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PHYLOGENETIC ANALYSES OF DNA SEQUENCES REVEAL A VASTLY UNDERESTIMATED RADIATION OF AMAZONIAN SALAMANDERS (PLETHODONTIDAE: BOLITOGLOSSA), WITH KEY IMPLICATIONS TO THE STUDY OF PLETHODONTID DIVERSIFICATION

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Resumo

O reconhecimento da biodiversidade é um dos desafios mais gratificantes na biologia, porque quando mais conhecemos sob as coisas que nos rodeiam mais entendemos os processos e mudam nossa percepção de ver as coisas. As salamandras da família Plethodontidae sofreram processos complexos de especiação onde principalmente tem diversificado em América do Norte e Mesoamerica, resultando no 66 % da diversidade de Caudata. Por outro lado, as salamandras da América do Sul albergam uma pequena diversidade (37 espécies) onde a maioria pertence ao gênero Bolitoglossa, este padrão é o resultado de uma colonização tardia onde as espécies não tiverem o suficiente tempo para especiar. Não obstante, trabalhos recentes sugerem que a diversidade de salamandras na América do Sul está sendo subestimada, refletindo nosso conhecimento e ignorância deste grupo. Neste contexto, nós estudamos as salamandras amazônicas fornecendo uma explicação filogenética da variação molecular de diferentes amostras de Bolitoglossa no contexto da radiação do gênero, e avaliar as implicações destes resultados no entendimento da diversidade de espécies de Bolitoglossa na Amazônia, como também sua diversificação e biogeografia. Foram obtidos dados de oito das nove espécies distribuídas na Amazônia seja perto e/ou da localidade tipo, além disso, foram geradas novas sequencias para 177 terminais da maioria das populações amazônicas desde Venezuela até Bolívia, e as analisamos junto com as sequencias disponíveis em bases de dados públicas. Nosso estudo representa a amostragem mais completa de taxa do gênero (~ 75 % da diversidade atual) para avaliar as relações de Bolitoglossa. Os dados foram analisados usando máxima verossimilhança com um alinhamento por similaridade e incluindo os indels como caráter binário e com parcimônia com homologia dinâmica e indels como quinto estado de caráter. Apesar de ambas as análises mostrarem diferenças nas topologias ótimas, os resultados são

incompatíveis com a presença de só 9 espécies de Bolitoglossa na bacia amazônica (a diversidade atualmente reconhecida). Usando métodos objetivos de delimitação de espécies, calculamos um aumento da riqueza de espécies de 300–400 %. A reconstrução de áreas ancestrais em ambas as topologias indica que uma única colonização da Amazônia desde os Andes é responsável da grande radiação de salamandras em América do Sul. Nossos resultados mudam o paradigma atual sobre a diversificação das salamandras neotropicais.

Palavras chaves: neotropico, especiação, diversidade críptica, delimitação de especies, biogeografia, anfíbios.

Abstract

The recognition of biodiversity is one of the most compelling challenges in biology, because more we know about the things that surrounding more understand about the processes and change our perspective to see the things. Plethodontid salamanders suffer a complex speciation process which radiate mainly in North America and Mesoamerica resulting in 66% of Caudata diversity. For other hand, South American salamanders harbor a little diversity (37 species) which most of them belong to *Bolitoglassa*, this pattern result by the latter colonization that not allowed to have sufficient time to speciate. However, recent works suggest that South American salamanders diversity are been underestimate, reflecting our knowledge and ignorance of this group. In this context, we study the Amazonian salamanders providing a phylogenetic explanation of molecular variation of different *Bolitoglossa* samples in the context of radiation of the genus, and evaluate the implications of our results to our understanding of *Bolitoglossa* species diversity in the Amazon, as well as its diversification and biogeography. Was obtained data from the eight of the nine species distributed in Amazon from near or type locality, generating new sequences from 177 terminals from most amazon populations between Venezuela and Bolivia, and including those previously published. Our sampling represents one of the most complete for the genera (~ 75 % of diversity) to evaluated the phylogenetic relationship of *Bolitoglossa*. The data was analyzed using Maximum Likelihood with similarity alignment incorporating the indels as binary characters, and Parsimony with dynamic homology and indels as fifth state. The topologies show discordances in the species relationships, but support that the Amazonian salamander diversity are vastly underestimated. Also with the incorporation of species delimitation methods suggest an increase of 300 – 400 % of diversity. The ancestral area reconstruction in both topologies show a unique colonization from Andes to Amazon

and was the responsible of the radiation of South American salamanders. Our results changes the current paradigm about the diversity and diversification of neotropical salamanders.

Key words: neotropics, speciaton, cryptic diversity, species delimitation, biogeography, amphibians.

Comentario

A presente dissertação é parte dos requisitos necessários para a obtenção do título de Mestrado em Ecologia E Evolução Da Biodiversidade, ainda que está disponível publicamente, sem restrições, não deve ser vista como uma publicação no sentido do Código Internacional de Nomenclatura Zoológica, pelo que não constitui um ato valido de nomenclatura (ICZN, Quarta edição, capitulo 2, Artigo 8,2 e 8,3). Desta forma qualquer informação inédita, opiniões, hipóteses e conceitos novos aqui não estão disponíveis na literatura zoológica. As pessoas interessadas devem estar cientes que as referências públicas do conteúdo deste trabalho devem ser feitas com a aprovação previa dos autores

Finalmente, a formatação e estrutura da presente dissertação estão baseada nas regras de edição da revista Molecular Phylogenetics and Evolution (https://www.elsevier.com/journals/molecular-phylogenetics-and-evolution/1055-7903/guide-for-authors#20000), onde vai ser publicada a presente dissertação.

Phylogenetic analyses of DNA sequences reveal a vastly underestimated radiation of Amazonian salamanders (Plethodontidae: *Bolitoglossa*), with key implications to the study of plethodontid diversification

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1. Introduction

The study of species richness patterns is a very active research program in biology (e.g., Mittelbach et al., 2007; Weir & Schluter, 2007; Jetz et al., 2012; Mannion et al., 2014; Hutter et al., 2017; Wiens, 2018). A key assumption in these studies—which ultimately try to discover differences in diversification (= speciation - extinction) and dispersal, and their causes of variation among compared units, let them be clades or areas—is that species richness is sufficiently well known within each unit of comparison so that the observed pattern reflects the true proportion of species differences. However, biologists working in tropical regions may look at this premise as containing more wishful thinking than corroborated background knowledge. The task at hand is gargantuan, since Earth's diversity is estimated in more than one billion species (Locey & Lennon, 2016; Larsen et al., 2017), but only about 1.5 million have been formally described (Roskov et al., 2018). Even in birds, arguably the most studied group of animals, our understanding of their species richness seems to be so limited as to compromise the results of detailed inferences (Tobias et al., 2008; Barrowclough et al., 2016).

Within amphibians, plethodontid salamanders have been the focus of multiple studies addressing the causes of differences in species richness related to latitude and elevation (Kozak, 2017), to the point that they are considered a model radiation in evolutionary biology and ecology (Wake 2009). Plethodontidae (475 spp.; Frost, 2019) harbors about 66 % of the currently recognized species of Caudata. Most species occur in Central America and the Mesoamerican Highlands of Mexico, with a second center of diversity in the southern Appalachian Highlands of eastern North America.

This pattern of species richness is explained by a relatively late dispersal from the Nearctic into the Neotropics followed by an increase in diversification in Mesoamerica (Vieites et al., 2007, 2011; Kozak and Wiens, 2010; Rovito et al., 2015; Shen et al., 2016). According to current data (Frost, 2019), South America is particularly poor in plethodontid salamanders when compared to Central America (280 species distributed in 17 genera), with only 35 species of *Bolitoglossa* (all of them in the subgenus *Eladinea*) and two of the genus *Oedipina*. This comparatively low number of species in South America seems at odds with other variables that usually are good predictors of species richness, such as available area and environmental heterogeneity that favor isolation and speciation (Stein et al., 2014). The Amazon basin, with more than 7 million km^2 and a geological history that have resulted in topographical complexity that includes, among many other features, some superlatives such as the longest and second highest mountain chain and largest rivers in the world, seems to have no shortage of opportunities for large radiations to happen (Hoorn and Wesselingh, 2010). Not surprisingly, many groups reach their peak of species richness in Amazonia (Myers et al., 2000; Jenkins et al., 2013), even in clades that originated elsewhere (Hughes and Eastwood, 2006). A time-dependent diversification model (Stephens and Wiens, 2003) seems like a good explanation for the limited number of salamander species in South America. A priori, one may think that relatively small amphibians such as *Bolitoglossa* are poor dispersers, with little ability to cross oceanic barriers—such as the land gap postulated to exist between Central and South America until relatively recently (~ 3.2 mya). However, a series of discoveries during the last decade or so indicate that this paradigm of poor salamander diversification in South America needs further scrutiny.

On the one hand, geological and biogeographic breakthroughs open the possibility of an older colonization. For example, an older land bridge between Central and South

America (Montes et al., 2015), possible amphibian oceanic dispersals (Vences et al., 2003; Pyron, 2014), considerably older estimated dates for the colonization of South America than the ~ 3 my land-bridge (Elmer et al., 2013), and the first and only fossil of a Caribbean salamander, apparently a Bolitoglossini of at least 15 MYA (Poinar and Wake, 2015). On the other hand, the number species of *Bolitoglossa* may be more underestimated in South America than in other regions. Different studies indicate that, in Plethodontidae, the pattern of cladogenesis—as inferred from phylogenetic analyses of DNA sequences—is rarely accompanied by detectable morphological changes (Larson and Chippindale, 1993; Tilley and Bernardo, 1993; Adams et al., 2009). Independently of the causes of this morphological stasis, the implications for the systematics of these salamanders are obvious: species delimitation and phylogenetic relationships solely based on the variation of a handful of morphological characters traditionally used in the group is likely biased towards an under estimation of species richness. To date, no study has addressed the systematics of South American salamanders using DNA sequences of a collection of samples that truly reflects their distribution; the most extensive study, based on Ecuadorian samples, indicate high levels of cryptic species richness even at a moderate geographic scale (Elmer et al., 2013).

In summary, there may be many more species of plethodontid salamanders in South America than is currently known, because the group may have arrived earlier than previously thought and/or our understanding of the species richness of the group is, to say the least, superficial. Although both the tempo and extent of the radiation of Amazonian salamanders are obviously relevant topics, we consider that the quality of the inferences on the tempo and mode of diversification of a group are dependent on the quality of our knowledge of its systematics, and improving this knowledge is our main goal with this work.

Therefore, in this study we focus mostly on the potential problem of the underestimation of Amazonian *Bolitoglossa* species and their phylogenetic relationships.

Amazonian salamanders are relatively small (snout-venth length = 24.2–53.9 mm) and have hands and feet modified as pads, apparently to increase adherence, which seems convenient for their arboreal and epiphyllous life. Like other plethodontids, they are lungless, with the hyoid system modified to dart their tongue and capture prey. Females deposit terrestrial eggs and embryos undergo direct development so that a miniature version of the adult hatches from the egg (Brame and Wake, 1963; Wake, 1966). Currently, nine species of *Bolitoglossa* are recognized in the Amazon basin, from the lowlands in the mouth of the Amazon river to around 2000 m asl in the eastern Andean slopes. Four of them—*B. caldwellae* Brcko, Hoogmoed and Neckel-Oliveira, 2013, *B. madeira* Brcko, Hoogmoed and Neckel-Oliveira, 2013, *B. paraensis* (Unterstein, 1930), and *B. tapajonica* Brcko, Hoogmoed and Neckel-Oliveira, 2013—are relatively well characterized morphologically as the result of a recent taxonomic revision (Brcko et al., 2013). The other five—*B. altamazonica* (Cope, 1874), *B. digitigrada* Wake, Brame and Thomas, 1982, *B. equatoriana* Brame and Wake, 1972, *B. palmata* (Werner, 1897), and *B. peruviana* (Boulenger, 1883)—present more challenging situations (Wake et al., 1982; Acosta-Galvis and Gutiérrez-Lamus, 2012; Elmer et al., 2013).

The phylogenetic relationships among Amazonian salamanders are also poorly studied and the taxonomic identification of many terminals is problematic. Until 2004, they were grouped in different phenetic clusters. Parra-Olea et al. (2004) studied the phylogeny of 61 species of *Bolitoglossa* analyzing DNA sequences of the mitochondrial genes 16S and Cytb under parsimony, maximum likelihood, and posterior probability. They proposed the recognition of seven subgenera (*Bolitoglossa*, *Eladinea*, *Magnadigita*, *Mayamandra*,

Nanotriton, *Oaxakia*, and *Pachymandra*) compatible with the topologies resulting from the different evaluation criteria. All the South American species analyzed by them are part of the subgenus *Eladinea*. Parra-Olea et al. (2004) divided *Eladinea* into four species groups (*B. adspersa*, *B. epimela*, *B. schizodactyla*, and *B*. *subpalmata*), with all South American species placed in the *B. adspersa* group. Following the work of Parra-Olea et al. (2004), several studies published phylogenetic hypotheses including DNA sequences of South American salamanders (García-Gutiérrez et al., 2013; Pyron and Wiens 2011; Acevedo et al., 2013; Elmer et al., 2013; Batista et al., 2014), although with limited taxon sampling, as they were designed to study either particular species-level systematics issues within salamanders or very broad phylogenetic questions among amphibians. Most of the phylogenetic hypotheses cited above agree that the Amazonian species included in their respective analyses are paraphyletic with respect to other species of the *B. adspersa* group. However, they all differ about the details of the relationships. Given than these studies vary in their combination of characters, terminals, and optimization criteria—among other variables that influence the result of phylogenetic analysis—their results cannot be meaningfully compared. In other words, one cannot elucidate which are the causes of the observed differences. Thus, besides compiling all available information to evaluate the current state of knowledge of South American salamanders, it is necessary to add most needed new data.

Considering the situation outlined above, the objective of this study is to provide a phylogenetic explanation (i.e., hypothesis) of observed nucleotide variation among specimens of *Bolitoglossa* from the Amazon basin in the context of the radiation of the genus, and to evaluate the implications of our results to our understanding of *Bolitoglossa* species diversity in the Amazon, as well as its diversification and biogeography.

2. Material and methods

2.1 Taxon sampling

Given our objective, we aimed to include as many specimens as possible of *Bolitoglossa* salamanders from the Amazon basin, including representatives of all currently recognized subgenera and species groups within *Eladinea*. Considering the current difficulty in assigning available names to specimens, we made an especial effort to include data from type material and/or topotypes so that binomials could be assigned to clades. Representatives of other genera of Plethodontidae (*Aquiloeurycea*, *Chiropterotriton*, *Ixalotriton*, *Parvimolge*, and *Pseudoeurycea*) were used as outgroups, and *Thorius* was set as the root in all analyses (Rovito et al., 2015). Our final dataset includes 366 terminals, 189 terminals of 89 non-Amazonian nominal species and 177 Amazonian specimens, including types or topotypes of eight of the nine recognized species in the region (Supplementary data 1). By including representatives of all the known species of Amazonian *Bolitoglossa*, except for *B. digitigrada*, and 73 % of the known diversity of the genus, our dataset represents the broadest sample of species, specimens, and geographic localities studied to date.

2.2 DNA sequences collection

We worked with the most used molecular markers of previous studies of *Eladinea* to be able to incorporate as much published data as possible. After a perusal of GenBank and the relevant literature (Parra-Olea et al., 2004; Rovito et al., 2012; Batista et al., 2014; Elmer et al., 2013), we selected three mitochondrial and two nuclear markers—16S rRNA (16S),

cytochrome c oxidase subunit I (COI), cytochrome b (cytb), and fragments of the nuclear genes proopiomelanocortin (POMC) and recombination activating gene 1 (RAG1). Laboratory protocols for newly generated sequences followed standard procedures described by Palumbi et al. (1991), Moritz et al. (1992), Ivanova et al. (2006), Vieites et al. (2007), and Elmer et al. (2013). The primers used are listed in Supplementary data 2.

Sequences were obtained from samples listed in Supplementary data 1. Positive PCR products (determined by the presence of bands of the expected size in agarose gels) were sequenced in both directions. The resulting chromatograms were analyzed in Sequencher 4.1.4 to trim unwanted edges and correct errors or ambiguous nucleotides. Additionally, we downloaded homologous sequences from GenBank of ingroup and outgroup taxa (up to 27 November 2017). We filtered all terminals from population and phylogeography studies from non-South American salamanders (i.e., García-París et al., 2000; Boza-Oviedo et al., 2012; Rovito et al., 2012), incorporating only those terminals that had genetic distances above 1 % in 16S and cytb (these genes are represented by more than 85 % of terminals), to reduce search space during phylogenetic analyses (Wilkinson, 1995; Kearney, 2002; Brower, 2018). In order to reduce wildcard terminals, incomplete sequences from different individuals of the outgroup (i.e., *B. colonnea*, *B. engelhardti*, *B. helmrichi*, *B. occidentalis*, *B. orestes*, *B. rufescens*; Supplementary data 1) were merged with sequences from other individuals of the same species to construct a single complete composite sequence. This last approach was applied after checking that genetic distances in 16S and/or cytb fragments were < 1.0 %. In total, 353 sequences were generated, including the first sequences of six South American species: *B. altamazonica*, *B. hypacra*, *B. madeira*, *B. peruviana*, *B. tapajonica* and *B. walkeri*. Nine terminals from GenBank were re-identified (Supplementary data 3) based on two criteria: (i) secondary literature, for recently described species with

sequences submitted to GenBank as belonging to undescribed taxa (i.e., sp.); and (ii) discordance in the species name between the GenBank database and the original publication.

2.3 Phylogenetic analyses

2.3.1 Theoretical considerations

Phylogenetic analyses can be understood from the perspective that transformation series, as well as organisms, species, and clades are progressively more inclusive individuals (Grant and Kluge, 2004). In other words, each is a real entity at a different level of organization so that transformation series are parts of organisms, organisms of species, and species of clades. In this context, the objective of a phylogenetic analysis of empirical data is to provide the best possible historical and evolutionary explanation of the observed variation. As such, transformations are the things to be explained through historical connections (i.e., homology) during the evolution of species, which are connected by speciation events. Therefore, and as simply stated by Farris (1967), our trees imply hypotheses of both transformations and the relative order of speciation or patristic and cladistic, respectively. Under the parsimony criterion, the best explanation of the observed variation is the one that requires fewer transformations (Kluge and Grant, 2006; Grant and Kluge, 2009). In other words, the explanatory content of the hypothesis maximizes by minimizing the number of necessary transformations.

Different and currently most popular approaches consider that the best phylogenetic hypothesis of observed variation among studied organisms is either the one that maximizes the likelihood of observing the data or the one that maximizes the likelihood of the

hypothesis considering the data. In either case, a probabilistic model of character change is needed, together with several further assumptions, to analyze the data.

We performed two types of phylogenetic analyses that reflect the two views outlined above. An equally weighted parsimony analysis, which is consistent with the first view, and a ML analysis compatible with the second perspective (details of both analyses are provided in the next section). The purpose of these analyses is twofold. First, we want to evaluate the sensitivity (sensu Giribet and Wheeler 2007) of our results to the different optimality criteria. Second, we wanted to foment collegiality among colleagues (including the authors of this study), some of which may have preferences over one of the analytical approaches outlined above. In any case, it should be clear that we do not consider sensitivity (or its absence) as an optimality criterion to select among competing hypotheses.

Regardless of optimality criterion, we interpret that a phylogenetic hypothesis is supported if it is not refuted by the critical evidence (i.e., it is the optimal solution according to a justified optimality criterion, parsimony or ML in this study) or contradicted by other, equally optimal hypotheses (i.e., evidence is ambiguous, such as when multiple most-parsimonious cladograms are obtained). Frequency of clades based on resampling measures (i.e., Jackknife and Bootstrap) are interpreted as a proxy of the relative amount of favorable and contradictory evidence for each group present in the optimal topology inferred from a specific dataset when frequency ≥ 50 % (Goloboff et al., 2003; Ramírez, 2005).

