,59
CCS13	QCAZ 25294	Ecuador: Napo: Estación Biológica Jatun Sacha (Inner Vision Lodge)	396	-1,10	-77,59
CCS13	QCAZ 25317	Ecuador: Napo: Cando	685	-1,10	-77,92
CCS13	QCAZ 25318	Ecuador: Napo: Cando	685	-1,10	-77,92
CCS13	QCAZ 25319	Ecuador: Napo: Cando	685	-1,10	-77,92
CCS13	QCAZ 25320	Ecuador: Napo: Cando	685	-1,10	-77,92
CCS13	QCAZ 25355	Ecuador: Pastaza: Sta Clara (finca de Tapia)	690	-1,35	-77,86
CCS13	QCAZ 25455	Ecuador: Napo: Estación Biológica Jatun Sacha	412	-1,08	-77,61
CCS13	QCAZ 25593	Ecuador: Orellana: Auca 14 Rd near Coca	255	-0,70	-76,73
CCS13	QCAZ 25747	Ecuador: Napo: Estación Biológica Jatun Sacha	450	-1,08	-77,61
CCS13	QCAZ 25753	Ecuador: Napo: Estación Biológica Jatun Sacha (Inner Vision Lodge)	431	-1,10	-77,60
CCS13	QCAZ 25758	Ecuador: Napo: Estación Biológica Jatun Sacha (Inner Vision Lodge)	410	-1,10	-77,59
CCS13	QCAZ 25771	Ecuador: Napo: Estación Biológica Jatun Sacha (Inner Vision Lodge)	417	-1,10	-77,60
CCS13	QCAZ 25872	Ecuador: Napo: Cando (Serena North side)	516	-1,09	-77,93
CCS13	QCAZ 29424	Ecuador: Pastaza: Colonia Mariscal Sucre	957	-1,36	-77,86
CCS13	QCAZ 35372	Ecuador: Napo: Cando (Pimpilala)	2650	-1,07	-77,93

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
CCS13	QCAZ 35373	Ecuador: Napo: Cando (Pimpilala)	2650	-1,07	-77,93
CCS13	QCAZ 53347	Ecuador: Orellana: Payamino	298	-0,48	-77,29
CCS13	QCAZ 53356	Ecuador: Orellana: Payamino	298	-0,48	-77,29
CCS13	QCAZ 54218	Ecuador: Pastaza: Kurintza (Campo Villano, Bloque 10-Agip Oil)	299	-2,06	-76,80
CCS13	QCAZ 57198	Ecuador: Napo: Cotundo (Huasquila Lodge)	804	-0,82	-77,80
UCS1	QCAZ 45254	Ecuador: Santo Domingo de los Tsáchilas: 6,9 km S Santo Domingo	432	-0,19	-79,13
UCS2 *B.* cf. *peruviana*	QCAZ 60027 (CORBIDI 6332)	Perú: Lamas: Pinto Recodo-Cerro Escalera (Río Mayo)	688	-6,25	-76,66
UCS2 *B.* cf. *peruviana*	QCAZ 60034 (CORBIDI 9206)	Perú: Picota: Chambirillo (Cordillera Azul)	1122	-7,07	-76,02
UCS3	QCAZ 53501	Ecuador: Pastaza: Juyuintza	200	-2,11	-76,19
UCS3	QCAZ 53539	Ecuador: Pastaza: Juyuintza	164	-2,11	-76,16
UCS3	QCAZ 56147	Ecuador: Pastaza: Lorocachi	200	-1,60	-76,00
UCS3	QCAZ 60026 (CORBIDI 4666)	Perú: Loreto: Andoas	187	-2,35	-75,82
UCS4	QCAZ 60029 (CORBIDI 10709)	Perú: La Convención: Bajo Poyentimari	1103	-12,21	-73,01
UCS5	QCAZ 60035 (CORBIDI 15488)	Perú: Leoncio Prado: Parque Nacional Tingo María (3 de Mayo)	948	-9,42	-75,99
UCS5	QCAZ 60036 (CORBIDI 15489)	Perú: Leoncio Prado: Parque Nacional Tingo María (3 de Mayo)	948	-9,42	-75,99
B. cf. *equatoriana*	DFCH-2730	Ecuador: Napo: Estación Biológica Jatun Sacha	420		