2.3.2 Parsimony

Analyses were performed under direct optimization in POY 5.1.1 (Varón et al., 2010; Wheeler et al., 2014), which evaluates hypotheses of nucleotide homology dynamically by

optimizing unaligned DNA sequences directly onto alternative topologies (Wheeler et al., 2006). First, sequences of each marker were individually aligned using the MUSCLE algorithm in AliView 1.17.1 (Larsson, 2014) under default parameters. Each aligned gene fragment was partitioned into smaller blocks so that within each block, length variation among DNA sequences was only attributable to insertions and/or deletions of nucleotides and never to missing data (Wheeler et al., 2006). Each block was flanked by conserved regions with no gaps and few or no nucleotide substitutions. Before tree searches in POY, all gaps were removed from each block. Tree searches were conducted using the cluster Amazonia, from the *Laboratório de Alto Desempenho* (LAD)-PUCRS high performance computing. The Amazonia cluster consists of an enclosure HP Blade System C3000 with 4 blades L620cG7 and a dedicated storage with access through Fiber Channel Protocol (8 Gib/s). It is composed by two Intel Xeon E7-2850 2.0 GHz Hyper-Threading processors with 160 GB and 512 GB of memory, respectively, and 20 cores (40 threads) for each processor (160 threads in total for the cluster). Three searches of 50 hours each on 40 CPUs (giving a total of 6024 CPU-hours) were run using the command "search", which implements an algorithm based on random addition sequence Wagner builds, subtree pruning and regrafting (SPR), and tree bisection and reconnection (TBR) branch swapping (Goloboff, 1996, 1999), parsimony ratcheting (Nixon, 1999), and tree fusing (Goloboff, 1999), storing the shortest trees from each independent run and performing a final round of Tree Fusing on the pooled trees. Next, 3000 rounds of Tree Fusing of the optimal trees from driven searches were performed, using the standard direct optimization algorithm. Then, we used the exact iterative pass algorithm (Wheeler, 2003a) to improve the cost of the optimal trees identified in the previous analyses. Finally, tree-alignment matrices of all the optimal trees were generated (i.e., the implied alignment; Wheeler, 2003b). To search for additional

optimal trees for each tree-alignment, we run searches using the New Technologies algorithms (Sectorial Search, Ratchet, Drift, Tree Fusing) in their default modes in TNT 1.5 (Goloboff et al., 2008; Goloboff and Catalano, 2016). Searches were set for all taxa, at level 70, with minimum tree length set to be found 100 times and random seed = 1. Finally, we visually compared the resulting consensus trees from each tree-alignment. Jackknife frequencies (JK) were calculated in TNT from the implied alignment for 1000 pseudoreplicate searches with the Traditional Search option with 50 replicates and 50 trees saved per replication, gaps as a fifth state, and removal probability of 0.36 (~ e-1) to render bootstrap and JK values comparable (Farris et al., 1996).

Given the heterogeneity of gene coverture (1–5 loci per terminal) in our data set, we analyzed the potential wildcard behavior of terminals (Simmons, 2012a, b; Simmons and Norton, 2013; Simmons and Goloboff, 2013; Padial et al., 2014) for all terminals with YBYRÁ (Machado, 2015) using all optimal topologies resulted from the parsimony analyses. Briefly, this analysis ranks all terminals according to the number of clades shared by all topologies when one terminal is pruned. Then, it prunes each terminal, one at a time, from all optimal topologies to calculate the average matching split distances among each set of pruned optimal topologies and compares it with the average matching split distance among the original topologies (Bogdanowicz and Giaro, 2012; Machado, 2015).

2.3.3 Maximum likelihood

We combined the similarity alignments mentioned above into a single matrix using SequenceMatrix 1.8 (Vaidya et al., 2011). We used the greedy algorithm of PARTITIONFINDER v.1.1.1 (Lanfear et al., 2016) and the Bayesian information criterion to

select the optimal combination of partition schemes and DNA substitution models for the concatenated matrix. We followed Simmons and Ochoterena (2000) and coded continuous indels as the largest possible single events as implemented in the option "simple coding" of SeqState (Müller 2005, 2006), which was included as an independent data partition. The best-fitting partition scheme and DNA substitution models were applied to search the ML tree. As indel characters were coded as binary (0 or 1), we used Mkv model of evolution for discrete morphological data (Lewis, 2001), which assumes that the data collected contains only variable characters. Tree searches of the final matrix (DNA sequences with gaps as unknown nucleotides and indels as binary characters) were performed in Garli (Zwickl, 2006) on XSEDE (CIPRES Science Gateway; Miller et al., 2010). We conducted 500 independent searches using a random tree ("streefname = random"), 100.000 generations without topology improvement required for termination (genthreshfortopoterm), tree rejection threshold at 50 (treerejectionthreshold), and the maximum number of branches away from original location that a branch may be reattached during a limited SPR move was 10 (limsprrange). The best tree from these independent searches was selected according to the highest value of log likelihood score. Bootstrap frequencies were calculated with 1000 pseudoreplicates under the same tree search parameters outlined above. The replicates were compiled in a single tree file using the R package Ape 4.1 (Paradis et al., 2004), and bootstrap frequencies were assigned to the corresponding clades of the optimal tree using SumTrees 4.3.0 (Sukumaran and Holder, 2010a) of the DendroPy 4.3.0 package (Sukumaran and Holder, 2010b).

2.4 Species: Conceptual and operational considerations

We consider a species as the single lineage segment of ancestor-descendant populations or metapopulations delimited by a splitting event (Simpson, 1951; Wiley, 1978; de Queiroz, 1998; Wiley and Lieberman, 2011). Under this theoretical perspective, species exist (i.e., they are ontological historical individuals, regardless of our ability to discover them), evolve, and are discoverable to the degree that footprints of their evolutionary history marked as characters observed on organisms allow us to infer their existence (Ghiselin, 1975; Hull, 1976; Wiley, 1978; Frost and Kluge, 1994). We used two criteria to infer the existence of distinct species using DNA data and to guide the recognition of candidate species: monophyly and genetic distances within and between monophyletic groups. Reciprocal monophyly supported by the congruent phylogenetic optimization of nucleotides of different markers can be considered evidence of species divergence (Avise and Ball 1990; Sites and Marshall 2004; Vences and Wake 2007). In addition, fixed diagnostic traits across populations are indicative of lineage divergence, because character fixation across populations requires limited or absent gene flow (see review by Padial et al., 2010). Therefore, reciprocally monophyletic groups recovered by the total evidence analysis of DNA sequences, and for which distinct phenotypic characters have been described in the literature, are herein considered distinct species. Paraphyly of species inferred by total evidence analyses of DNA sequences that, yet, include morphologically distinct groups is considered indicative of the presence of more than one species. The second criterion, based on genetic divergences, assumes that genetic divergence among populations within a species tends to be relatively small because of gene flow, whereas divergence among species increases with time due to lack of gene flow (reviewed by Avise 2000). When large gaps in genetic divergences were detected between populations of the same nominal species, morphological evidence was revised to determine whether genetic divergences

were indicative of otherwise overlooked divergence in phenotypic traits and hence of the presence of unnamed species. However, for the reasons exposed by Padial et al. (2009) and Padial and De la Riva (2010), we refrain from using thresholds of genetic divergences to avoid recognizing species artificially established (or candidate species). We calculated genetic distances for the mitochondrial markers 16S and cytb because they are the best represented in our dataset (sequenced for 130 and 115 Amazonian samples, respectively). Uncorrected p-distances were estimated in Mega 7 (Kumar et al. 2018) for each marker independently using a similarity alignment (453 and 528 bp for 16S and cytb, respectively). Indels were considered as characters, although Mega invariably eliminates 5 % of them. All potential new species in the ingroup are indicated by adding sp. (= species).

As a complement to our integrative approach, we used two objective species delimitation methods based on analyses of DNA sequences: Automated Barcode Gap Discovery (ABGD; Puillandre et al. 2012) and multi-rate Poisson Tree Processes (mPTP; Zhang et al.2013; Kapli et al. 2017). The ABGD analyses were performed using the online server http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. We used the simple distance method, with a relative gap width of 0.01, and intraspecific distance of 0.001 to 0.029 (16S) or 0.057 (cytb). The upper value used for both genes corresponds to the maximum intraspecific distance found between the two terminals of *Bolitoglossa tapajonica*. We used this species because it has the highest intraspecific distance among the currently recognized species. The other parameters were set according to the default configuration. We used the software mPTP 0.2.4 v. (Kapli et al. 2017) on the ML tree, inasmuch as it contains information about the nucleotide substitution rate that is used by the algorithm to identified speciation events (Kapli et al. 2017). The analyses were

conducted with the MCMC method and multi-rate command with 50.000.000 generations, sample every 10.000 generations and burning of 1.000 generations.

To evaluate the performance of the used delimitation methods, we used the Relative Taxonomic Resolving Power Index (R_{tax}) and the Taxonomic Index of Congruence (C_{tax}) following Miralles and Vences (2013). The R_{tax} quantifies the relative power of a method to infer all estimated speciation events present in a data set (large R_{tax} means small type II error), but does not necessarily imply correct delimitations (i.e., it can lead to oversplitting). On the other hand, the C_{tax} measures the congruence in delimitation assignments between two methods, with a value of 1 indicating complete congruence. For details of calculation of both indexes, see Miralles and Vences (2013).

2.5 *Biogeographic analysis*

We restricted the analysis to the subgenus *Eladinea*, which comprises all South American species and some Mesoamerican species. Based on the known species distribution and the South American geomorphological domains proposed by Ab'Saber (1977), we selected three biogeographic units: Chocó, Andes, and Amazonia. Also, we considered Mesoamerica in a broad sense for the outgroup. To separate Andean and non-Andean species, we compiled data of the elevation ranges for all species with South American distribution from IUCN's web page, new data published here, and recent taxonomic and species descriptions (Brcko et al 2013; Acevedo et al. 2013; Meza-Joya et al. 2017). Based on the elevation ranges plot (Fig. 1), we considered 1200 m a.s.l. as the elevational limit to separate Andean and no-Andean species. The break between highland and lowland taxa roughly coincides with the lower limit of mountain rain forest belts (c. 800–1200 m a.s.l.

depending on specific local conditions; Hooghmiestra et al., 2006 and references therein) and only two of the evaluated species overpass this elevational “barrier”.

To identify dispersal, vicariance and extinction events between geographic areas, we used dispersal-vicariance analysis (DIVA; Ronquist 1997), as implemented in RASP v.4.0 (Yu et al., 2010). The reconstruction was performed on a simplified pruned species tree (resulting from the species delimitation mentioned above) of one of the most parsimonious trees and the best topology of maximum likelihood. *Bolitoglossa* (*Magnadigita*) *rostrata* was used as outgroup.

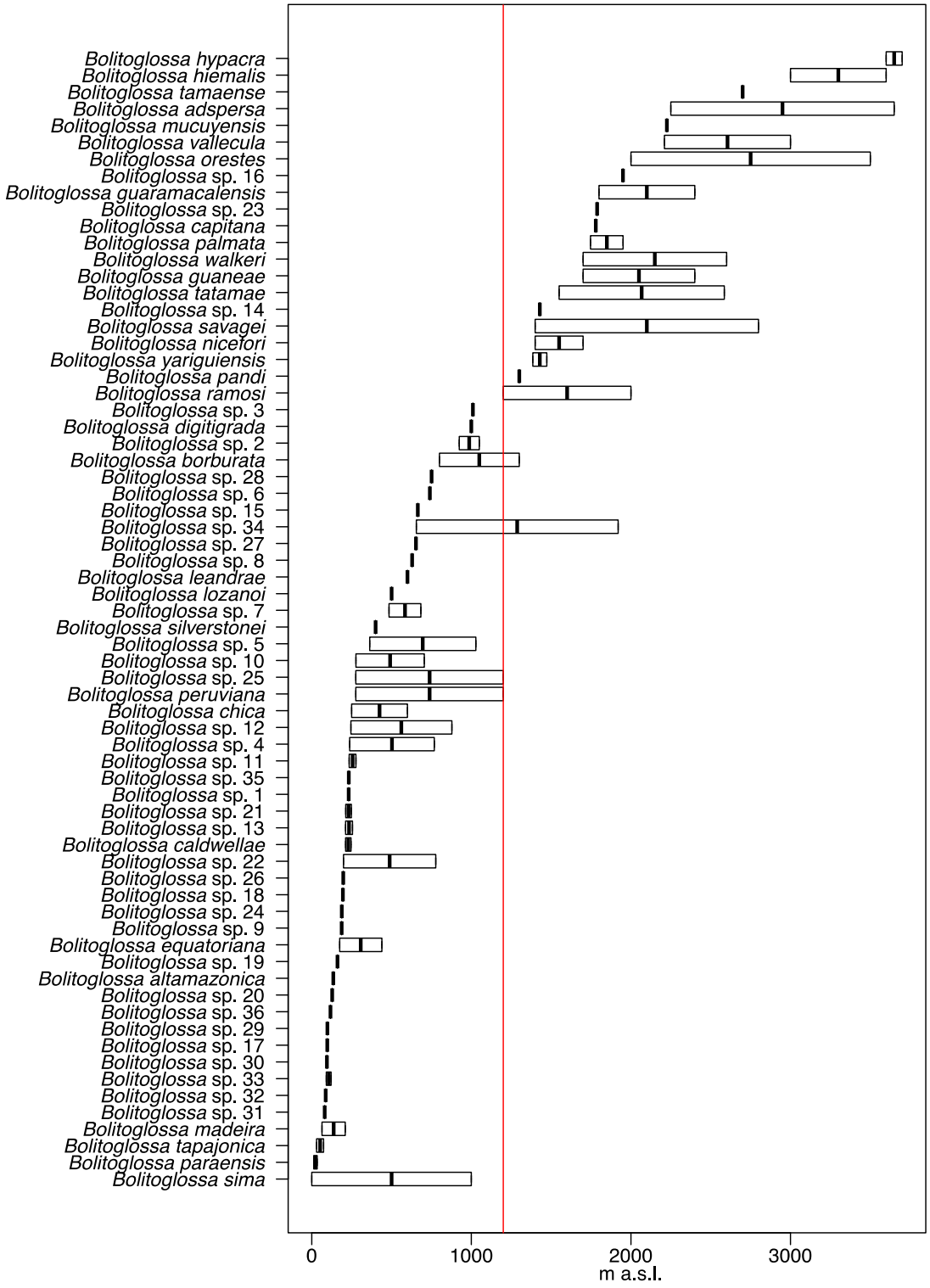


Figure 1. Know elevational ranges of species of the subgenus *Eladinea,* following the species taxonomy resulting from this work. The red line indicates 1,200 m a.s.l., which we identified as the limit between lowland and Andean taxa. Horizontal rectangles indicate minimum and maximum recorded elevation, while the thicker black line marks the midelevation between the known range.

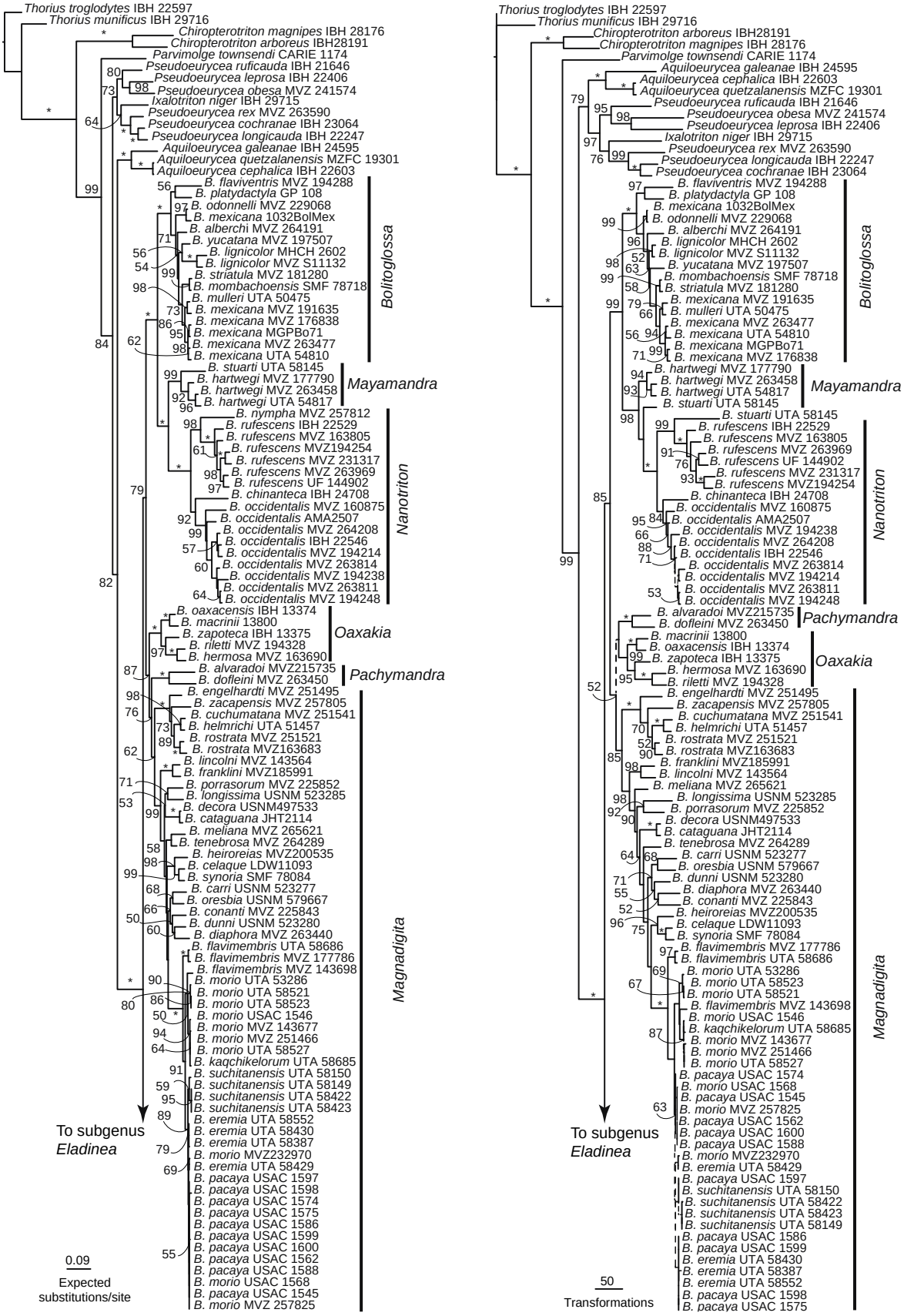
3. Results

3.1 Parsimony

Tree searches of the complete dataset in POY yielded six most parsimonious trees (12,610 steps). A final round of swapping using iterative pass optimization on these trees further reduced the cost (12,597 steps). The implied alignment contains 3,441 molecular transformation series (Supplementary data 4). Tree searches of this static dataset in TNT found 1,751 most parsimonious trees. The strict consensus (Fig. 2, 3) is well resolved with 34 % polytomies of 381 possible nodes of a fully bifurcating tree. All polytomies correspond to shallow clades involving specimens of the same species. Jackknife values are ≥ 75 in 59 clades and ≤ 50 in 31 clades.

The results recovered all sampled genera monophyletic except *Pseudoeurycea*, which is paraphyletic with respect to *Ixalotriton niger*—the latter is sister of a clade formed by *P. cochranae*, *P. longicauda*, and *P. rex*. Within the genus *Bolitoglossa* (JK = 100), all currently recognized subgenera are monophyletic but *Mayamandra*, which is paraphyletic in relation with *Nanotriton* (JK = 100), because *M. stuarti* is more closely related to *Nanotriton* than to *M. hartwegi* (JK ≤ 50). The first split within *Bolitoglossa* separates a clade (JK = 85) containing the subgenera *Bolitoglossa*, *Magnadigita*, *Mayamandra*, *Nanotriton*, *Oaxakia*, and *Pachymandra* from the subgenera *Eladinea* (JK = 100). The subgenus *Bolitoglossa* (JK = 100) is sister to *Mayamandra* and *Nanotriton* (JK = 98). Within the subgenus *Bolitoglossa*, *B. mexicana* is non-monophyletic because the sample *B. mulleri* UTA 50475 is embedded within five samples of *B. mexicana* and because *B. mexicana* 1032 is more closely related to *B. odonnelli* MVZ 229068 than to the other samples of *B. mexicana*. The sister clade of *Bolitoglossa*, *Mayamandra*, and *Nanotriton* contains the subgenera *Oaxakia* (JK = 99), *Pachymandra* (JK = 100) and *Magnadigita* (JK = 85). Within the latter subgenus, samples of

B. morio are rampantly non-monophyletic. The species *B. eremia*, *B. flavimembris*, and *B. pacaya* are also non-monophyletic.



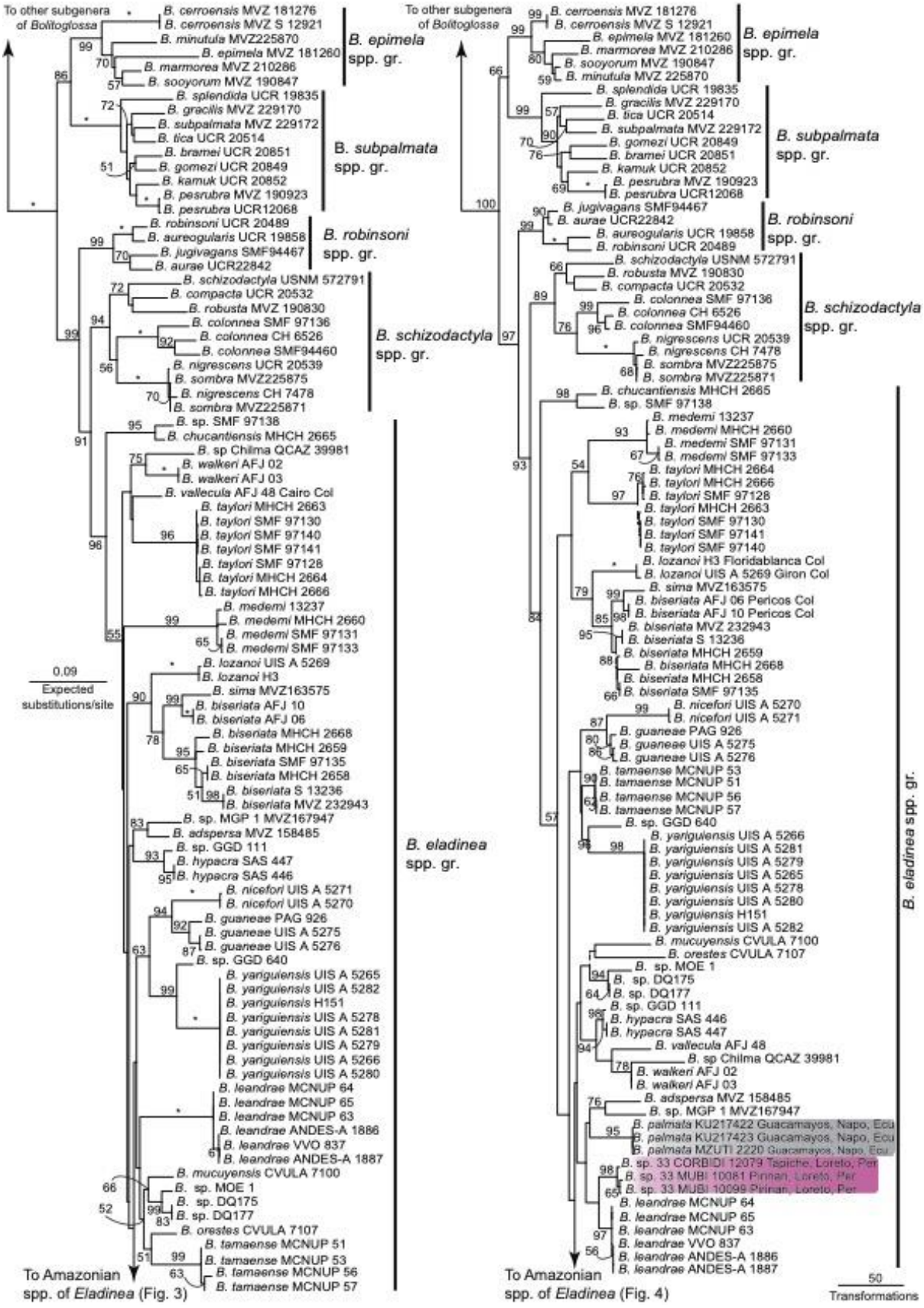


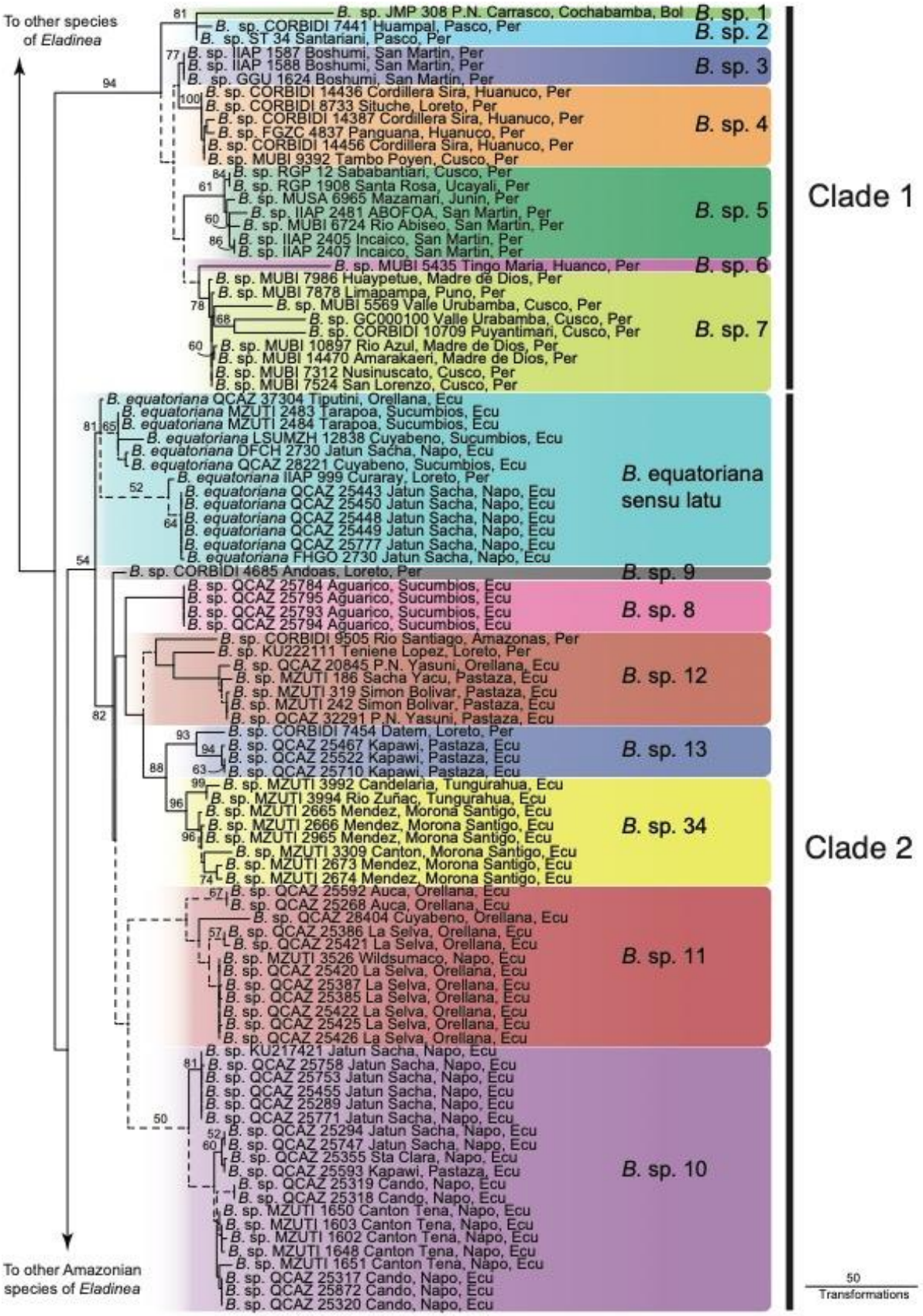
Figure 2. Phylogenetic relationships of *Bolitoglossa* and outgroups inferred from up to three mitochondrial (16S, COI, and cytb) and two nuclear (POMC and Rag1) genes. On the left, maximum likelihood tree (log likelihood = - 5,9863.1) from a similarity alignment and

considering indels as the longest possible binary characters. On the left, one of the 1,752 shortest trees (12,597 transformations) from a tree-alignment parsimony analysis, coding indels as a fifth character, with dashed lines indicating collapsed clades in the strict consensus. Numbers on branches are bootstrap (left) and jackknife (right) frequencies of 1,000 searches. For both trees, the relationships among Amazonian *Eladinea* are shown in Figs. 3 and 4.

Within *Eladinea*, all species groups currently recognized are monophyletic with the exception of *B. schizodactyla* and *B. adspersa* groups due to the position of *B. compacta*. This species is currently considered part of the *B. adspersa* group based on similarity to other species (Parra-Olea et al., 2004); however, our results indicate that it is nested within the *B. schizodactyla* group. The *B. epimela* (JK = 99) and *B. subpalmata* (JK = 99) groups are sister taxa (JK = 66). This clade is sister to the *B. robinsoni* (JK = 99), *B. schizodactyla* (JK = 89), and *B. adspersa* (JK = 84) groups. *Bolitoglossa nigrescens*, of the *B. schizodactyla* group, is non-monophyletic with respect to *B. sombra*.

The *Bolitoglossa adspersa* group includes all South American species of the genus plus a few species from the Chocó and Darién of Panama, such as *B. biseriata*, *B. chucantiensis*, *B. medemi*, and *B. taylori*. Our results indicate that samples identified as *B. biseriata* are non-monophyletic because the two samples from Pericos, Colombia (AFJ 06 and 10) are more closely related to *B. sima* than to the other samples of *B. biseriata*. *Bolitoglossa walkeri* is also non-monophyletic because one of our samples is more closely related to a sample of a putative new species from Chilma, Ecuador. All of our 177 samples of *Bolitoglossa* from the Amazon basin, but six, form an exclusive monophyletic group. The exception includes specimens of *B. palmata* (highlands of Ecuador) and three samples of a putative new species (*B*. sp. 33) from the Amazonian lowlands of Loreto, Peru; both taxa are more closely related to Andean species from outside the Amazon basin such as *B. adspersa* (from the western flank of the Cordillera Oriental of Colombia) and *B. leandrae* (from the

Andes of the Orinoco basin). To facilitate comparisons and discussions among results of parsimony and ML, we labelled seven clades (numbers 1 to 7, Figs. 3–5) that are identical in content in both analyses but for Clade 2 because the parsimony optimal trees do not include *B. palmata*. Nevertheless, the optimal evolutionary relationships among these historical units have in general JK and BS ≤ 50, indicating that there is plenty of conflicting evidence (i.e., transformations that are against the optimal clades) in both alignments, few transformations supporting the clades (regardless of conflict) or a high proportion of missing data.


To other species of Eladinea
B. sp. JMP 308 P.N. Carrasco, Cochabamba, Bol
B. sp. 1
B. sp. CORBIDI 7441 Huampal, Pasco, Per
B. sp. ST 34 Santariani, Pasco, Per
B. sp. 2
B. sp. IIAP 1587 Boshumi, San Martin, Per
B. sp. IIAP 1588 Boshumi, San Martin, Per
B. sp. GGU 1624 Boshumi, San Martin, Per
B. sp. 3
B. sp. CORBIDI 14436 Cordillera Sira, Huanuco, Per
B. sp. CORBIDI 8733 Situche, Loreto, Per
B. sp. CORBIDI 14387 Cordillera Sira, Huanuco, Per
B. sp. FGZC 4837 Panguana, Huanuco, Per
B. sp. CORBIDI 14456 Cordillera Sira, Huanuco, Per
B. sp. MUBI 9392 Tambo Poyen, Cusco, Per
B. sp. 4
B. sp. RGP 12 Sababantiari, Cusco, Per
B. sp. RGP 1908 Santa Rosa, Ucayali, Per
B. sp. MUSA 6965 Mazamari, Junin, Per
B. sp. IIAP 2481 ABOFOA, San Martin, Per
B. sp. MUBI 6724 Rio Abiseo, San Martin, Per
B. sp. IIAP 2405 Incaico, San Martin, Per
B. sp. IIAP 2407 Incaico, San Martin, Per
B. sp. 5
B. sp. MUBI 5435 Tingo Maria, Huanco, Per
B. sp. 6
B. sp. MUBI 7986 Huaypetue, Madre de Dios, Per
B. sp. MUBI 7878 Limapampa, Puno, Per
B. sp. MUBI 5569 Valle Urubamba, Cusco, Per
B. sp. GC000100 Valle Urabamba, Cusco, Per
B. sp. CORBIDI 10709 Puyantimari, Cusco, Per
B. sp. MUBI 10897 Rio Azul, Madre de Dios, Per
B. sp. MUBI 14470 Amarakaeri, Madre de Dios, Per
B. sp. MUBI 7312 Nusinuscato, Cusco, Per
B. sp. MUBI 7524 San Lorenzo, Cusco, Per
B. sp. 7
Clade 1
B. equatoriana QCAZ 37304 Tiputini, Orellana, Ecu
B. equatoriana MZUTI 2483 Tarapoa, Sucumbios, Ecu
B. equatoriana MZUTI 2484 Tarapoa, Sucumbios, Ecu
B. equatoriana LSUMZH 12838 Cuyabeno, Sucumbios, Ecu
B. equatoriana DFCH 2730 Jatun Sacha, Napo, Ecu
B. equatoriana QCAZ 28221 Cuyabeno, Sucumbios, Ecu
B. equatoriana IIAP 999 Curaray, Loreto, Per
B. equatoriana QCAZ 25443 Jatun Sacha, Napo, Ecu
B. equatoriana QCAZ 25450 Jatun Sacha, Napo, Ecu
B. equatoriana QCAZ 25448 Jatun Sacha, Napo, Ecu
B. equatoriana QCAZ 25449 Jatun Sacha, Napo, Ecu
B. equatoriana QCAZ 25777 Jatun Sacha, Napo, Ecu
B. equatoriana FHGO 2730 Jatun Sacha, Napo, Ecu
B. equatoriana sensu latu
B. sp. CORBIDI 4685 Andoas, Loreto, Per
B. sp. 9
B. sp. QCAZ 25784 Aguarico, Sucumbios, Ecu
B. sp. QCAZ 25795 Aguarico, Sucumbios, Ecu
B. sp. QCAZ 25793 Aguarico, Sucumbios, Ecu
B. sp. QCAZ 25794 Aguarico, Sucumbios, Ecu
B. sp. 8
B. sp. CORBIDI 9505 Rio Santiago, Amazonas, Per
B. sp. KU222111 Teniene Lopez, Loreto, Per
B. sp. QCAZ 20845 P.N. Yasuni, Orellana, Ecu
B. sp. MZUTI 186 Sacha Yacu, Pastaza, Ecu
B. sp. MZUTI 319 Simon Bolivar, Pastaza, Ecu
B. sp. MZUTI 242 Simon Bolivar, Pastaza, Ecu
B. sp. QCAZ 32291 P.N. Yasuni, Pastaza, Ecu
B. sp. 12
B. sp. CORBIDI 7454 Datem, Loreto, Per
B. sp. QCAZ 25467 Kapawi, Pastaza, Ecu
B. sp. QCAZ 25522 Kapawi, Pastaza, Ecu
B. sp. QCAZ 25710 Kapawi, Pastaza, Ecu
B. sp. 13
B. sp. MZUTI 3992 Candelaria, Tungurahua, Ecu
B. sp. MZUTI 3994 Rio Zuñac, Tungurahua, Ecu
B. sp. MZUTI 2665 Mendez, Morona Santigo, Ecu
B. sp. MZUTI 2666 Mendez, Morona Santigo, Ecu
B. sp. MZUTI 2965 Mendez, Morona Santigo, Ecu
B. sp. MZUTI 3309 Canton, Morona Santigo, Ecu
B. sp. MZUTI 2673 Mendez, Morona Santigo, Ecu
B. sp. MZUTI 2674 Mendez, Morona Santigo, Ecu
B. sp. 34
B. sp. QCAZ 25592 Auca, Orellana, Ecu
B. sp. QCAZ 25268 Auca, Orellana, Ecu
B. sp. QCAZ 28404 Cuyabeno, Orellana, Ecu
B. sp. QCAZ 25386 La Selva, Orellana, Ecu
B. sp. QCAZ 25421 La Selva, Orellana, Ecu
B. sp. MZUTI 3526 Wildsumaco, Napo, Ecu
B. sp. QCAZ 25420 La Selva, Orellana, Ecu
B. sp. QCAZ 25387 La Selva, Orellana, Ecu
B. sp. QCAZ 25385 La Selva, Orellana, Ecu
B. sp. QCAZ 25422 La Selva, Orellana, Ecu
B. sp. QCAZ 25425 La Selva, Orellana, Ecu
B. sp. QCAZ 25426 La Selva, Orellana, Ecu
B. sp. 11
B. sp. KU217421 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25758 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25753 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25455 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25289 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25771 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25294 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25747 Jatun Sacha, Napo, Ecu
B. sp. QCAZ 25355 Sta Clara, Napo, Ecu
B. sp. QCAZ 25593 Kapawi, Pastaza, Ecu
B. sp. QCAZ 25319 Cando, Napo, Ecu
B. sp. QCAZ 25318 Cando, Napo, Ecu
B. sp. MZUTI 1650 Canton Tena, Napo, Ecu
B. sp. MZUTI 1603 Canton Tena, Napo, Ecu
B. sp. MZUTI 1602 Canton Tena, Napo, Ecu
B. sp. MZUTI 1648 Canton Tena, Napo, Ecu
B. sp. MZUTI 1651 Canton Tena, Napo, Ecu
B. sp. QCAZ 25317 Cando, Napo, Ecu
B. sp. QCAZ 25872 Cando, Napo, Ecu
B. sp. QCAZ 25320 Cando, Napo, Ecu
B. sp. 10
Clade 2
To other Amazonian species of Eladinea
50
Transformations