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
B. cf. *equatoriana*	QCAZ 25443	Ecuador: Napo: Estación Biológica Jatun Sacha	419	-1,08	-77,61
B. cf. *equatoriana*	QCAZ 25448	Ecuador: Napo: Estación Biológica Jatun Sacha	412	-1,08	-77,61
B. cf. *equatoriana*	QCAZ 25449	Ecuador: Napo: Estación Biológica Jatun Sacha	412	-1,08	-77,61
B. cf. *equatoriana*	QCAZ 25450	Ecuador: Napo: Estación Biológica Jatun Sacha	412	-1,08	-77,61
B. cf. *equatoriana*	QCAZ 25777	Ecuador: Napo: Estación Biológica Jatun Sacha (Inner Vision Lodge)	412	-1,10	-77,60
UCS6	KU 222111	Perú: Loreto: 1.5 km N Teniente López	187		
UCS6	QCAZ 20845	Ecuador: Orellana: Estación Científica Yasuní (PUCE)	240	-0,68	-76,40
UCS6	QCAZ 32291	Ecuador: Orellana: Estación Científica Yasuní (PUCE)	235	-0,67	-76,40
UCS6	QCAZ 38739	Ecuador: Pastaza: Villano (Campamento K4, Bloque 10-Agip Oil)	352	-1,47	-77,49
UCS6	QCAZ 51131	Ecuador Orellana: Parque Nacional Yasuní (km 66 Pompeya Rd)	270	-0,81	-76,40
UCS6	QCAZ 54656	Ecuador: Orellana: Parque Nacional Yasuní (Transecto BOR)	232	-0,67	-76,44
UCS6	QCAZ 55138	Ecuador: Pastaza: Oglán (Bloque 10-Agip Oil)	817	-1,35	-77,67
UCS6	QCAZ 55188	Ecuador: Pastaza: Oglán (Bloque 10-Agip Oil)	817	-1,35	-77,67

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
UCS6	QCAZ 56556	Ecuador: Pastaza: Tarangaro (Campo Villano, Bloque 10-Agip Oil)	349	-1,39	-77,39
B. alberchi	MVZ 264191	Mexico: Chiapas: Armando Zebadua, 23.2 km N Ocozocoautla	924		
B. alvaradoi	MVZ 215735	Costa Rica: Heredia: El Plástico	370		
B. bramei	UCR 20483	Costa Rica: Coordillera de Talamanca	2100		
B. carri	USNM 523277	Honduras: Cerro Cantagallo	1955		
B. cerroensis	MVZ S 12921	Costa Rica: San José: Salsipuedes	2740		
B. colonnea	No voucher	Panamá: Chiriquí: Reserva Forestal Fortuna	1200		
B. compacta	UCR 20532	Costa Rica: Coordillera de Talamanca, Valle del Silencio	2215		
B. dofleini	MVZ 263450	Honduras: Cortés: Aldea Buenos Aires, 18.7 km from Cofradía	1113		
B. dunni	USNM 523280	Honduras: Cortés: San Pedro SulaHonduras: Cortés: San Pedro Sula	1300		
B. engelhardti	MVZ 251495	Guatemala: Depto. San Marcos: Finca Insula, km 263 on RN1	2057		
B. epimela	MVZ 181260	Costa Rica: Cartago: Turrialba	915		
B. flavimembris	MVZ 177786	Mexico Chiapas: Volcán Tacaná	1850		
B. franklini	MVZ 185991	Mexico: Chiapas: Volcán Tacaná	1885		
B. hartwegi	MVZ 263458	Mexico: Chiapas: Zoológico San José, 5.5 km W San Cristobal de las Casas	2260		