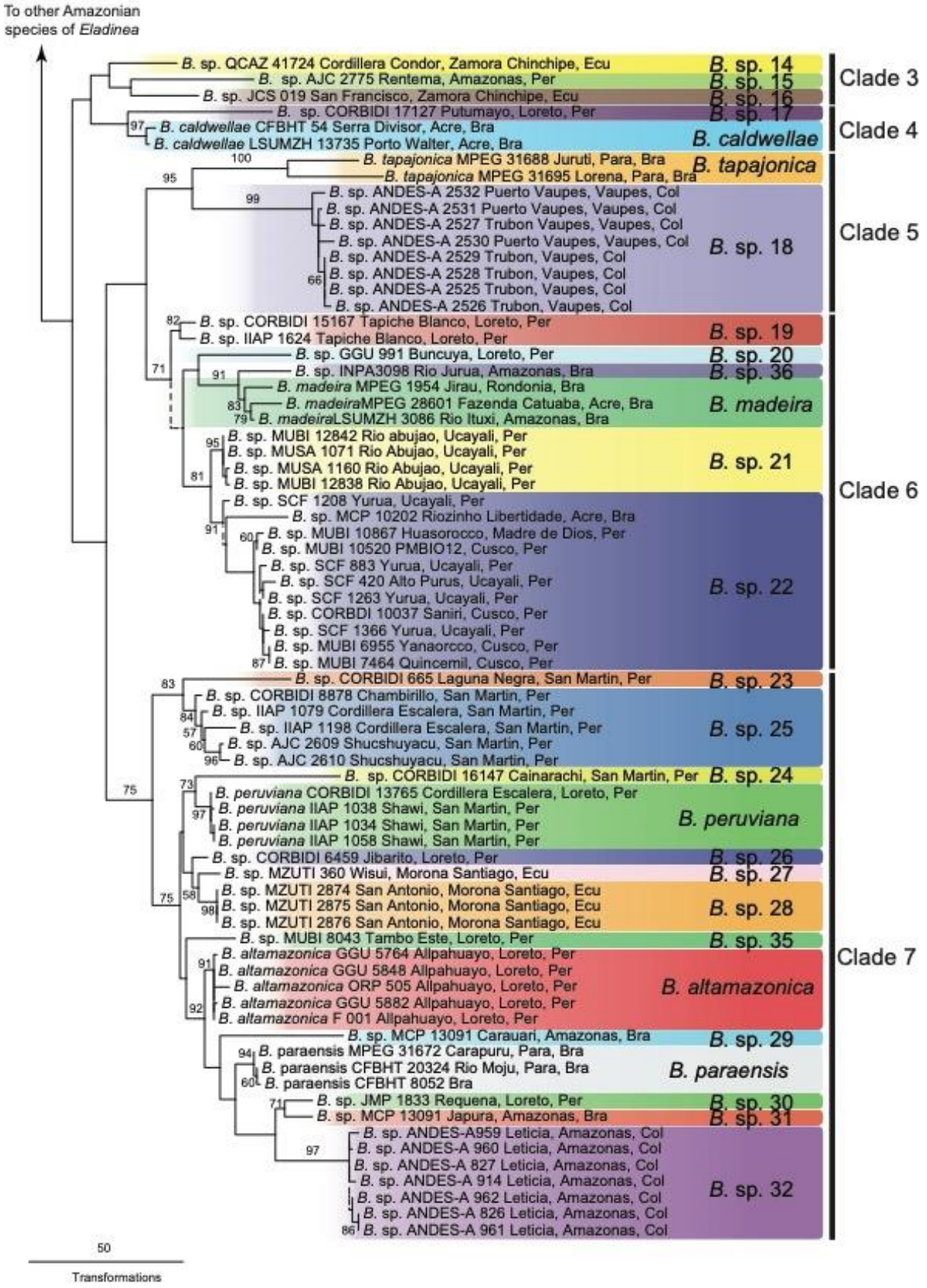
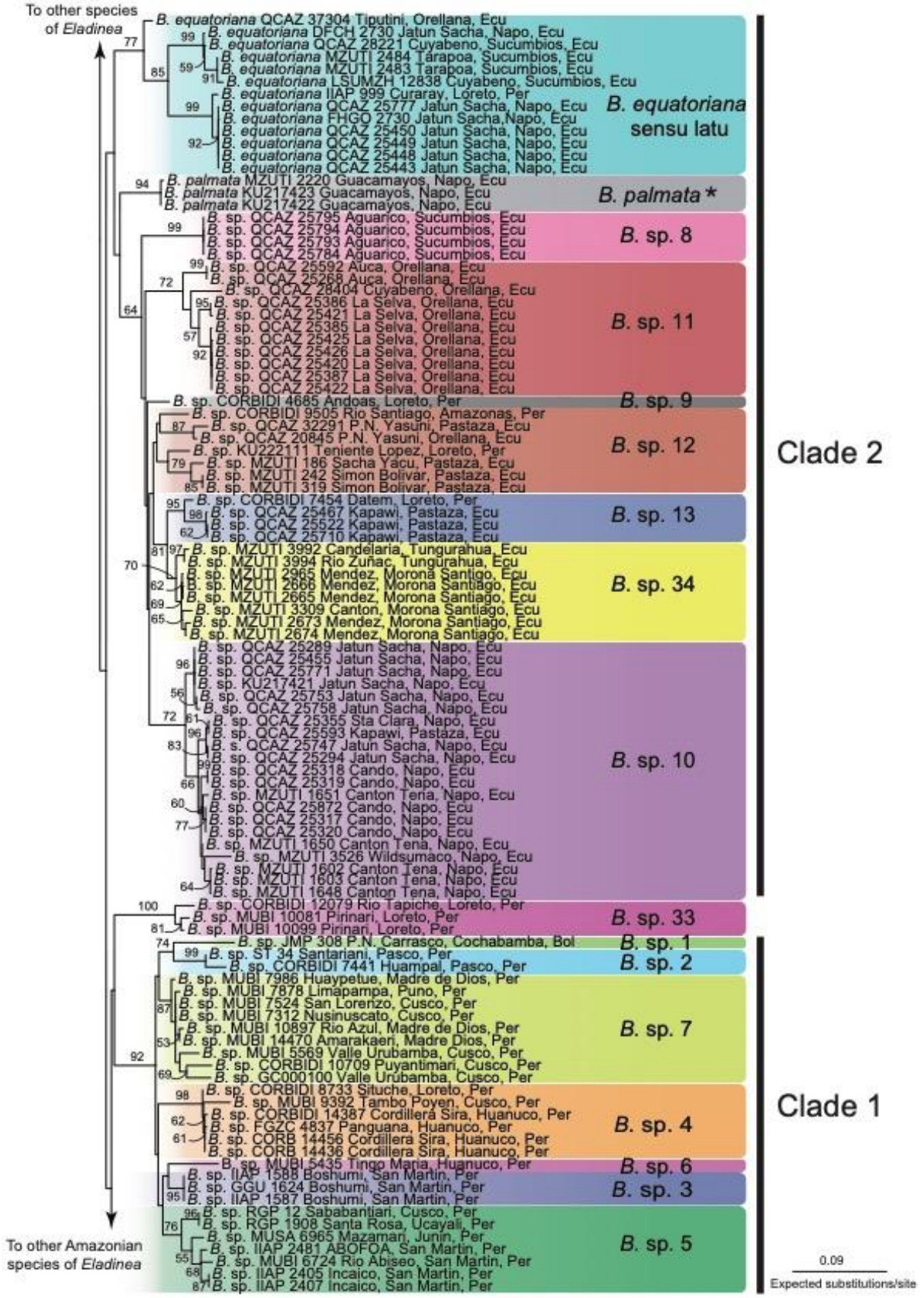


Figure 3. One of the 1,752 shortest trees (12,597 transformations) illustrating the relationships among Amazonian *Eladinea* and inferred from up to three mitochondrial (16S, COI, and cytb) and two nuclear (POMC and Rag1) genes from a tree-alignment parsimony analysis coding indels as a fifth character. Dashed lines indicate collapsed clades in the strict

consensus. Numbers on branches are jackknife frequencies of 1,000 searches. Nominal and candidate species according to this work are indicated with color rectangles. Clades 1 to 7 indicate groups with equal content in the parsimony analysis (except for *Bolitoglossa palmata*), see main text for discussion. This tree is a continuation of Fig. 2.



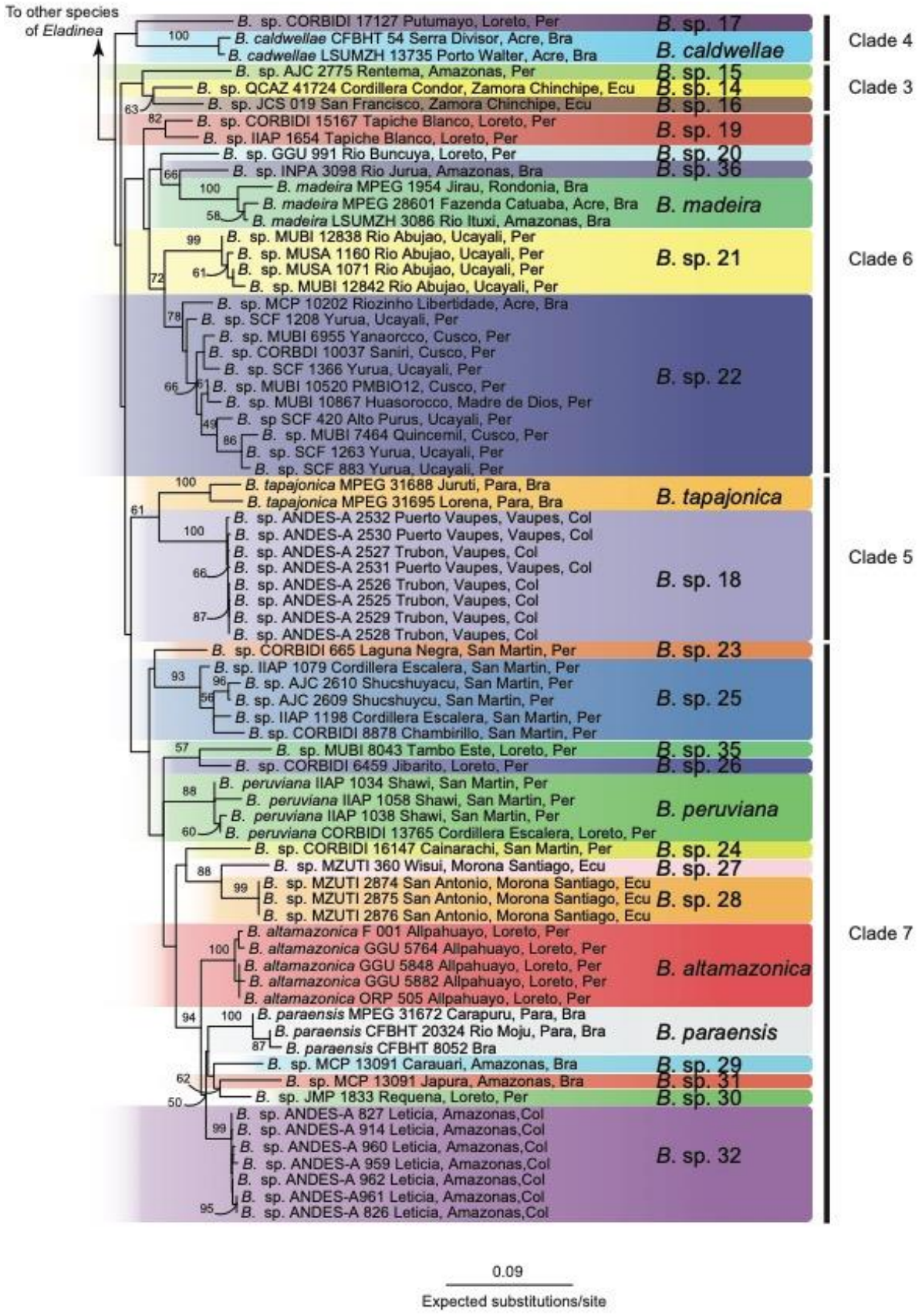


Figure 4. Maximum likelihood tree (log likelihood = - 5,9863.1) of Amazonian *Eladinea* inferred from up to three mitochondrial (16S, COI, and cytb) and two nuclear (POMC and Rag1) genes from a similarity alignment and considering indels as the longest possible binary characters. Numbers on branches are bootstrap frequencies of 1,000 pseudoreplicates.

Nominal and candidate species according to this work are indicated with color rectangles. Clades 1 to 7 indicate groups with equal content in the parsimony analysis (except for *Bolitoglossa palmata* marked with an asterisk); see main text for discussion. This tree is a continuation of Fig. 2.

Clade 1 (JK = 94) is sister to a clade formed by all other six clades (JK ≤ 50) and includes seven putative new species labelled *Bolitoglossa* sp. 1 to 7 (Fig. 3). None of the currently recognized species of *Bolitoglossa* from the Amazon is part of this clade. All specimens that are part of Clade 1 were found on the western Amazon basin between 236–1050 m a.s.l. following the arc described by the eastern slopes of the Andes from central Bolivia in the south to the Peruvian border with Ecuador in the North (Fig. 5). Relationships among them are not resolved (i.e., polytomy in the strict consensus). Clade 2 in parsimony (JK = 54) includes seven putative new species and *B. equatoriana sensu lato*. The samples that are part of Clade 2 are restricted to Ecuador and northern Peru between 187–1920 m a.s.l. (Fig. 5). Clade 3 (JK ≤ 50) includes *B.* sp. 14 to 16, with each species known from its own single locality on the eastern slopes of the Andes (664–1953 m a.s.l.) of southern Ecuador and northern Peru (Fig. 5). The sisters *B. caldwellae* and *B*. sp. 17, Clade 4 (JK ≤ 50), are known from one or two localities on the lowlands of the Jurua and Putumayo rivers, respectively. Clade 5 (JK = 95) is exclusively represented by lowland taxa (30–195 m a.s.l.); *B*. sp. 18 is from the Vaupés River in Colombia and *B. tapajonica* from the Tapajos River in eastern Amazonia. Clade 6 (JK = 71) contains *B. madeira* and five candidate species and is exclusively represented by lowland taxa (64–777 m a.s.l.) from Rio Tapiche in Loreto, Peru (*B*. sp. 19 and 20) to the foothills of the Andes in Madre de Dios, Peru following a north-south axis, and from there to the Rivers Juruá (*B*. sp. 36), Purús, and Madeira in Brazil (*B. madeira*). Clade 7 (JK = 75) includes *B*. *altamazonica*, *B*. *paraensis*, *B*. *peruviana*, and 11 candidate species from 12–1788 m a.s.l (Fig. 5). It includes western taxa associated with the

eastern slopes of the Andes of northern Peru and southern Ecuador—*B. peruviana*, *B*. sp. 23 and *B*. sp. 24 to 28—and seven species distributed along the axis of the Amazon from Requena, Loreto, Peru (*B*. sp. 30) in the west to the mouth of the Tocantins in Belém, Pará, Brazil (*B*. *paraensis*).

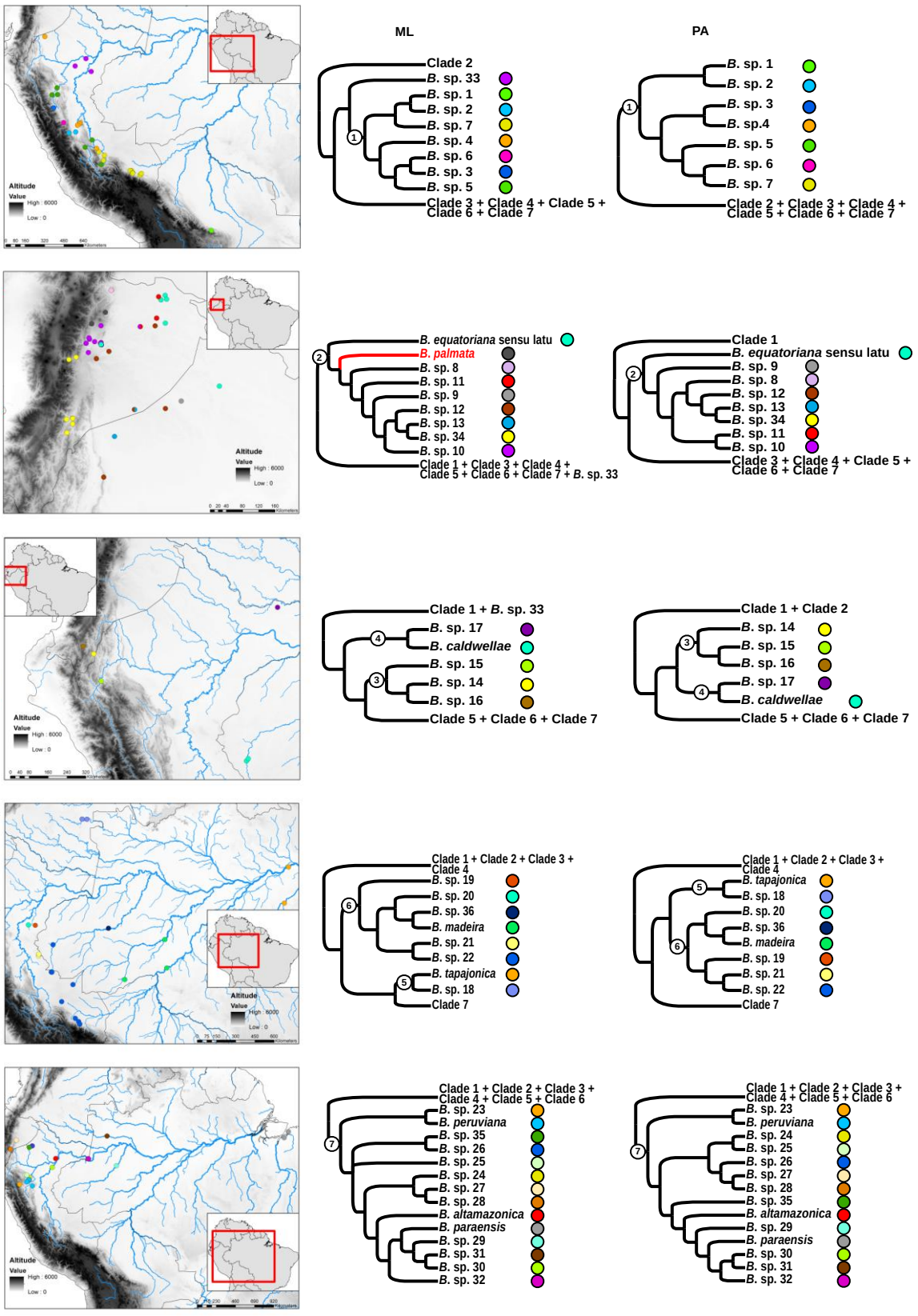


Figure 5. Maps illustrating the known localities of nominal and candidate species of *Bolitoglossa* from the Amazon basin according to the results of this study. Trees represent

schematic relationships according to maximum likelihood (ML) and parsimony analyses (see Figs. 2–4) and clade numbers follow those illustrated in Figs. 3 and 4.

Among outgroup taxa, the YBYRÁ analysis identified as the top wildcards (Supplementary data 5) the terminals of Bolitoglossa pacaya (all USAC series) and B. morio (USAC 1568 and MVZ 257825). These terminals are responsible for most of the incongruence among optimal topologies, resulting in a large polytomy that also includes the terminals of B. eremia and B. suchitanensis (Fig. 2). Most of these terminals are represented in our dataset only by 16S and cytb, indicating that, at this level of universality, either there is not enough information in these markers or that the information is contradictory. Other terminals represented in our dataset by these markers alone (i.e., B. adspersa MVZ 158485, B. aurae UCR 22842, B. palmata KU 217422, B. robusta MVZ 190830, B. tica UCR 20514 and B. zapoteca IBH 13375) were not recovered as wildcards. Within the ingroup, the terminal B. sp. MZUTI 3526 was the top potential wildcard. This terminal is only represented by sequences of 16S and Rag1 and causes the polytomy of B. sp. 10 and B. sp. 11 (Fig. 3). The terminal B. equatoriana QCAZ 37304, only represented by Rag1 in the dataset, was recovered as the 9th top wildcard terminal (Supplementary data 5) and causes the collapse of the B. equatoriana complex. Other wildcard terminals seem to rather collapse conspecific relationships, such as B. sp. MZUTI 1603 and 1650 within B. sp. 11 or the terminals belonging to B. yariguiensis.

3.2 Maximum likelihood

The similarity alignment of DNA sequences includes 3,252 transformation series and the binary block codifying indels an additional 126 (Supplementary data 6). The selected models and partition scheme are indicated in Table 1. Tree searches of the complete dataset in Garli found a single most likely tree (log likelihood = - 5,9863.1). The optimal tree

is shown in Figs. 2 and 4. As in the parsimony strict consensus, several shallow clades corresponding to intraspecific relationships are collapsed. Nonetheless, the optimal tree is well resolved with 17 % polytomies of 381 possible nodes of a fully bifurcating tree. Bootstrap values are ≥ 75 % in 50 clades and ≤ 50 % in 23 clades.

The optimal tree recovered all sampled genera monophyletic except *Pseudoeurycea*, which is paraphyletic with respect to *Ixalotriton niger*—as in parsimony, the latter species is sister to a clade formed by *P. cochranae*, *P. longicauda*, and *P. rex*. Relationships among the subgenera of *Bolitoglossa* (BS = 100) are similar to those of parsimony except that all currently recognized subgenera are monophyletic in ML (*Mayamandra* is non-monophyletic in parsimony) and that *Pachymandra* (BS = 100) is sister of *Magnadigita* (BS = 76)—sister of *Oaxakia* in parsimony). As in parsimony, *B*. (*Bolitoglossa*) *mexicana*, *B*. (*Magnadigita*) *eremia*, *B*. (*Magnadigita*) *flavimembris*, *B*. (*Magnadigita*) *morio*, and *B*. (*Magnadigita*) *pacaya* are non-monophyletic.

Table 1. Partition scheme, models of nucleotide substitution, and number of sites per partition selected by the PARTITIONFINDER analysis.