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
B. helmrichi	MVZ 257804	Guatemala Depto. Zacapa: Finca Planada de Margot	1647		
B. lincolni	MVZ 143564	Guatemala: San Marcos	2377		
B. longissima	USNM 523285	Honduras: Olancho: Pico La Picucha	1900		
B. nympha	MVZ 257812	Guatemala: Zacapa: 5.2km SE by road from center of La Union	1000		
B. oaxacensis	IBH 13374	México: Oaxaca: 40 km N San Gabriel Mixtepec	1920		
B. occidentalis	MVZ 194254	México: Chiapas: Berriozabal	1100		
B. odonnelli	MVZ 229068	Honduras: Olancho	480		
B. platydactyla	GP 108	México: Veracruz	1000		
B. riletti	MVZ 194328	México: Oaxaca: 20.9 km NE Putla	1030		
B. robusta	MVZ 190830	Costa Rica: Alajuela: Salto El Angel	1360		
B. schizodactyla	No voucher	Panamá: Coclé: Parque Nacional El Copé	425		
B. sombra	MVZ 225875	Costa Rica: Puntarenas, Aquaduct Trail along Rio Coton, below Las Tablas	1760		
B. striatula	MVZ 181280	Costa Rica: Cartago	600		
B. subpalmata	MVZ 229172	Costa Rica: Puntarenas: Monteverde	1500		
Outgroup					
Batrachoseps attenuatus	MVZ 230761	United States: Oregon: Winchuck Campground	45,72		

Species	Voucher	Locality	Altitud	Geographical Coordinates	
				Latitude	Longitude
Desmognathus fuscus	MVZ 224931	United States: Massachusetts, S. Shelbourne Rd., Smead Brook, vicinity Greenfield; Franklin Co.	202		
Eurycea bislineata	MVZ 225074	United States: Pennsylvania, Hauger Rd., ca. 0.2-0.5 mi W junction Four Mile Run Rd., vicinity Donegal; Westmoreland Co.	556		
Hemidactylium scutatum	MVZ 225077	United States: New York, Clarence Fahnestack State Park, vicinity Pelton Pond Natural Area; Putnam Co.	321		
Hydromantes brunus	MVZ 230641	United States: California, Mariposa: 0.6 mi NE (by road) Briceburg	575		
Ixalotriton niger	IBH 29715	Mexico: Chiapas: Cerro Baúl	1100		
Oedipina taylori	MVZ 267200	Honduras: Francisco Morazán: near Sabana Grande	912		
Parvimolge townsendi	CARIE 1174	Mexico: Puebla: Hueyapan; Veracruz: Rancho San Gabriel, Teocelo	1300		
Plethodon cinereus	MVZ 225101	United States: Pennsylvania, State Game Lands No. 122, Centerville Rd.; Crawford Co.	399		
Pseudoeurycea galeanae	IBH 24595	Mexico: Nuevo León: Puerto de Cieneguillas	2526		

Species	**Voucher**	**Locality**	**Altitud**	**Geographical Coordinates**	
				Latitude	**Longitude**
Pseudoeurycea rex	MVZ 263590	Guatemala: San Marcos: 3 km E Flores de Ixchiguán	3240		

Table S2. Quantitative morphological measurements. Clades UCS2, 4-5 were not included because we did not have access to the specimens. Mean ± SD is given along with the range in parenthesis (only when n>2). Sample size is specified unless n=1. Abbreviations are: Premaxillary teeth (PTM); Maxillary teeth (MT); Vomerine teeth (VT); Snout-vent length (SVL); Distance from the gular fold to the tip of the snout (SG); Snout width (SW); Head width at the greatest width of the head (HW); Head depth at the posterior angle of the jaw (HD); Jaw length (JL); Body width at the level of the axilla (BW); Right forearm length (RFL); Right thigh length (RTL); Right leg length (RLL); Right foot width (RFW); Right foot length (RFL); Axilla-going distance (AX). All measurements are in mm.

	CCS1	**UCS1**	**CCS2**		***B. sima***		**CCS3: *B.* aff. *palmata***		**CCS5**	**CCS6**	**CCS7**
Variable	**Female**	**Male**	**Female**	**Male**	**Female n=5**	**Male n=2**	**Female n=2**	**Male**	**Female**	**Female**	**Female n=3**
PMT	0	0	0	0	0−2	1	3	3	1	3	0−1
MT	14	2	4	1	40−48	19−26	53−54	37	33	39	9−25
VT	16	15	18	18	12−26	15−20	26−33	27	25	23	10−16
SVL	50.62	28.53	41.67	38.15	44.68 ± 4.11 (39.85−47.94)	28.41−32.58	46.81−51.31	39.92	53.58	49.35	34.65 ± 3.2 (32.06−38.23)
SG	10.65	7.78	10.05	8.83	10.99 ± 1.3 (9.51−12.74)	7.02−8	11.03−12.29	9.82	12.39	10.26	7.96 ± 0.65 (7.26−8.56)
HW	6.47	3.78	5.07	4.68	6.63 ± 0.7 (5.65−7.42)	4.54−4.87	6.07−6.22	5.52	7.54	5.61	4.63 ± 0.3 (4.36−4.57)
HD	3.60	2.29	2.90	2.51	2.83 ± 0.35 (2.46−3.29)	2.34−2.58	3.4−3.57	3.02	3.85	2.71	2.47 ± 0.11 (2.35−2.54)
JL	5.66	3.57	4.90	4.62	5.14 ± 0.63 (4.29−5.77)	3.85−3.96	6.22−7.38	4.91	5.25	5.17	4.3 ± 0.46 (3.77−4.63)
BW	6.13	2.97	5.13	3.85	5.5 ± 0.59 (5.45−5.89)	3.27−3.69	5.64−5.73	4.14	5.84	4.27	4.14 ± 0.96 (3.58−5.24)
RFL	4.03	2.68	3.03	2.80	3.66 ± 0.2 (3.5−3.93)	2.19−2.77	3.81−3.88	3.70	5.01	3.87	3.04 ± 0.23 (2.9−3.31)
RTL	5.72	3.94	4.91	5.21	5.97 ± 0.5 (5.44−6.52)	3.95−4.52	5.49−5.83	4.62	6.73	4.47	4.53 ± 0.38 (4.14−4.89)
RLL	4.52	2.60	3.59	3.65	4.34 ± 0.24 (4.01−4.59)	2.48−2.5	4.22−4.62	3.64	5.02	3.76	2.99 ± 0.67 (2.41−3.72)
RFW	4.40	2.45	3.48	2.92	3.95 ± 0.49 (3.17−4.32)	2.43−2.51	4.33−4.68	3.33	4.02	3.69	2.31 ± 0.38 (2.07−2.75)
RFL	5.41	2.64	3.58	2.92	4.49 ± 0.52 (3.69−4.92)	2.75−3.31	4.55−5.59	4.14	5.26	3.77	2.37 ± 0.24 (2.11−2.59)
AX	28.69	15.53	23.64	22.62	27.33 ± 2.99 (23.5−30.28)	17.93−19.7	26.86−28.36	21.85	27.73	28.18	18.97 ± 2.31 (17.25−21.6)