Partition	**Substitution model**	**# sites**
16S	GTR+I+G	560
COI, first position	TRN+I	196
COI, second position	TRN+G	196
COI, third position	SYM+G	195
Cytb, first position	TRN+I+G	269
Cytb, second position	GTR+G	269
Cytb, third position	SYM+I+G	269

POMC, first position	TRN+G	161
POMC, second position	GTR+I+G	160
POMC, third position	TRN+I	160
Rag1, first position	GTR+G	273
Rag1, second position	SYM+I+G	272
Rag1, third position	GTR+I+G	272

Within *Eladinea* (BS = 100), all species groups currently recognized are monophyletic with the exception of *B. schizodactyla* and *B. adspersa* groups due to the position of *B. compacta*. As in parsimony, this species of the *B. adspersa* group (Parra-Olea et al., 2004) is nested within the *B. schizodactyla* group (BS = 94). The relationships among the species groups of *Eladinea* are the same as in the parsimony results. Bootstrap values for the species groups are 94–100, while BS for the relationships among the species groups are 86–99 (Fig. 2).

As in parsimony, within the *adspersa* species group *B. biseriata* is non-monopyletic; however, and differently than in parsimony, *B. walkeri* is monophyletic. Regarding the salamanders of the Amazon basin, ML recovers all of them as a monophyletic group (BS ≤ 50) exclusive of salamanders from other regions. In this regard, it differs from the parsimony trees, where *B. palmata* and *B*. sp. 33 are more closely related to species from outside the Amazon basin. Although the parts of Clades 1 to 7 are identical between the results of parsimony and ML (except for the aforementioned inclusion of *B*. *palmata* in Clade 2 in the optimal ML tree), there are important differences regarding how these five clades are

related among them. The best ML tree recovers Clade 2 (BS ≤ 50) as sister to a group that includes Clades 3 to 7.

3.3 Species diversity of Amazonian salamanders

The clade containing all the Amazonian *salamanders* or their vast majority (ML and parsimony results, respectively) shows high levels of hierarchic structure corresponding to clades with relatively long branches and JK/BS ≥ 75 %. However, within each of them (shaded clades of Figs. 3 and 4), we found a combination of poorly resolved relationships (i.e., polytomies), shorter branches, and JK/BS ≤ 50 %. Furthermore, these monophyletic subdivisions show consistent geographic patterns (Fig. 5) and clades containing sequences of type specimens and/or topotypes of currently recognized species, many of which show phenotypic diagnostic characters (Brcko et al., 2013), clearly corresponding to some of these clades. Thus, our results are not compatible with the recognition of just nine independently evolving lineages at the population level—the current number of recognized species in the region—unless one is ready to consider rampantly non-monophyletic species of very large ranges across important geographic barriers (e.g., the Amazon and its main tributaries) and encompassing levels of interspecific morphological variation unknown in other clades of plethodontids. We prefer to explain the observed historical (i.e., topologies) and phenetic (i.e., genetic distances) patterns of nucleotide variation in consilience with the geographic distribution of the samples and the known morphological variation (e.g., Brcko et al., 2013) as compatible with the existence of up to 33 new species of *Bolitoglossa* in the Amazon basin. Within these 33 candidate species, the pairs *B*. sp. 1 and *B*.sp. 2 and *B*. sp. 10 and *B*. sp. 11 are not reciprocally monophyletic in the parsimony analysis.

In the first case, our single sample from Bolivia (*B*. sp. 1 JMP 308) forms a polytomy with two samples from Pasco, in central Peru in the parsimony consensus tree (JK = 81). On the other hand, the ML optimal tree recovers the two samples from Pasco as monophyletic (BS = 99) and sister of the sample from Bolivia (BS = 74). The branch corresponding to the Bolivian sample is much longer than those of the Pasco samples (in both parsimony and ML) and the genetic distance between the Pasco samples is 1.1 % for 16S (the only shared marker between them), while is 11.0 % between the Bolivian and the Pasco sample for cytb (the only shared marker between them). Taking into account the reciprocal monophyly in ML, the longer branch length and larger genetic distance of the Bolivian sample, and the large geographic gap between Carrasco, Bolivia and Pasco, Peru, we consider these specimens as part of two unconfirmed candidate species rather than of a single biological entity.

In the second case, the strict consensus of the most parsimonious trees collapses samples labelled *B*. sp. 10 and *B*. sp. 11 into a large polytomy. However, the ML optimal tree recovers them not only as reciprocally monophyletic but also as non-sister taxa, although the branches separating these clades in ML are short and with BS ≤ 50. It is also relevant that the samples forming clade *B*. sp. 10 are all from the Andean foothills (277–705 m a.s.l.) of Napo and Pastaza, Ecuador, while those within *B*. sp. 11 are all from the lowlands (≤ 277 m a.s.l.) of Orellana, Ecuador. Genetic distances for cytb (the only shared mitochondrial marker) within *B*. sp. 10 = 0.0–3.6 % and within *B*. sp. 11 = 0.0–5.5 %, while distances between samples of *B*. sp. 10 and *B*. sp. 11 = 8.7–12.1 %. The wildcard analysis recovered the terminal *B*. sp. MZUTI 3526 as the top potential wildcard within the ingroup. This terminal changes position between different places within *B*. sp. 10 and *B*. sp. 11 in the different most parsimonious trees, apparently causing the polytomy in the strict consensus.

The sample *B*. sp. MZUTI 3526 contains information just for 16S and Rag1 and this may be the cause of its wildcard behavior because samples of *B*. sp. 11 only share Rag1. Considering all the aforementioned factors, we preferred to maintain *B*. sp. 10 and *B*. sp. 11 as two different unconfirmed candidate species.

The situation with *Bolitoglossa equatoriana* is also partially unresolved because the sample *B. equatoriana* QCAZ 37304 from Tiputini, Napo (about 43 km in straight line to the type locality in Limón Cocha, Napo) causes a polytomy on the strict consensus of the parsimony optimal trees. This sample is one of the top 10 wildcard terminals of the ingroup, which is probably caused by being represented just by Rag1. The ML optimal tree places this sample as sister to a clade of samples from Cuyabeno, Jatun Sacha and Tarapoa (BS = 99) and another one with samples just from Jatun Sacha (BS = 99). Given the current situation and until more data are gathered for sample QCAZ 37304, we prefer to consider all the aforementioned samples as *B. equatoriana* sensu lato, although it is obvious that at least two independent lineages at the population level are present under this name.

The uncorrected genetic p-distances do not show a clear threshold value, neither for 16S nor for cytb, to differentiate intra and interspecific variation (Supplementary data 7). Among the nominal species, the minimal distance of type or topotype samples (excluding *B. equatoriana* sensu lato for the reasons outlined above) with other Amazonian salamanders ranges from 1.6–3.2 % and 5.1–10.4 % in 16S and cytb respectively (Supplementary data 7). Interestingly, the topotype samples of *B. altamazonica* and the sample *B. sp*. MCP 13091 from Japura, Brazil (*B*. sp. 29) show the lowest genetic distance, but both parsimony and ML recovered this specimen as more closely related to specimens of *B. paraensis* and candidate species from Requena, Peru and Leticia, Colombia (Figs. 3 and 4).

The ABGD analyses did not find a barcoding gap for 16S or cytb (Fig. 6). Based on this distribution of genetic distances, the program calculated the number of potential species using four threshold values of intraspecific divergences (Table 2). These thresholds are also inferred by the program from the data.

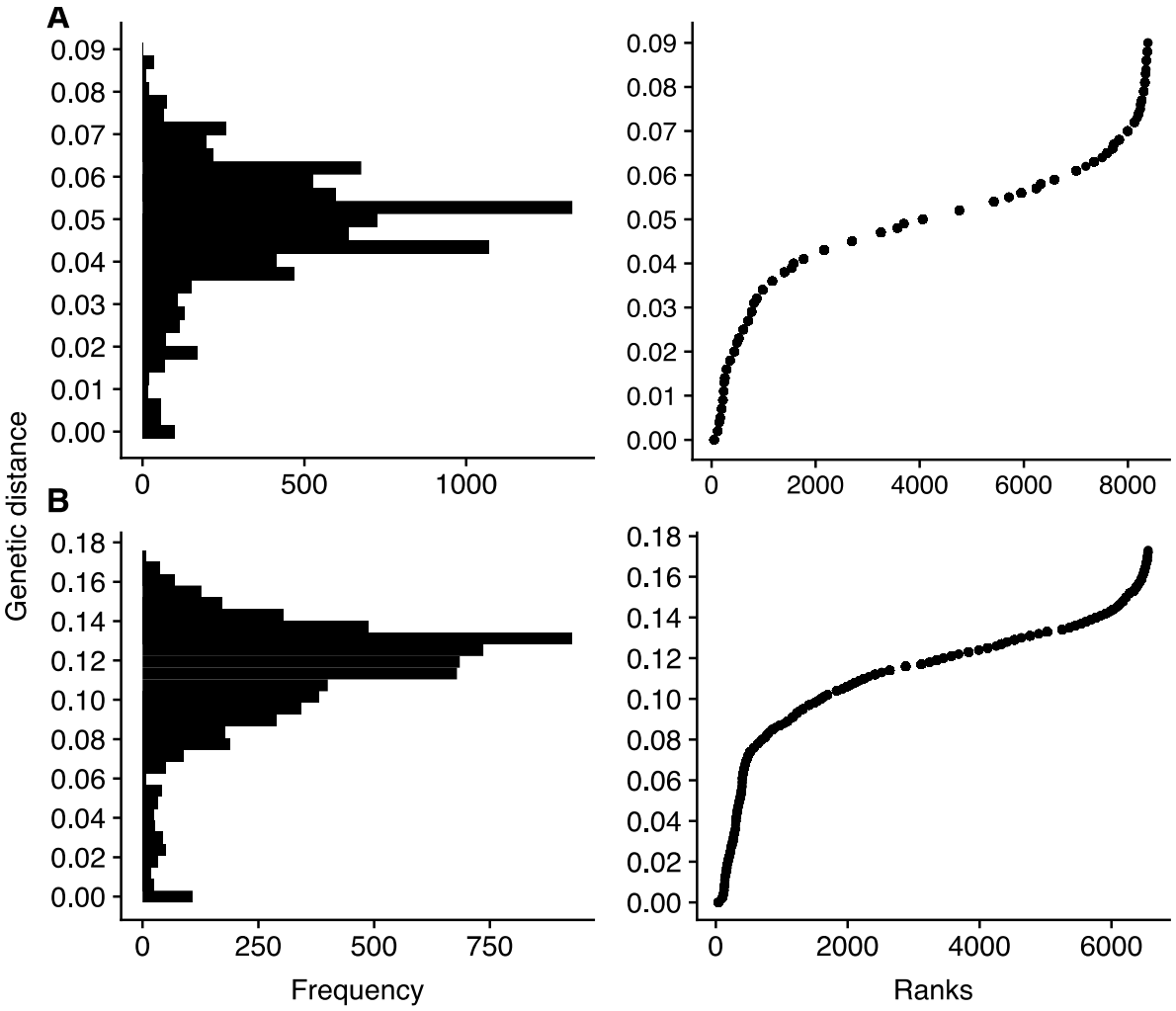


Figure 6. Results of the ABGD analyses for a similarity alignment of 16S (top row) and cytb (bottom row). Left column shows the frequency of genetic distances, while the right column illustrates the number of observations per value of genetic distances. Note the absence of a gap in all graphics.

Table 2. Number of sample clusters resulting from the ABGD analyses of a similarity alignment of 16S and cytb according to different values of intraspecific diversity (P) assigned by the program. Number of clusters can be used as a proxy to number of species, notwithstanding important assumptions.

	16S

Intraspecific diversity	P = 1.0 x 10^{-3}–4.5 x 10^{-3}	P = 6.5 x 10^{-3}	P = 9.4 X 10^{-3}	P = 1.4 x 10^{-2}	P = 2.0 x 10^{-2}
# clusters	53	41	25	23	2
	cytb				
Intraspecific diversity	P = 1.0 x 10^{-3}–6.0 x 10^{-3}	P = 9.4 x 10^{-3}–1.5 x 10^{-2}	P=2.3 x 10^{-2}	P=3.6 x 10^{-2}	P=5.7 x 10^{-2}
# clusters	52–55	49	41	29	1

However, according to Puillandre et al. (2012) and Pardo et al. (2014) the lowest and highest thresholds of an analysis can lead to trivial delimitations, where every terminal is considered a species or all terminals are included into a single one. Thus, we focused on the two intermediate values. It is worth noting that not all samples are represented by these markers so that some candidate species could not be evaluated by the ABGD analysis and that several of the putative species proposed by this approach are incompatible with the inferred evolutionary history represented by the optimal phylogenetic trees (Fig. 7). The mPTP analyses recognized 43 candidate species, including eight singletons, with the best score of multi coalescent rate of 1039.7 (Fig. 7).