	UCS3		**CCS8**		***B.* cf. *equatoriana***		**CCS9**		**CCS10**	
Variable	**Female n=2**	**Male**	**Female**	**Male n=2**	**Female n=2**	**Male**	**Female n=9**	**Male n=4**	**Female n=3**	**Male**
PMT	0−2	0	1	1	1	0	0−2	0−1	1	1
MT	27−34	12	25	12−13	29	14	10−31	10−24	20−25	13
VT	9−16	11	19	10−15	28−29	12	8−20	10−17	13−14	10
SVL	39.11−42.54	36.54	49.26	28.43−29.5	46.76−48.37	32.86	32.57 ± 2.26 (29.6−35.42)	26.66 ± 2.75 (23.86−29.91)	30.97 ± 1.98 (28.72−32.57)	29.36
SG	8.61−9.5	8.53	10.28	7.82−9.85	10.44−11.84	5.84	7.45 ± 0.52 (6.58−8.21)	6.75 ± 0.72 (5.93−7.43)	7.17 ± 0.49 (6.75−7.7)	7.69
HW	4.43−4.67	4.80	6.80	4.69−5.79	7.71−7.79	5.29	4.34 ± 0.43 (3.52−4.9)	3.94 ± 0.28 (3.65−4.31)	4.4 ± 0.07 (4.32−4.47)	4.92
HD	2.49−4.25	2.61	4.17	2.52−3.04	3.65−3.88	3.34	2.46 ± 0.11 (2.27−2.59)	2.29 ± 0.26 (2.07−2.66)	2.28 ± 0.03 (2.25−2.31)	2.28
JL	5.29−6	5.21	5.36	4.38−4.8	5.24−5.54	4.61	4.09 ± 0.39 (3.55−4.72)	3.65 ± 0.85 (2.59−4.48)	3.93 ± 0.13 (3.81−4.06)	4.03
BW	3.8−4.4	3.68	6.16	3.4−4.75	5.99−6.43	4.17	3.69 ± 0.31 (3.14−4.18)	2.53−3.13	3.75 ± 0.36 (3.47−4.14)	3.10
RFL	3.59−3.84	3.64	4.21	2.55−4.5	4.34−4.44	2.80	2.98 ± 0.29 (2.57−3.35)	2.34 ± 0.37 (2.09−2.88)	2.81 ± 0.22 (2.56−2.97)	2.68
RTL	3.87−5.18	4.95	6.05	4.04−6.27	6.45−6.55	4.85	4.03 ± 0.52 (3.21−4.76)	3.56 ± 0.45 (2.92−3.99)	3.64 ± 0.68 (2.85−4.01)	4.11
RLL	3.57−3.65	3.55	4.49	2.54−4.15	3.97−4.95	3.40	2.99 ± 0.33 (2.59−3.44)	2.35 ± 0.27 (2−2.66)	3.31 ± 0.68 (2.83−4.09)	2.76
RFW	2.74−2.75	2.70	3.69	1.78−3.07	3.40−3.36	2.47	2.4 ± 0.25 (1.88−2.74)	2.09 ± 0.37 (1.84−2.63)	2.18 ± 0.24 (1.91−2.37)	2.46
RFL	3.17−3.37	3.50	4.41	2.21−2.93	4.27−4.36	2.68	2.79 ± 0.29 (2.36−3.18)	2.27 ± 0.35 (2.02−2.79)	2.4 ± 0.16 (2.27−2.57)	2.30
AX	21.95−24.06	19.94	26.60	14.15−20.45	25.57−26.76	16.15	18.97 ± 1.33 (17.2−21.37)	15.27 ± 1.94 (13.36−17.97)	18.27 ± 0.82 (17.33−18.87)	17.24

	CCS11		CCS12	CCS13		UCS6	
Variable	**Female n=4**	**Male**	**Female n=3**	**Female n=12**	**Male n=6**	**Female n=7**	**Male**
PMT	0−4	2	1−2	0−1	1−2	0−2	2
MT	20−52	33	30−33	20−43	21−43	21−45	21
VT	11−25	18	14−22	10−23	13−29	13−24	17
SVL	38.38 ± 2.93 (38.8−42.37)	31.85	42.2 ± 3.12 (39.42−45.57)	35.86 ± 1.9 (33.2−39.43)	30.54 ± 4.39 (25.16−38.12)	40.39 ± 3.1 (35.31−44.33)	29.63
SG	8.2−8.91	7.04	8.87 ± 0.7 (8.16−9.56)	8.48 ± 0.33 (7.78−8.97)	7.61 ± 0.6 (6.82−8.67)	8.94 ± 0.68 (7.85−9.82)	6.69
HW	4.81 ± 0.52 (4.33−5.54)	3.86	4.98 ± 0.42 (4.58−5.46)	4.86 ± 0.57 (3.68−5.89)	4.31 ± 0.22 (4.14−4.73)	4.99 ± 0.4 (4.36−5.58)	4.24
HD	2.72 ± 0.05 (2.68−2.78)	2.21	2.85 ± 0.17 (2.67−3.01)	2.66 ± 0.16 (2.26−2.85)	2.52 ± 0.26 (2.3−2.88)	2.77 ± 0.31 (2.53−3.38)	2.70
JL	4.31 ± 0.32 (3.95−4.73)	3.73	4.95 ± 1.23 (3.52−5.72)	4.69 ± 0.4 (4.11−5.52)	3.66 ± 0.38 (3.34−4.39)	4.96 ± 0.55 (4.14−5.6)	3.92
BW	4.18 ±0.37 (3.68−4.53)	2.80	4.27 ± 0.48 (3.95−4.83)	3.92 ± 0.31 (3.47−4.37)	3.34 ± 0.25 (3−3.69)	4.11 ± 0.61 (3.23−5.15)	3.40
RFL	3.48 ± 0.72 (2.9−4.51)	2.24	3.46 ± 0.07 (3.39−3.54)	3.2 ± 0.34 (2.47−3.7)	2.83 ± 0.34 (2.47−3.46)	3.47 ± 0.29 (3.1−4.04)	2.66
RTL	5.14 ± 0.63 (4.58−5.96)	3.75	4.35 ± 0.33 (4.01−4.67)	4.48 ± 0.67 (2.79−5.47)	4.04 ± 0.36 (3.72−4.57)	4.68 ± 0.72 (3.38−5.37)	3.83
RLL	3.46 ± 0.33 (3.08−3.85)	2.85	3.31 ± 0.85 (2.43−4.12)	3.24 ± 0.24 (2.85−3.73)	2.89 ± 0.5 (2.51−3.54)	3.71 ± 0.39 (3.29−4.23)	2.86
RFW	3.03 ± 0.36 (2.54−3.39)	2.50	3.21 ± 0.35 (2.8−3.43)	2.72 ± 0.28 (2.23−3.05)	2.51 ±0.27 ±2.29−3.04)	3.06 ± 0.38 (2.55−3.72)	2.27
RFL	3.48 ± 0.3 (3.15−3.84)	2.62	3.7 ± 0.38 (3.31−4.07)	3 ± 0.34 (2.49−3.4)	2.76 ± 0.47 (2.29−3.67)	3.69 ± 0.49 (3.03−4.56)	2.83
AX	23.08 ± 1.65 (21.66−25.11)	17.50	24.27 ± 1.15 (23.36−25.56)	21.2 ± 1.38 (18.02−23.13)	17.52 ± 1.53 (16.43−20.58)	23.65 ± 1.8 (21.45−27.11)	15.71

Table S3. Qualitative morphological variation. Clades UCS2, 4-5 were not included because we did not have access to the specimens. Coloration corresponds to preserved specimens. Sample size is specified unless n=1.

Clade	Dorsal coloration	Ventral Coloration	Feet structure	Mental gland (males)	Snout coloration	Feet coloration
CCS1	Dark brown	Light brown	Slightly webbed	–	White from the tip to the eyes	Similar to the ventral coloration
USC1	Light brown with dark brown stripes	Light brown with a lighter middle stripe	Completely webbed	Absent	White from the tip to the eyes	Similar to the ventral coloration
CCS2 n=2	Dark brown with a lighter middle bar	Dark brown	Moderately webbed	Absent	White from the tip to the eyes	Light brown
***B. sima* n=7**	From dark to light brown with darker dots	Light brown with few dark dots	Completely webbed	Absent	Similar to the dorsal coloration	Similar to the ventral coloration
CCS3: *B*. aff. *palmata* n=4	From dark to light brow with spots	Dark brown with lighter stripes or dots	Extensively webbed	Present	White from the tip to the eyes	Light brown
CCS4	Light brown with a darker middle stripe	Light brown extensively white-spotted	Completely webbed	–	White from the tip to the eyes	Similar to the ventral coloration
CCS5	Dark brown with darker spots	Brown extensively white-spotted	Extensively webbed	Present	White from the tip to the eyes	Similar to the ventral coloration
CCS6	Dark brown	Dark brown with white dots	Completely webbed	–	White contour	Light brown
CCS7 n=3	Dark brown	Dark brown with a darker middle stripe	Completely webbed	–	Similar to the dorsal coloration	Similar to the ventral coloration
UCS3 n=3	Dark grey uniformly or with white stripes	Dark brown with white dots	Completely webbed	Present	White from the tip to the eyes	Similar to the ventral coloration
CCS8 n=4	From dark brown to dark grey	Dark brown with white dots	Completely webbed	Present	Similar to the dorsal coloration	Similar to the ventral coloration