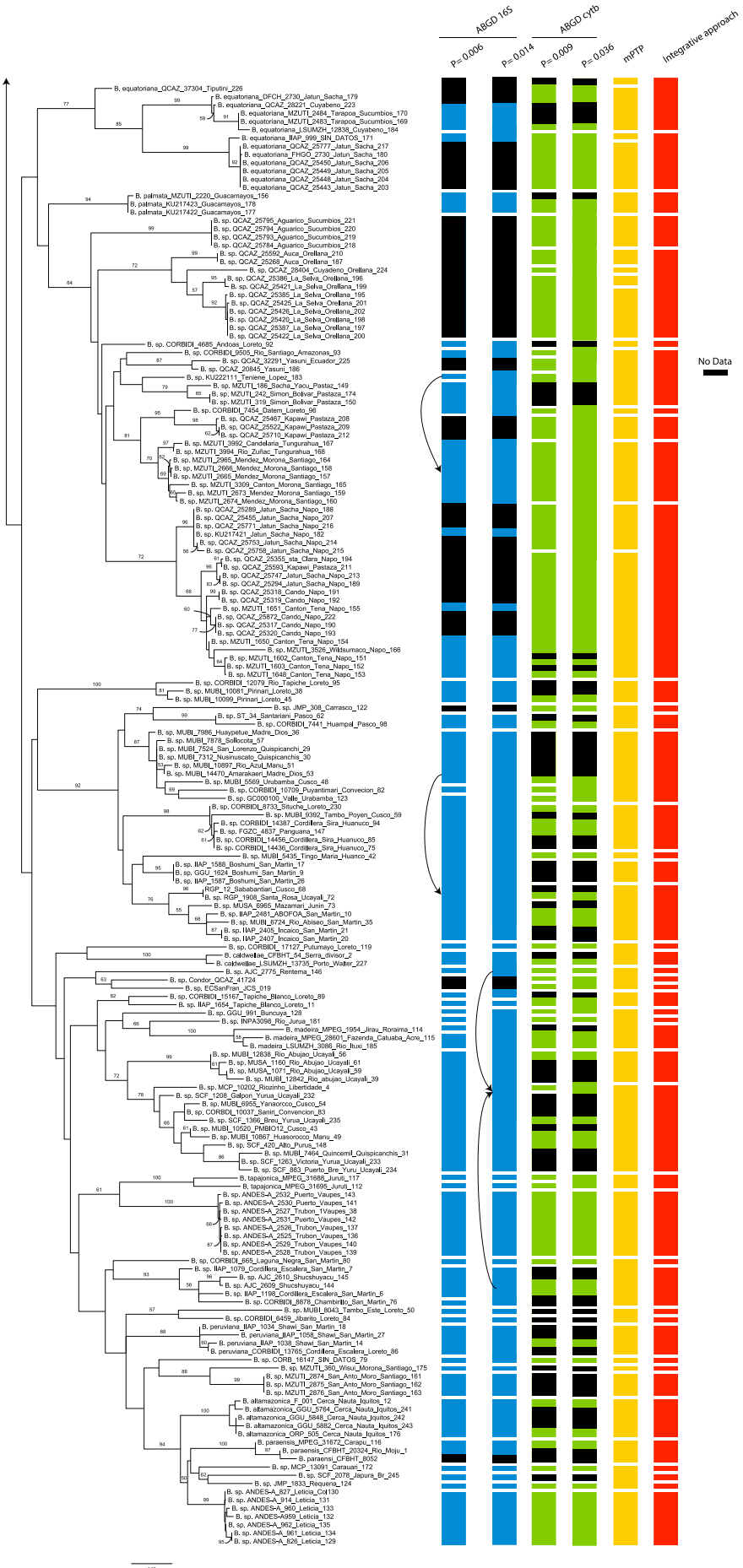

ABGD 16S
ABGD cytb
P= 0.006
P= 0.014
P= 0.009
P= 0.036
mPTP
Integrative approach
No Data
B. equatoriana_QCAZ_37304_Tiputini_226
B. equatoriana_DFCH_2730_Jatun_Sacha_179
B. equatoriana_QCAZ_28221_Cuyabeno_223
B. equatoriana_MZUTI_2484_Tarapoa_Sucumbios_170
B. equatoriana_MZUTI_2483_Tarapoa_Sucumbios_169
B. equatoriana_LSUMZH_12838_Cuyabeno_184
B. equatoriana_IIAP_999_SIN_DATOS_171
B. equatoriana_QCAZ_25777_Jatun_Sacha_217
B. equatoriana_FHGO_2730_Jatun_Sacha_180
B. equatoriana_QCAZ_25450_Jatun_Sacha_206
B. equatoriana_QCAZ_25449_Jatun_Sacha_205
B. equatoriana_QCAZ_25446_Jatun_Sacha_204
B. equatoriana_QCAZ_25443_Jatun_Sacha_203
B. palmata_MZUTI_2220_Guacamayos_156
B. palmata_KU217423_Guacamayos_178
B. palmata_KU217422_Guacamayos_177
B. sp. QCAZ_25795_Aguarico_Sucumbios_221
B. sp. QCAZ_25794_Aguarico_Sucumbios_220
B. sp. QCAZ_25793_Aguarico_Sucumbios_219
B. sp. QCAZ_25784_Aguarico_Sucumbios_218
B. sp. QCAZ_25592_Auca_Orellana_210
B. sp. QCAZ_25268_Auca_Orellana_187
B. sp. QCAZ_28404_Cuyadeno_Orellana_224
B. sp. QCAZ_25386_La_Selva_Orellana_196
B. sp. QCAZ_25421_La_Selva_Orellana_199
B. sp. QCAZ_25385_La_Selva_Orellana_195
B. sp. QCAZ_25425_La_Selva_Orellana_201
B. sp. QCAZ_25426_La_Selva_Orellana_202
B. sp. QCAZ_25420_La_Selva_Orellana_198
B. sp. QCAZ_25387_La_Selva_Orellana_197
B. sp. QCAZ_25422_La_Selva_Orellana_200
B. sp. CORBIDI_4685_Andoas_Loreto_92
B. sp. CORBIDI_9505_Rio_Santiago_Amazonas_93
B. sp. QCAZ_32291_Yasuni_Ecuador_225
B. sp. QCAZ_20845_Yasuni_186
B. sp. KU222111_Teniente_Lopez_183
B. sp. MZUTI_186_Sacha_Yacu_Pastaz_149
B. sp. MZUTI_242_Simon_Bolivar_Pastaza_174
B. sp. MZUTI_319_Simon_Bolivar_Pastaza_150
B. sp. CORBIDI_7454_Datem_Loreto_96
B. sp. QCAZ_25467_Kapawi_Pastaza_208
B. sp. QCAZ_25522_Kapawi_Pastaza_209
B. sp. QCAZ_25710_Kapawi_Pastaza_212
B. sp. MZUTI_3992_Candelaria_Tungurahua_167
B. sp. MZUTI_3994_Rio_Zuñac_Tungurahua_168
B. sp. MZUTI_2965_Mendez_Morona_Santiago_164
B. sp. MZUTI_2666_Mendez_Morona_Santiago_158
B. sp. MZUTI_2665_Mendez_Morona_Santiago_157
B. sp. MZUTI_3309_Canton_Morona_Santiago_165
B. sp. MZUTI_2673_Mendez_Morona_Santiago_159
B. sp. MZUTI_2674_Mendez_Morona_Santiago_160
B. sp. QCAZ_25289_Jatun_Sacha_Napo_188
B. sp. QCAZ_25455_Jatun_Sacha_Napo_207
B. sp. QCAZ_25771_Jatun_Sacha_Napo_216
B. sp. KU217421_Jatun_Sacha_Napo_182
B. sp. QCAZ_25753_Jatun_Sacha_Napo_214
B. sp. QCAZ_25758_Jatun_Sacha_Napo_215
B. sp. QCAZ_25355_sta_Clara_Napo_194
B. sp. QCAZ_25593_Kapawi_Pastaza_211
B. sp. QCAZ_25747_Jatun_Sacha_Napo_213
B. sp. QCAZ_25294_Jatun_Sacha_Napo_189
B. sp. QCAZ_25318_Cando_Napo_191
B. sp. QCAZ_25319_Cando_Napo_192
B. sp. MZUTI_1651_Canton_Tena_Napo_155
B. sp. QCAZ_25872_Cando_Napo_222
B. sp. QCAZ_25317_Cando_Napo_190
B. sp. QCAZ_25320_Cando_Napo_193
B. sp. MZUTI_1650_Canton_Tena_Napo_154
B. sp. MZUTI_3526_Wildsumaco_Napo_166
B. sp. MZUTI_1602_Canton_Tena_Napo_151
B. sp. MZUTI_1603_Canton_Tena_Napo_152
B. sp. MZUTI_1648_Canton_Tena_Napo_153
B. sp. CORBIDI_12079_Rio_Tapiche_Loreto_95
B. sp. MUBI_10081_Pirinari_Loreto_38
B. sp. MUBI_10099_Pirinari_Loreto_45
B. sp. JMP_308_Carrasco_122
B. sp. ST_34_Santariani_Pasco_62
B. sp. CORBIDI_7441_Huampal_Pasco_98
B. sp. MUBI_7986_Huaypetue_Madre_Dios_36
B. sp. MUBI_7878_Sollocota_57
B. sp. MUBI_7524_San_Lorenzo_Quispicanchi_29
B. sp. MUBI_7312_Nusinuscato_Quispicanchis_30
B. sp. MUBI_10897_Rio_Azul_Manu_51
B. sp. MUBI_14470_Amarakaeri_Madre_Dios_53
B. sp. MUBI_5569_Urubamba_Cusco_48
B. sp. CORBIDI_10709_Puyantimari_Convecion_82
B. sp. GC000100_Valle_Urubamba_123
B. sp. CORBIDI_8733_Sturiche_Loreto_230
B. sp. MUBI_9392_Tambo_Poyen_Cusco_59
B. sp. CORBIDI_14387_Cordillera_Sira_Huanuco_94
B. sp. FGZC_4637_Panguana_147
B. sp. CORBIDI_14456_Cordillera_Sira_Huanuco_85
B. sp. CORBIDI_14436_Cordillera_Sira_Huanuco_75
B. sp. MUBI_5435_Tingo_Maria_Huanco_42
B. sp. IIAP_1588_Boshumi_San_Martin_17
B. sp. GGU_1624_Boshumi_San_Martin_9
B. sp. IIAP_1587_Boshumi_San_Martin_26
RGP_12_Sababantari_Cusco_68
B. sp. RGP_1908_Santa_Rosa_Ucayali_72
B. sp. MUSA_6965_Mazamari_Junin_73
B. sp. IIAP_2481_ABOFOA_San_Martin_10
B. sp. MUBI_8724_Rio_Abiaso_San_Martin_35
B. sp. IIAP_2405_Incaico_San_Martin_21
B. sp. IIAP_2407_Incaico_San_Martin_20
B. sp. CORBIDI_17127_Putumayo_Loreto_119
B. caldwellae_CFBHT_54_Serra_divisor_2
B. caldwellae_LSUMZH_13735_Porto_Walter_227
B. sp. AJC_2775_Rentema_146
B. sp. Condor_QCAZ_41724
B. sp. ECSanFran_JCS_019
B. sp. CORBIDI_15167_Tapiche_Blanco_Loreto_89
B. sp. IIAP_1654_Tapiche_Blanco_Loreto_11
B. sp. GGU_991_Buncuya_128
B. sp. INPA3098_Rio_Jurua_181
B. madeira_MPEG_1954_Jirau_Roraima_114
B. madeira_MPEG_28601_Fazenda_Catuaba_Acre_115
B. madeira_LSUMZH_3086_Rio_Ituxi_185
B. sp. MUBI_12838_Rio_Abujao_Ucayali_56
B. sp. MUSA_1160_Rio_Abujao_Ucayali_61
B. sp. MUSA_1071_Rio_Abujao_Ucayali_59
B. sp. MUBI_12842_Rio_abujao_Ucayali_39
B. sp. MCP_10202_Riozinho_Libertidade_4
B. sp. SCF_1208_Gatpon_Yurua_Ucayali_232
B. sp. MUBI_6955_Yanarocco_Cusco_54
B. sp. CORBIDI_10037_Saniri_Convencion_83
B. sp. SCF_1366_Breu_Yurua_Ucayali_235
B. sp. MUBI_10520_PMBIO12_Cusco_43
B. sp. MUBI_10867_Huisorocco_Manu_49
B. sp. SCF_420_Alto_Purus_148
B. sp. MUBI_7464_Quincemil_Quispicanchis_31
B. sp. SCF_1263_Victoria_Yurua_Ucayali_233
B. sp. SCF_883_Puerto_Bre_Yuru_Ucayali_234
B. tapajonica_MPEG_31688_Juruti_117
B. tapajonica_MPEG_31695_Juruti_112
B. sp. ANDES-A_2532_Puerto_Vaupes_143
B. sp. ANDES-A_2530_Puerto_Vaupes_141
B. sp. ANDES-A_2527_Trubon_1Vaupes_38
B. sp. ANDES-A_2531_Puerto_Vaupes_142
B. sp. ANDES-A_2526_Trubon_Vaupes_137
B. sp. ANDES-A_2525_Trubon_Vaupes_136
B. sp. ANDES-A_2529_Trubon_Vaupes_140
B. sp. ANDES-A_2528_Trubon_Vaupes_139
B. sp. CORBIDI_665_Laguna_Negra_San_Martin_80
B. sp. IIAP_1079_Cordillera_Escalera_San_Martin_7
B. sp. AJC_2610_Shucshuyacu_145
B. sp. AJC_2609_Shucshuyacu_144
B. sp. IIAP_1198_Cordillera_Escalera_San_Martin_6
B. sp. CORBIDI_8878_Chambintin_San_Martin_76
B. sp. MUBI_8043_Tambo_Este_Loreto_50
B. sp. CORBIDI_6459_Jibarito_Loreto_84
B. peruviana_IIAP_1034_Shawi_San_Martin_16
B. peruviana_IIAP_1056_Shawi_San_Martin_27
B. peruviana_IIAP_1038_Shawi_San_Martin_14
B. peruviana_CORBIDI_13765_Cordillera_Escalera_Loreto_86
B. sp. CORB_16147_SIN_DATOS_79
B. sp. MZUTI_360_Wisui_Morona_Santiago_175
B. sp. MZUTI_2874_San_Anto_Moro_Santiago_161
B. sp. MZUTI_2875_San_Anto_Moro_Santiago_162
B. sp. MZUTI_2876_San_Anto_Moro_Santiago_163
B. altamazonica_F_001_Cerca_Nauta_Iquitos_12
B. altamazonica_GGU_5764_Cerca_Nauta_Iquitos_241
B. altamazonica_GGU_5848_Cerca_Nauta_Iquitos_242
B. altamazonica_GGU_5882_Cerca_Nauta_Iquitos_243
B. altamazonica_ORP_505_Cerca_Nauta_Iquitos_176
B. paraensis_MPEG_31672_Carapu_116
B. paraensis_CFBHT_20324_Rio_Moju_1
B. paraensi_CFBHT_8052
B. sp. MCP_13091_Carauari_172
B. sp. SCF_2078_Japura_Br_245
B. sp. JMP_1833_Requena_124
B. sp. ANDES-A_827_Leticia_Col130
B. sp. ANDES-A_914_Leticia_131
B. sp. ANDES-A_960_Leticia_133
B. sp. ANDES-A959_Leticia_132
B. sp. ANDES-A_962_Leticia_135
B. sp. ANDES-A_961_Leticia_134
B. sp. ANDES-A_826_Leticia_129

Figure 7. Maximum likelihood phylogenetic relationships of Amazonian *Bolitoglossa* with numbers on branches indicating bootstrap values. Bars on the right indicate inferred species according to different criteria, separated by white space. Arrows indicate terminals that were clustered inside other clades according to genetic distances.

Regarding nominal species, most of the analyses recovered as independent units the samples that are restricted to type localities or near them. The exceptions were *B. tapajonica* and *B. equatoriana*, where more than half of the analyses show at least two independent lineages due to high intraspecific genetic distances or long branches. The R_{tax} values for our data set was low for ABGD 16S 2 (R_{tax} = 0.37) and high in ABGD cytb 1 (R_{tax} = 0.79) and integrative approach (R_{tax} = 0.73), Table 3. Congruence among methods (C_{tax}) was highest between integrative approach and ABGD cytb 1 (C_{tax} = 0.80) and lowest between ABGD 16S2 and ABGD cytb 1 (C_{tax} = 0.48) Table 3. According to the aforementioned results, we opted to use the delimitation resulted from the integrative approach indicating the perceived confidence on the different candidate species by using the adjective unconfirmed when dealing with singletons and ambiguous monophyly, such as *B*. sp. 1, *B*. sp. 2, *B*. sp. 10 and *B*. sp. 11.

Table 3. Summary of performance of methods using the Relative Taxonomic Resolving Power Index (R_{tax}) and the Taxonomic Index of Congruence (C_{tax}).

Delimitation method	**Nº. species**	**Rtax**	**Mean Ctax**	**Ctax**				
				ABGD 16S 1	ABGD 16S 2	ABGD CYTB 1	ABGD CYTB 2	mPTP
ABGD 16S 1	41	0.66	0.74					
ABGD 16S 2	23	0.37	0.56	0.63				
ABGD Cytb 1	49	0.79	0.65	0.78	0.48			
ABGD Cytb 2	29	0.47	0.65	0.74	0.63	0.59		
mPTP	43	0.69	0.64	0.64	0.50	0.65	0.55	
Integrative	45	0.73	0.72	0.74	0.56	0.80	0.72	0.71
All speciation events	62							

3.4 Ancestral area reconstruction

The different species relationships inferred by both methods resulted in some differences in the ancestral area reconstructions of parsimony and ML (Fig. 8). However, the incongruences are minor and the most important biogeographic events are shared between reconstructions. Both biogeographic histories show a unique dispersion event from Central America to Chocó, explaining the presence of salamanders in South America. This was followed by one (parsimony) or two (ML) dispersions into the Andes from the Chocó and two dispersions from the Andes to the Amazon (Fig. 8). One contributed with just one (ML) or two species (parsimony), while the second dispersal event was followed by an impressive radiation of Amazonian salamanders (37–38 spp. according to our results) that went back into the highlands of the Andes in three (parsimony) or four occasions (ML).

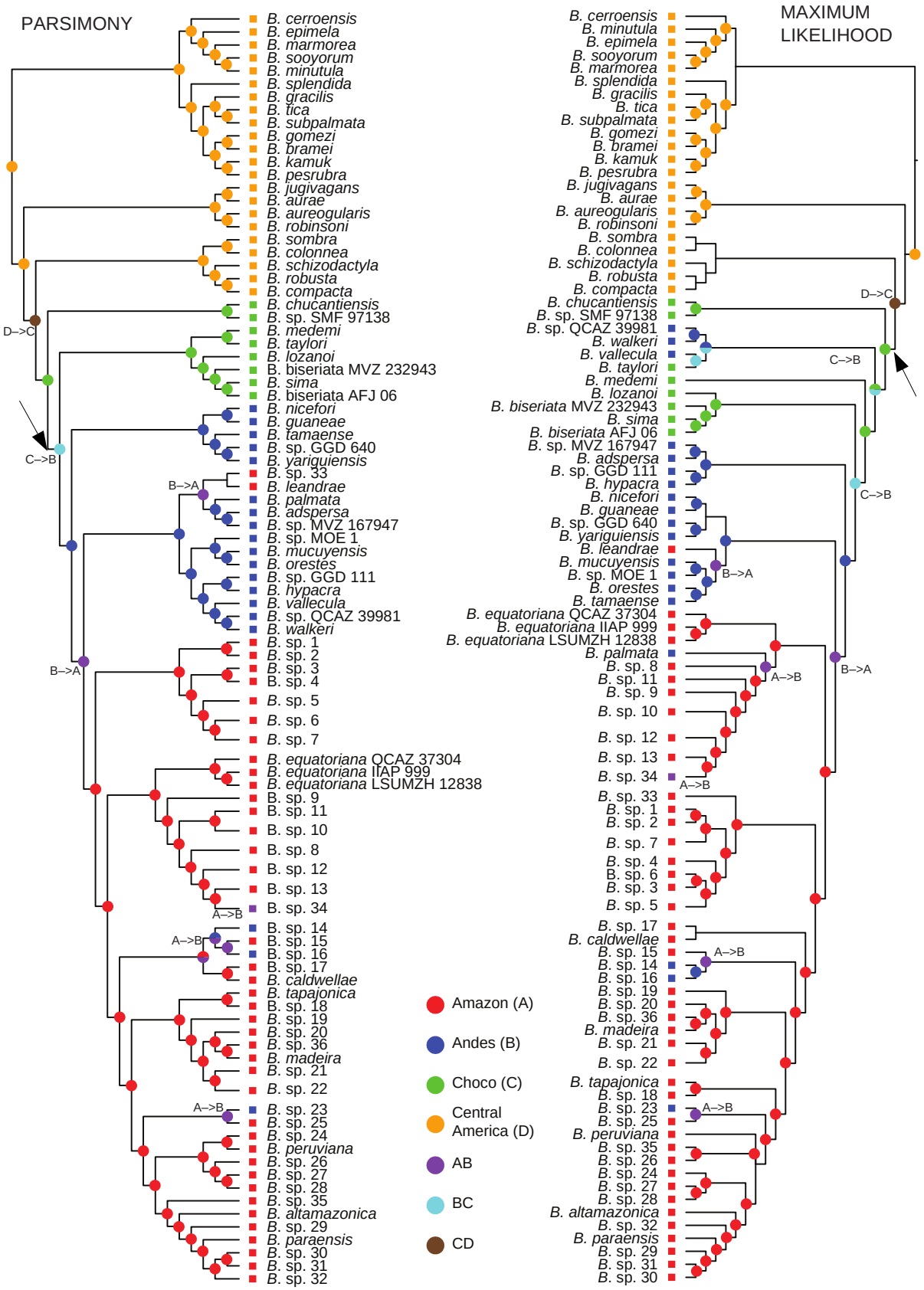


Figure 8. Ancestral area reconstruction of the subgenus *Eladinea* using one of the most parsimonius trees (left) and the most likely tree (right). In both cases, the tree was pruned to keep one terminal per species, except for *Bolitoglossa equatoriana* (see main text).

Polytomies were solved randomly, but the alternatives do not affect the result. Colors and letters indicate presence of a taxon in an area. Squares indicate distribution of terminals and circles inferred distribution of ancestors. Dispersals are marked with letters on the corresponding branches. Arrow indicates the inferred colonization of South America from Central America.

4. Discussion

4.1 *Phylogenetic relationships of* Bolitoglossa *within Bolitoglossini*

Two topologies resulted from each optimization method, *Bolitoglossa* sister of *Aquiloeurycea* (ML) or of a clade including *Aquiloeurycea*, *Pseudoeurycea*, and *Ixalotriton* (parsimony). Both alternatives have been inferred in previous studies using different analytical premises and datasets. For example, Rovito et al. (2015: Figs 4, 5) found *Bolitoglossa* as sister taxa of *Aquiloeurycea* + *Isthmura* (the latter not represented in our dataset), whereas Wiens et al. (2007), Pyron and Wiens (2011), Rovito et al. (2015: Figs 2, 3, 6) found *Bolitoglossa* as sister of more complex clades, in terms of supraspecific taxa, that at least include *Aquiloeurycea*, *Pseudoeurycea*, and *Ixalotriton*. A cursory review of the relevant literature reveals non-mutually exclusive factors that could be behind these differences—such as taxon and character sampling, optimality criteria, exhaustiveness of tree searches, treatment of indels, data partition schemes, model selection, and alignment parameters—and, without detailed sensitivity analysis, one cannot tell apart, by mere comparison of the results of previous studies, which factor or combination of them is causing the incongruence observed among different studies. Our study was designed with different objectives and our only germane contribution is that relationships among Bolitoglossini should be revisited in light of our new data.

4.2 *Phylogenetic relationships within Bolitoglossa*

After Parra-Olea et al. (2004), only a few studies have a comparable taxon sampling of *Bolitoglossa* (Wiens et al. 2007; Pyron and Wiens, 2011; Elmer et al. 2013). Nonetheless, all of them differ in important aspects of the relationships among subgenera and species within them, and even the monophyly of some subgenera has been questioned. For example, some *Magnadigita* species were nested within *Pachymandra* (Wiens et al. 2007; Elmer et al. 2013) or *Oaxakia* (Pyron and Wiens 2011). Our study, with 73 % of the currently described species and sequences from up to five genes, constitutes the largest effort to address the evolutionary relationship of *Bolitoglossa*. Despite important differences in our analytical assumptions regarding nucleotide homology, indel coding, and optimization criterion, the results of both analyses are very much congruent (although not identical) regarding the relationships among subgenera of *Bolitoglossa*. Both analyses agree in placing a monophyletic *Eladinea* as sister of a clade with the other six subgenera. Within the latter clade, the subgenera *Bolitoglossa* is sister of *Mayamandra* + *Nanotriton*, although in parsimony, *B*. (*Mayamandra*) *stuarti* is more closely related to *Nanotriton* than to other species of *Mayamandra*. Regarding the position of *B*. (*Mayamandra*) *stuarti*, it is relevant to note that this is the first time that this species is included in a large scale phylogenetic study of *Bolitoglossa* and that in our dataset is only represented by a single marker (609 nucleotides of cytb). We think that the amount of evidence is still too limited to implement nomenclatural changes, although future studies should revisit the generic placement of this taxon. The relationships among the three remaining subgenera are also different between the two analyses, with *Oaxakia* as sister of *Pachymandra* + *Magnadigita* in ML while in parsimony we retrieved a polytomy. Current implementations of ML and Bayesian posterior

probability perform a limited number of lower quality heuristic searches than advance search strategies in programs such as TNT (Goloboff and Pol, 2005; Goloboff, 2014), holding only one tree for every tree search and pseudoreplicate, at least in ML. As a result, unsupported clades may be resolved and a high BS value or clade posterior probability assigned to them (Goloboff and Pol, 2005; Simmons and Goloboff, 2013, 2014; Simmons and Randle, 2014; Sanderson et al., 2015; Dobrin et al., 2018). This undersamplig artifacts are more likely when analyzing supermatrices, consisting mostly or entirely of locally sampled characters, but that can also affect smaller and more complete matrices (Simmons and Goloboff, 2013). Thus, clades recovered as a polytomy by parsimony analyses and completely resolved by ML or Bayesian analyses must be interpreted cautiously. For example, Padial *et al*. (2014) provided a clear empirical case of such artifact with Terraranan frogs. At least for some ML implementations, new approaches are being developed to evaluate some of these cases (Biczok et al., 2018), although one needs a root with fully sampled characters, which our dataset lacks. Also, it could be argued that the increase in resolution observed in our ML results, when compared to parsimony, could be related to the expectations of homogeneity incorporated in the used models of our ML analysis. These expectations could count as evidence nucleotides that would be rendered uninformative under parsimony—the so called “multiple hits”. These two explanations are non-mutually exclusive and both could be behind the observed pattern of more polytomies in the strict consensus of the parsimony optimal trees.

Regardless of operational implementations, the core of the units currently recognized as subgenera within *Bolitoglossa* are stable across analyses of different studies and, although one should expect some changes as currently non-sampled species are included into phylogenetic analysis and more data are added (as in any clade of similar size),

perhaps systematists working with salamanders may consider in the near future a taxonomy where subgenera are treated as genera. It is not only that we found trinomens cumbersome (e.g., *Bolitoglossa* (*Bolitoglossa*) *lignicolor*), although favored by some (e.g., Pauly et al., 2009; Duellman and Trueb 2015), but *Bolitoglossa* is part of Plethodontidae and with 132 currently recognized species (many more awaiting description as reported herein) is a clear outlier with regards to its species richness. Current number of species per genera within Plethodontidae varies from one (e.g., *Phaeognathus*, *Stereochilus*) to 55 (i.e., *Plethodon*), with an average of about 16 spp. (see Frost, 2019). Obviously, neither taxonomies nor phylogenetic trees need to be balanced with regards to their contents, but considering that since Parra-Olea et al. (2004) more than 42 species of *Bolitoglossa* have been described and all studies dealing with the diversity of *Bolitoglossa* have focused on either species-level systematics of a restricted area (e.g., Acevedo-Rincón et al., 2013; Brcko et al., 2013) or macro-ecology and biogeographic questions (e.g., Wiens et al., 2007; Rovito et al., 2012), we suspect that the current and peculiar taxonomy of *Bolitoglossa* within its family—132 spp. in seven subgenera—reflects the absence of updated and detailed systematic reviews rather than any intrinsic property of the salamanders of this clade. Stability has an important role in taxonomy, but so does monophyly and comparative biology (Frost et al., 2009). If we agree that the canon of monophyly is achieved either way, it seems logical that as information (e.g., phenotypic synapomorphies, biogeographic history, conservation challenges) about different clades accumulates, the community chooses to carve smaller chunks as genera. After all, systematists have a responsibility to produce and communicate advances in the scientific knowledge of biological diversity to those outside of systematics; those non-systematic biologists—probably the vast majority—rely on formal evolutionary history translated into taxonomies to design their studies and interpret their results. This is

why systematists have moved from *Caecilia*, *Rana*, and *Salamandra* of Linnaeus (monophyletic units promoting maximum stability) into more than five hundred genera of amphibians to represent a diversity larger than 8000 species of amphibians (Frost et al., 2009; Frost 2019).

4.3 *Species richness of Amazonian salamanders*

With more than 6 million Km^2 (more than twice the area of India), the Pan-Amazonian lowlands constitute the largest uninterrupted stretch of tropical rainforest in the world. It also seems to be the most species diverse region, with amphibians as a clear example of this pattern. With reports of more than 100 species in less than 6 Km^2 (Bass et al., 2010), these amphibian communities have no rivals among other tropical ecosystems (Jenkins et al., 2013). However, this already outstanding amphibian species richness is drastically underestimated. Several studies with anurans document an unexpected high diversity of new species, representing an increase of 22–350 % of the known diversity (e.g., Fouquet et al., 2007a; Funk et al., 2012; Jungfer et al., 2013). Elmer et al. (2013) provided evidence in the form of DNA sequences that the diversity of Amazonian salamanders in Ecuador was higher than previously thought. Our results not only corroborate the findings of Elmer et al. (2013), but provide outstanding levels of previously unlooked species richness of salamanders. If we considered all candidate species in the Amazon basin alone, there will be 36 more species, an increase of 400 %. This result surpasses any previous estimation of amphibian cryptic diversity (Fouquet et al., 2007a; Fouquet et al., 2007b; Padial and De la Riva, 2009; Angulo and Icochea, 2010; Funk et al., 2012; Jungfer et al., 2013; Caminer and Ron, 2014; Fouquet et al., 2014; Gehara et al., 2014; Lourenço et al., 2015), and confirms

that *Bolitoglossa* is one of the most poorly studied amphibian groups. Even if the number of new species is smaller than our current inferences—after all, species are hypothesis that try to explain observed differences among organisms and as such they are prone to change as new data and theories are developed—one has to keep in mind than large portions of the Andes and the Amazonian lowlands remain to be explored (Mayer et al., 2019) and new species are likely to be discovered.

Our results have also important implications for the currently recognized Amazonian species of *Bolitoglossa*. The type locality of *B. altamazonica* is Nauta, Loreto, Peru, and our samples assigned to this species are from just ~ 50 km (straight line) from the type locality on a continuous stretch of forest without barriers. These samples of *B. altamazonica* are sister of a clade, in both ML and parsimony, that includes topotypes of *B. paraensis* and four candidate species (*B.* sp. 29, *B.* sp. 30, *B.* sp. 31, and *B.* sp. 32), showing genetic distance of 1.6–3.6 % in 16S and 5.1–6.8 % in cytb. However, the nearest samples (in a buffer radius of 250 km) to the type locality of *B. altamazonica* correspond to *B.* sp. 19, *B.* sp. 20, *B.* sp. 30, and *B.* sp.33. These lineages are all more distantly related to *B. altamazonica* (except the aforementioned *B*. sp. 30) than to other nominal species in both analyses and with larger genetic distances (3.2–7.7 % in 16S and 6.3–10.4 % in cytb). The only sample in the literature with DNA sequences identified as *B. altamazonica* (KU 222111 from Loreto, Peru; Parra-Olea et al., 2004; Elmer et al., 2013) is also distantly related to our samples of *B. altamazonica* (4.3–5.4% in 16S and 10.3–13.6% in cytb) and is herein considered part of *B.* sp. 12. With the evidence at hand, *B. altamazonia* has changed from a catchall name used for specimens from Venezuela to Bolivia and from Ecuador to Brazil into a micro-endemic species restricted to the terra firme forests between the rivers Nanay in the north, Tigre-Marañón in the south, and Amazonas in the west.

The type locality of *B. peruviana* is Moyobamba, San Martín, Peru. Our samples of *B. peruviana* come from Shawi, San Martín, Peru, located at approximately 41 km (straight line) from the region of the type locality. *Bolitoglossa peruviana* is sister of a putative new species from Cainarachi, San Martin, Peru (*B.* sp. 24) in parsimony (Fig. 3), while in ML is part of a polytomy (Fig. 4). The geographically nearest samples (in a buffer radius of 140 km) to the type locality of *B. peruviana* are those of *B.* sp. 5, *B.* sp. 23, *B.* sp. 24, and *B.* sp. 25, all distantly related except samples of *B. sp*. 24, with genetic distances of 2.9–3.2% in 16S and 8.7% in cytb. The Ecuadorian samples identified as *B*. cf*. peruviana* by Elmer et al. (2013) are also distantly related to our samples of *B. peruviana* and with considerable large genetic distances (5.4–6.5% in 16S and 10.6–14.0% in cytb). The Ecuadorian samples *B. cf. peruviana* of Elmer et al. (2013) are herein considered as part of three candidate new species (*B.* sp. 8, *B.* sp. 10, and *B.* sp. 11).

4.4 *Biogeography and diversification of South American salamanders*

Our results agree with previous studies that suggest or infer that *Bolitoglossa* colonized South America from Mesoamerica (Dunn, 1926; Brame and Wake, 1963; Wake and Lynch 1976; Parra-Olea et al. 2004; Elmer et al. 2013). However, our most relevant inferences relate to the biogeographic events within South America. First, we infer a clear pattern of dispersals between adjacent areas from Mesoamerica to the Chocó, from the Chocó into the Andes, and from the Andes into the Amazon. The presence of *Bolitoglossa* in the Amazonian lowlands is due to two independent dispersal events from the Andes. One is rather anecdotic in terms of diversification because it only explains the presence of one (*B. leandrae*, ML) or two species (*B. leandrae* and *B*. sp. 33, parsimony) in the Amazon. The

other dispersal originated a large radiation of Amazonian species (41 or 42 species, supported by parsimony and ML respectively). Contrary to other studies of amphibians (Castroviejo-Fisher et al. 2014; Mendoza et al. 2015; Santos et al. 2009), the Amazon was the source of more dispersals into the Andes (three to four) than the opposite. Similarly, Faivovich et al. (2005) suggested at least three hylid clades that may have radiated into the Andes after a dispersal event from lowland regions (i: *Hyloscirtus*, ii: *Boana pulchella* [as *Hypsiboas pulchellus*] group, and iii: the clade conformed by *Dendropsophus colombianus* and *B. labialis* groups).

The discovery of this large radiation of lowland salamanders in the Amazon bears important implications into the study of the mechanisms behind observed differences in species richness between regions and among clades. Plethodontids have been used as a model radiation to test general hypotheses of differences in species richness over space and time (review in Kozak, 2017). Generally, these studies rest upon two key assumptions related to the geographical pattern of species of plethodontids: (i) Species are concentrated in two hotspots, Appalachian and Mesoamerica highlands and (ii) most species are concentrated in midelevation habitats. Our study reports data that question these premises. First, the number of South American species currently recognized is vastly underestimated. From 35 nominal species of *Bolitoglossa* (Frost, 2019), we report up to 41 new species. If confirmed by future studies, South America would move from harboring 37 species of plethodontids (7.4 % of the current 475 species) to 78 species (14 % of 553 species). Furthermore, the greatest species richness within South America would concentrate in the lowland rainforest below 1,000 m a.s.l. (Fig. 9).

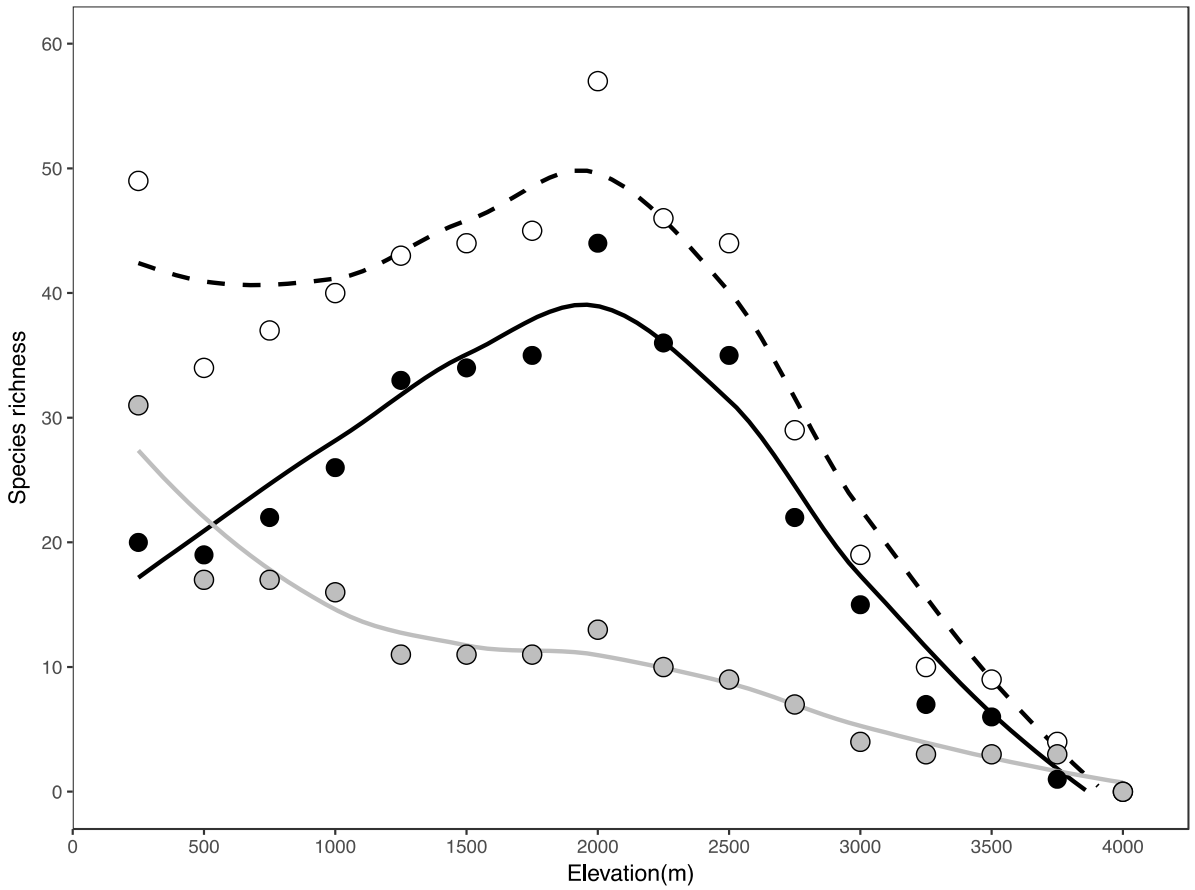


Figure 9. Elevational pattern of cumulative species richness of Plethodontidae globally (white), in Mesoamerica (black), and in South America (grey).

Researchers oriented to macro-ecological questions may find these new patterns of species richness of *Bolitoglossa* as intriguing and exciting, as we do. However, what we think is the great task ahead is to continue the study of the species-level systematics of *Bolitoglossa* and to provide additional information to evaluate and refine all these species hypotheses. Furthermore, although our study has greatly increased the sampling of salamanders in the Amazonian lowlands and midlands, the Andes of Colombia remain poorly sampled. Even our meager sampling of Andean salamanders indicates the presence of eight new species from Colombia, Ecuador, and Venezuela. All these facts taken together, clearly point out that species richness of salamanders in the Neotropics is not sufficiently

well known within each unit of comparison (regions or clades), which means that observed patterns have great potential to reflect our ignorance rather than our knowledge.

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Supplementary Information

Supplementary 1. List of samples used used in for GenBank with it respective assession number, and the new sequences generated (highlight in bold) with it respective information about collected locality

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa altamazonica	**F 1**	**Peru: Departamento Loreto: Cerca de Nauta**	**-4.097464**	**-73.478389**	**113**	**560 (46 indels)**	**-**	**742**	**481**	**817**	**2600 bp**
Bolitoglossa altamazonica	**GGU 5764**	**Peru: Departamento Loreto: Cerca de Nauta**	**-4.097464**	**-73.478389**	**113**	**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa altamazonica	**GGU 5848**	**Peru: Departamento Loreto: Cerca de Nauta**	**-4.097464**	**-73.478389**	**113**	**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa altamazonica	**GGU 5882**	**Peru: Departamento Loreto: Cerca de Nauta**	**-4.097464**	**-73.478389**	**113**	**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa altamazonica	**ORP 505**	**Peru: Departamento Loreto: Cerca de Nauta**	**-4.439165**	**-73,576,839**	**113**	**560 (46 indels)**	**-**	**782**	**481**	**754**	**2577 bp**
Bolitoglossa caldwellae	**CFBHT 54**	**Brazil: Estado Acre: Serra do Divisor**	**-8.35**	**-72.843**	**246**	**559 (46 indels)**	**-**	**-**	**-**	**-**	**559 bp**
Bolitoglossa caldwellae	LSUMZH 13735	Brazil: Estado Acre: 5 km N de Porto Walter	-8.258667	-72.776972	212	AY526129	-	AY526168	-	-	1168 bp
Bolitoglossa equatoriana sensu latu	DFCH 2730	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.08067	-77.60837	439	-	-	DQ353845	-	-	728 bp
Bolitoglossa equatoriana sensu latu	FHGO 2730	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.06499	-77.6142	117	-	-	DQ353846	-	-	722 bp
Bolitoglossa equatoriana sensu latu	**IIAP 999**	**Peru: Departamento Loreto: Provincia Maynas: río Curaray**	**-2.018356667**	**-74.96975889**	**175**	**560 (45 indels)**	**-**	**732**	**-**	**-**	**1292 bp**
Bolitoglossa equatoriana sensu latu	LSUMZH 12838	Ecuador: Provincia Sucumbios: Estación Científica University Católica, Cuyabeno	-0.00184	-76.17555	226	AY526130	-	AY526169	-	-	1142 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa equatoriana sensu latu	**MZUTI 2483**	**Ecuador: Provincia Sucumbios: PEER: Tara2, 11 km on Tarapoa road to Bloque Mariann 4**	**-0.09985**	**-76.26749**	**232**	**560 (42 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa equatoriana sensu latu	**MZUTI 2484**	**Ecuador: Provincia Sucumbios: PEER: Tara2, 11 km on Tarapoa road to Bloque Mariann 4**	**-0.09985**	**-76.26749**	**232**	**560 (42 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa equatoriana sensu latu	QCAZ 25443	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.08067	-77.60837	439	-	-	DQ353841	-	-	773 bp
Bolitoglossa equatoriana sensu latu	QCAZ 25448	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.06499	-77.6142	406	-	-	DQ353842	-	KC614451	1577 bp
Bolitoglossa equatoriana sensu latu	QCAZ 25449	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.06499	-77.6142	406	-	-	DQ353843	-	-	773 bp
Bolitoglossa equatoriana sensu latu	QCAZ 25450	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.08117	-77.60685	431	-	-	DQ353844	-	-	755 bp
Bolitoglossa equatoriana sensu latu	QCAZ 25777	Ecuador: Provincia Napo: Inner Vision Lodge, Río Arajuno	-1.095	-77.59917	430	-	-	DQ353840	-	-	762 bp
Bolitoglossa equatoriana sensu latu	QCAZ 28221	Ecuador: Provincia Sucumbios: Puerto Bolívar	-0.0886	-76.14204	221	-	-	KC614428	-	-	667 bp
Bolitoglossa equatoriana sensu latu	QCAZ 37304	Ecuador: Provincia Orellana: Reserva Tiputini	-0.61809	-76.17194	234	-	-	-	-	KC614452	804 bp
Bolitoglossa madeira	LSUMZH 3086	Brazil: Estado Amazonas: Rio Ituxi at the Madeireira Scheffer	-7.264519	-64.795298	64	AY526128	-	AY526167	-	-	1126 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa madeira	**MPEG 28601**	**Brazil: Estado Acre: Fazenda experimental Catuaba, Rio Branco**	**-10.076657**	**-67.616506**	**210**	**560 (45 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa madeira	**MPEG 1954**	**Brazil: Estado Rondonia: UHE-Jirau**	**-9.261841667**	**-64.66849167**	**107**	**534 (44 indels)**	**-**	**-**	**481**	**817**	**1832 bp**
Bolitoglossa palmata	KU 217422	Ecuador: Provincia Napo: Cordillera de los Guacamayos, 31 km to Baeza	-0.6338889	-77.8080556	1951	AY526125	-	AY526164	-	-	1205 bp
Bolitoglossa palmata	KU 217423	Ecuador: Provincia Napo: Cordillera de los Guacamayos, 31 km to Baeza	-0.6338889	-77.8080556	1951	AY526126	-	AY526165	-	-	1205 bp
Bolitoglossa palmata	**MZUTI 2220**	**Ecuador: Provincia Napo: Cordillera de los Guacamayos, La Virgen**	**-0.37693**	**-77.50488**	**1747**	**560 (46 indels)**	**-**	**-**	**-**	**817**	**1377 bp**
Bolitoglossa paraensis	CFBHT 8052					KU495161	KU494368	-	-	-	894 bp
Bolitoglossa paraensis	**CFBHT 20324**	**Brazil: Estado Para: Municipio Moju: Rio Moju**	**-1.854750288**	**-48.7513469**	**12**	**559 (45 indels)**	**-**	**-**	**-**	**-**	**559 bp**
Bolitoglossa paraensis	**MPEG 31672**	**Brazil: Estado Para: Municipio Santa Izabel: Sítio Semente Etérea, Vila do Carapuru**	**-1.202913889**	**-48.300675**	**34**	**560 (45 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa sp. 25	**CORBIDI 8878**	**Peru: Departamento San Martin: Provincia Picota: Chambirillo, Puesto de control 16, Parque Nacional Cordillera azul**	**-7.069138889**	**-76.01533333**	**1122**	**560 (44 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 25	**IIAP 1079**	**Peru: Departamento San Martin: Provincia Maynas: Distrito San Antonio de Cumbaza: Area de Conservacion Regional Cordillera Escalera**	**-6.387362**	**-76.372379**	**1200**	**526 (36 indels)**	**-**	**-**	**-**	**-**	**526 bp**