Clade	**Dorsal coloration**	**Ventral Coloration**	**Feet structure**	**Mental gland (males)**	**Snout coloration**	**Feet coloration**
***B.* cf. *equatoriana* n=5**	From dark brown to dark grey	Dark brown extensively white-spotted	Completely webbed	Absent	Similar to the dorsal coloration	Similar to the ventral coloration
CCS9 n=16	From dark brown to dark grey uniformly or with white stripes	From brown to dark brown uniformly or with a white middle stripe	Completely webbed	Present	Similar to the dorsal coloration	Light brown
CCS10 n=5	Dark brown	Dark brown uniformly or with white dots	Completely webbed	Present	White contour	Light brown
CCS11 n=5	From dark brown to dark grey with lighter bars at the sides	From brown to dark brown uniformly or with white dots	Completely webbed	Absent	White from the tip to the eyes	Similar to the ventral coloration
CCS12 n=3	Dark brown	Dark brown	Completely webbed	–	White from the tip to the eyes	Similar to the ventral coloration
CCS13 n=19	Dark brown uniformly or with white stripes	Dark brown uniformly or with white dots	Completely webbed	Present	Similar to the dorsal coloration	Similar to the ventral coloration
UCS6 n=8	From dark brown to dark grey	Dark brown with white dots	Extensively webbed	Absent	White contour	Light brown

Table S4. Character loadings, eigenvalues, and percentage of explained variance for Principal Components I–IV. The analyses were made using 12 size-corrected variables for adults of A (CCS1−8, UCS1,2, *B. sima*, and *B.* cf. *equatoriana*) and B (CCS9−13 and UCS6). Bold figures indicate highest loadings.

Variable	**A**				**B**			
	PCI	**PCII**	**PCII**	**PCIV**	**PCI**	**PCII**	**PCIII**	**PCIV**
MT	0.096	0.343	0.221	0.173	0.009	**0.502**	-0.112	0.208
SG	0.300	0.221	-0.131	**0.428**	0.347	0.376	0.238	0.068
HW	**0.455**	-0.126	-0.089	-0.203	**0.425**	0.132	0.128	-0.206
HD	0.073	-0.291	**0.540**	-0.359	0.352	-0.115	-0.325	-0.317
JL	-0.052	0.133	**0.617**	0.190	-0.043	**0.517**	-0.114	0.354
BW	0.381	-0.054	0.004	**-0.500**	0.228	0.225	**0.529**	-0.166
RFL	0.184	**-0.410**	0.056	**0.502**	0.024	-0.244	0.230	**0.565**
RTL	**0.451**	-0.182	-0.186	0.052	**0.418**	-0.025	-0.068	-0.170
RLL	0.376	-0.167	0.041	0.173	-0.097	-0.181	**0.653**	-0.110
RFL	0.230	**0.453**	0.172	-0.125	0.337	-0.112	0.039	**0.447**
RFW	0.319	0.259	0.299	0.107	0.301	-0.385	-0.044	0.298
AX	0.088	**0.459**	-0.309	-0.137	0.363	-0.075	-0.167	0.091
Eigenvalue	3.355	2.783	1.435	1.150	3.688	1.849	1.397	1.250
%	27.957	23.189	11.962	9.585	30.731	15.406	11.641	10.416

DECLARACION Y AUTORIZACION

Yo, Francisca Hervas Sotomayor, CI. 1714914502 autora del trabajo de graduación intitulado: "Cryptic diversity and biogeography of South American salamanders", previa a la obtención del grado académico de **MAGISTER EN BIOLOGIA DE LA CONSERVACION** en la **Facultad de Ciencias Exactas y Naturales**:

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Francisca Hervas Sotomayor
1714914502