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 25	**IIAP 1198**	**Peru: Departamento San Martin: Provincia Maynas: Distrito San Antonio de Cumbaza: Area de Conservacion Regional Cordillera Escalera**	**-6.389512**	**-76.406655**	**485**	**560 (45 indels)**	**-**	**782**	**-**	**817**	**2159 bp**
Bolitoglossa sp. 25	**AJC 2609**	**Peru: Departamento San Martín: Shucshuyacu**	**-6.622**	**-76.615**	**537**	**560 (45 indels)**	**-**	**782**	**481**	**783**	**2606 bp**
Bolitoglossa sp. 25	**AJC 2610**	**Peru: Departamento San Martín: Shucshuyacu**	**-6.622**	**-76.615**	**537**	**560 (45 indels)**	**587**	**-**	**442**	**817**	**2406 bp**
Bolitoglossa sp. 1	**JMP 308**	**Bolivia: Departamento Cochabamba: Campamento los Guacharos, Parque Nacional Carrasco**	**-17.417339**	**-65.000847**	**924**	**-**	**587**	**724**	**481**	**817**	**2609 bp**
Bolitoglossa sp. 10	KU 217421	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.05	-77.60	406	AY526131	-	AY526170	-	-	1161 bp
Bolitoglossa sp. 10	**MZUTI 1602**	**Ecuador: Provincia Napo: Cantón Tena: Río Lupi, Hotel Establo de Don Tomás**	**-0.97654**	**-77.85875**	**546**	**560 (47 indels)**	**-**	**768 (2 'N')**	**-**	**808**	**2136 bp**
Bolitoglossa sp. 10	**MZUTI 1603**	**Ecuador: Provincia Napo: Cantón Tena: Río Lupi, Hotel Establo de Don Tomás**	**-0.97654**	**-77.85875**	**546**	**518 (46 indels)**	**-**	**-**	**-**	**-**	**518 bp**
Bolitoglossa sp. 10	**MZUTI 1648**	**Ecuador: Provincia Napo: Cantón Tena: Río Pashimbí, road to El Colonso**	**-0.94615**	**-77.86640**	**621**	**560 (48 indels)**	**-**	**782**	**-**	**817**	**2159 bp**
Bolitoglossa sp. 10	**MZUTI 1650**	**Ecuador: Provincia Napo: Cantón Tena: Parroquia Misahuallí: Río Quillayacu**	**-1.03012**	**-77.74275**	**430**	**560 (47 indels)**	**-**	**-**	**-**	**817**	**1377 bp**
Bolitoglossa sp. 10	**MZUTI 1651**	**Ecuador: Provincia Napo: Cantón Tena: Parroquia Misahuallí: Río Quillayacu**	**-1.03012**	**-77.74275**	**430**	**560 (47 indels)**	**-**	**782 (1 'N')**	**-**	**808 (1 'N')**	**2150 bp**
Bolitoglossa sp. 10	QCAZ 25289	Ecuador: Provincia Napo: Inner Vision Lodge, Río Arajuno	-1.10183	-77.593	389	-	-	DQ353826	-	-	740 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 10	QCAZ 25294	Ecuador: Provincia Napo: Inner Vision Lodge, Río Arajuno	-1.10183	-77.59317	393	-	-	DQ353816	-	-	762 bp
Bolitoglossa sp. 10	QCAZ 25317	Ecuador: Provincia Napo: Cando	-1.067	-77.93315	705	-	-	DQ353822	-	-	719 bp
Bolitoglossa sp. 10	QCAZ 25318	Ecuador: Provincia Napo: Cando	-1.067	-77.93315	705	-	-	DQ353824	-	-	719 bp
Bolitoglossa sp. 10	QCAZ 25319	Ecuador: Provincia Napo: Cando	-1.067	-77.93315	705	-	-	DQ353823	-	-	744 bp
Bolitoglossa sp. 10	QCAZ 25320	Ecuador: Provincia Napo: Cando	-1.067	-77.93315	705	-	-	DQ353821	-	KC614445	1548 bp
Bolitoglossa sp. 10	QCAZ 25355	Ecuador: Provincia Pastaza: Santa Clara, Finca de Tapia	-1.271231	-77.882846	680	-	-	DQ353818	-	-	734 bp
Bolitoglossa sp. 10	QCAZ 25455	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.06499	-77.6142	406	-	-	DQ353829	-	-	740 bp
Bolitoglossa sp. 10	QCAZ 25593	Ecuador: Provincia OrellanaDayuma: AUCA 14, EcoCiencia	-0.6975	-76.72983	277	-	-	DQ353819	-	KC614444	1517 bp
Bolitoglossa sp. 10	QCAZ 25747	Ecuador: Provincia Napo: Estación Biológica Jatun Sacha	-1.06499	-77.6142	406	-	-	DQ353817	-	-	757 bp
Bolitoglossa sp. 10	QCAZ 25753	Ecuador: Provincia Napo: Inner Vision Lodge, Río Arajuno	-1.10133	-77.59508	426	-	-	DQ353827	-	KC614446	1535 bp
Bolitoglossa sp. 10	QCAZ 25758	Ecuador: Provincia Napo: Inner Vision Lodge, Río Arajuno	-1.10133	-77.59508	426	-	-	DQ353825	-	-	728 bp
Bolitoglossa sp. 10	QCAZ 25771	Ecuador: Provincia Napo: Inner Vision Lodge, Río Arajuno	-1.0965	-77.59783	426	-	-	DQ353828	-	-	740 bp
Bolitoglossa sp. 10	QCAZ 25872	Ecuador: Provincia Napo: Cando, north of Serena	-1.067	-77.93315	705	-	-	DQ353820	-	-	751 bp
Bolitoglossa sp. 10/sp. 11	**MZUTI 3526**	**Ecuador: Provincia Napo: Wildsumaco Lodge**	**-0.67570**	**-77.60129**	**1485**	**560 (47 indels)**	**-**	**-**	**-**	**817**	**1377 bp**
Bolitoglossa sp. 11	QCAZ 25268	Ecuador: Provincia OrellanaDayuma: AUCA 14, EcoCiencia	-0.6975	-76.72983	277	-	-	DQ353830	-	KC614447	1491 bp
Bolitoglossa sp. 11	QCAZ 25385	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353835	-	KC614449	1534 bp
Bolitoglossa sp. 11	QCAZ 25386	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353833	-	-	762 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 11	QCAZ 25387	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353836	-	KC614450	1577 bp
Bolitoglossa sp. 11	QCAZ 25420	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353838	-	-	567 bp
Bolitoglossa sp. 11	QCAZ 25421	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353832	-	-	744 bp
Bolitoglossa sp. 11	QCAZ 25422	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353834	-	-	767 bp
Bolitoglossa sp. 11	QCAZ 25425	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353839	-	-	740 bp
Bolitoglossa sp. 11	QCAZ 25426	Ecuador: Provincia Orellana: La Selva Lodge	-0.50868	-76.36493	234	-	-	DQ353837	-	-	730 bp
Bolitoglossa sp. 11	QCAZ 25592	Ecuador: Provincia OrellanaDayuma: AUCA 14, EcoCiencia	-0.6975	-76.72983	277	-	-	DQ353831	-	KC614448	1571 bp
Bolitoglossa sp. 11	QCAZ 28404	Ecuador: Provincia Sucumbios: Monte Tour, Río Cuyabeno bridge	-0.0315	-76.32111	239	-	-	KC614429	-	KC614454	1353 bp
Bolitoglossa sp. 12	**CORBIDI 9505**	**Peru: Departamento Amazonas: Provincia Condorcanqui: Distrito Río Santiago: Quebrada Kampankis**	**-4.043083333**	**-77.54119444**	**325**	**560 (47 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa sp. 12	KU 222111	Peru: Departamento Loreto: 1.5 km N Teniente Lopez	-2.5167	-76.1667	263	AY526117	-	AY526160	-	-	1168 bp
Bolitoglossa sp. 12	**MZUTI 186**	**Ecuador: Provincia Pastaza: Comunidad Simón Bolivar, Sacha Yacu**	**-1.40712**	**-77.70351**	**841**	**560 (48 indels)**	**-**	**-**	**-**	**808**	**1368 bp**
Bolitoglossa sp. 12	**MZUTI 242**	**Ecuador: Provincia Pastaza: Comunidad Simón Bolivar, Sacha Yacu**	**-1.4097**	**-77.70396**	**816**	**560 (48 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 12	**MZUTI 319**	**Ecuador: Provincia Pastaza: Comunidad Simón Bolivar**	**-1.40712**	**-77.70351**	**878**	**560 (48 indels)**	**-**	**-**	**-**	**817**	**1377 bp**
Bolitoglossa sp. 12	QCAZ 20845	Ecuador: Provincia Orellana: Estación Científica Yasuní PUCE, km 7.5 to Tivacuno	-0.6785	-76.39633	247	-	-	KC614427	-	KC614453	1471 bp
Bolitoglossa sp. 12	QCAZ 32291	Ecuador: Provincia Pastaza: Kapawi Lodge	-2.53867	-76.85833	255	-	-	KC614430	-	KC614455	1471 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 13	**CORBIDI 7454**	**Peru: Departamento Loreto: Provincia Datem: Sector 3**	**-3.137429167**	**-77.30009833**	**212**	**560 (47 indels)**	**-**	**782**	**481**	**-**	**1823 bp**
Bolitoglossa sp. 13	QCAZ 25467	Ecuador: Provincia Pastaza: Kapawi Lodge	-2.53867	-76.85833	255	-	-	DQ353811	-	-	666 bp
Bolitoglossa sp. 13	QCAZ 25522	Ecuador: Provincia Pastaza: Kapawi Lodge	-2.53867	-76.85833	255	-	-	DQ353809	-	KC614442	1560 bp
Bolitoglossa sp. 13	QCAZ 25710	Ecuador: Provincia Pastaza: Kapawi Lodge	-2.53867	-76.85833	255	-	-	DQ353810	-	-	707 bp
Bolitoglossa sp. 14	QCAZ 41724	Ecuador: Provincia Zamora-Chinchipe: Parroquia Zurmi: Las Orquídeas, Tepuy, campamento dos	-4.26	-78.68	1429	-	-	KC614432	-	KC614456	1471 bp
Bolitoglossa sp. 15	**AJC 2775**	**Peru: Departamento Amazonas: Pongo de Rentema, Bagua-Sara Merisa road, stream before La Oliva**	**-5.301**	**-78.396**	**664**	**560 (46 indels)**	**-**	**768**	**481**	**817**	**2626 bp**
Bolitoglossa sp. 16	ECSanFran-JCS 19	Ecuador: Provincia Zamora Chinchipe: Estación Científica San Francisco	-3.97	-79.08	1953	-	-	KC699921	-	KC699927	1390 bp
Bolitoglossa sp. 17	**CORBIDI 17127**	**Peru: Departamento Loreto: Provincia Putumayo: Distrito Putumayo: Comunidad El Estrecho, Bufeo stream camp**	**-2.48025**	**-71.654139**	**97**	**526 (37 indels)**	**-**	**782**	**481**	**817**	**2606 bp**
Bolitoglossa sp. 18	**ANDES-A 2525**	**Colombia: Departamento Vaupes: Comunidad Trubón, Río Vaupes**	**1.21**	**-70.62**	**195**	**560 (46 indels)**	**587**	**782**	**-**	**817**	**2746 bp**
Bolitoglossa sp. 18	**ANDES-A 2526**	**Colombia: Departamento Vaupes: Comunidad Trubón, Río Vaupes**	**1.21**	**-70.62**	**195**	**560 (46 indels)**	**587**	**750**	**481**	**817**	**3195 bp**
Bolitoglossa sp. 18	**ANDES-A 2527**	**Colombia: Departamento Vaupes: Comunidad Trubón, Río Vaupes**	**1.21**	**-70.62**	**195**	**560 (46 indels)**	**541**	**782**	**443**	**817**	**3143 bp**
Bolitoglossa sp. 18	**ANDES-A 2528**	**Colombia: Departamento Vaupes: Comunidad Trubón, Río Vaupes**	**1.21**	**-70.62**	**195**	**560 (46 indels)**	**587**	**782**	**481**	**-**	**2410 bp**

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 18	ANDES-A 2529	Colombia: Departamento Vaupes: Comunidad Trubón, Río Vaupes	1.21	-70.62	195	560 (46 indels)	587	782	481	817	3227 bp
Bolitoglossa sp. 18	ANDES-A 2530	Colombia: Departamento Vaupes: Comunidad Puerto Vaupes, Río Vaupes	1.198	-70.281	177	560 (46 indels)	587	782 (3 indels)	481	817	3227 bp
Bolitoglossa sp. 18	ANDES-A 2531	Colombia: Departamento Vaupes: Comunidad Puerto Vaupes, Río Vaupes	1.198	-70.281	177	560 (46 indels)	587	782	481	817	3227 bp
Bolitoglossa sp. 18	ANDES-A 2532	Colombia: Departamento Vaupes: Comunidad Puerto Vaupes, Río Vaupes	1.198	-70.281	177	560 (46 indels)	587	782	481	817	3227 bp
Bolitoglossa sp. 19	CORBIDI 15167	Peru: Departamento Loreto: Provincia Requena: Distrito Tapiche: Tapiche - Blanco	-6.265	-73.91	161	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 19	IIAP 1654	Peru: Departamento Loreto: Provincia Requena: Distrito Tapiche: Tapiche - Blanco	-6.265	-73.91	161	560 (45 indels)	-	782	481	817	2640 bp
Bolitoglossa sp. 2	CORBIDI 7441	Peru: Departamento Pasco: Provincia Oxapampa: Pan de azucar, Huampal	-10.18416667	-75.57416667	1050	560 (45 indels)	-	782	481	817	2640 bp
Bolitoglossa sp. 2	ST 34	Peru: Departamento Pasco: Provincia Oxapampa: Santariani, Ciudad Constitución	-10.110183038	-75.08608192	231	560 (46 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 20	GGU 991	Peru: Departamento Loreto: Provincia Requena: Rio Buncuya	-6.23333	-74.4	128	560 (45 indels)	587	782	481	817	3227 bp
Bolitoglossa sp. 21	MUBI 12838	Peru: Departamento Ucayali: Provincia Coronel Portillo: Distrito Callaria: Cuenca del río Abujao, 95 Km E to Pucallpa	-8.352719	-73.680296	213	560 (44 indels)	-	782	481	817	2640 bp
Bolitoglossa sp. 21	MUBI 12842	Peru: Departamento Ucayali: Provincia Coronel Portillo: Distrito Callaria: Cuenca del río Abujao, 95 Km E to Pucallpa	-8.352719	-73.680296	213	560 (44 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 21	MUSA 1071	Peru: Departamento Ucayali: Provincia Coronel Portillo: Río Abujao	-8.412267377	-73.69093817	236	560 (44 indels)	-	-	-	-	560 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 21	MUSA 1160	Peru: Departamento Ucayali: Provincia Coronel Portillo: Río Abujao	-8.287816176	-73.67457545	248	560 (44 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 22	CORBIDI 10037	Peru: Departamento Cusco: Provincia La Convencion: Saniri, Malvinas	-11.63102778	-72.05938889	386	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 22	MCP 10202	Brazil: Estado Acre: Municipio Cruzeiro do Sul: Reserva Extravista Riozinho Libertidade	-7.7119444	-72.0036111	182	560 (45 indels)	-	782	481	-	1823 bp
Bolitoglossa sp. 22	MUBI 10520	Peru: Departamento Cusco: PMBIO 12, He - 03	-12.81422222	-71.10422222	471	526 (36 indels)	-	-	-	-	526 bp
Bolitoglossa sp. 22	MUBI 10867	Peru: Departamento Madre de Dios: Provincia Manu: Distrito Manu: near to Rio Huasorocco, Huasorocco	-13.040941	-70.867284	777	560 (45 indels)	-	782	481	817	2640 bp
Bolitoglossa sp. 22	MUBI 6955	Peru: Departamento Cusco: Provincia Quispicanchis: Distrito Camanti: betwee, Yanaorcco and Yanamayo streams, Yanaorcco	-13.21983333	-70.78994444	684	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 22	MUBI 7464	Peru: Departamento Cusco: Provincia Quispicanchis: Distrito Camanti: Quincemil	-13.21983333	-70.78994444	684	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 22	SCF 1208	Peru: Departamento Ucayali: Provincia: Distrito Yurua: 1.8-2.0 km from Puerto Breu, around Cocha Galpón	-9.54223	-72.77184	257	560 (45 indels)	-	-	481	817	1858 bp
Bolitoglossa sp. 22	SCF 1263	Peru: Departamento Ucayali: Provincia: Distrito Yurua: Track ca. 4 km West of Breu, on the road to Victoria	-9.54513	-72.79332	262	560 (45 indels)	-	-	481	817	1858 bp
Bolitoglossa sp. 22	SCF 1366	Peru: Departamento Ucayali: Provincia: Distrito Yurua: Track to Beu, 3.8 km south of Puerto Breu	-9.56625	-72.75499	267	526 (44 indels)	-	739	481	817	2563 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 22	SCF 420	Peru: Departamento Ucayali: a 12 km N of Puesto de control y vigilancia Cocama, P.N. Alto Purus	-10.43495	-71.269	278	560 (45 indels)	-	750	481	817 (1 'N')	2608 bp
Bolitoglossa sp. 22	SCF 883	Peru: Departamento Ucayali: Provincia: Distrito Yurua: Puerto Breu	-9.53198	-72.75893	245	560 (45 indels)	-	-	481	817	1858 bp
Bolitoglossa sp. 23	CORBIDI 665	Peru: Departamento San Martin: Provincia Mariscal Caceres: Laguna Negra	-6.891472222	-77.38841667	1788	560 (45 indels)	-	742 (1 'N')	481	817	2600 bp
Bolitoglossa sp. 24	CORBIDI 16147	Peru: Departamento San Martin: Provincia Lamas: Distrito Caynarachi: Concesión Palmito	-6.179206	-76.310367	188	560 (48 indels)	-	782	481	817	2640 bp
Bolitoglossa peruviana	CORBIDI 13765	Peru: Departamento Loreto: Provincia Datem del Marañon: Cordillera Escalera	-5.856111111	-76.76052778	1200	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa peruviana	IIAP 1034	Peru: Departamento Loreto: Provincia Alto Amazonas: Distrito Balsa Puerto: Shawi, Cordillera Escalera	-5.883944444	-76.60436111	276	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa peruviana	IIAP 1038	Peru: Departamento Loreto: Provincia Alto Amazonas: Distrito Balsa Puerto: Shawi, Cordillera Escalera	-5.883944444	-76.60436111	276	560 (45 indels)	-	732	481	817	2590 bp
Bolitoglossa peruviana	IIAP 1058	Peru: Departamento Loreto: Provincia Alto Amazonas: Distrito Balsa Puerto: Shawi, Cordillera Escalera	-5.883944444	-76.60436111	276	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 26	CORBIDI 6459	Peru: Departamento Loreto: Provincia Andoas: Jibarito	-2.735646944	-76.03177806	197	560 (46 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 27	MZUTI 360	Ecuador: Provincia Morona-Santiago: Estación Biológica Wisui	-2.11233	-77.74019	653	560 (46 indels)	-	-	-	817	1377 bp
Bolitoglossa sp. 28	MZUTI 2874	Ecuador: Provincia Morona Santiago: Cantón Limón: Parroquia San Antonio: San Jorge	-3.07693	-78.37351	751	560 (47 indels)	-	-	-	-	560 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 28	MZUTI 2875	Ecuador: Provincia Morona Santiago: Cantón Limón: Parroquia San Antonio: San Jorge	-3.07693	-78.37351	751	560 (47 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 28	MZUTI 2876	Ecuador: Provincia Morona Santiago: Cantón Limón: Parroquia San Antonio: San Jorge	-3.07693	-78.37351	751	560 (47 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 29	MCP 13091	Brazil: Estado Amazonas: Municipio Carauari	-4.831952	-66.940343	98	560 (45 indels)	-	782 (3 'N')	481	817	2640 bp
Bolitoglossa sp. 3	GGU 1624	Peru: Departamento San Martín: Provincia Tocache: Distrito Shunté: Boshumi	-8.32015	-76.70098333	1010	526 (37 indels)	-	-	-	-	526 bp
Bolitoglossa sp. 3	IIAP 1587	Peru: Departamento San Martín: Provincia Tocache: Distrito Shunté: Boshumi	-8.32015	-76.70098333	1010	560 (46 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 3	IIAP 1588	Peru: Departamento San Martín: Provincia Tocache: Distrito Shunté: Boshumi	-8.32015	-76.70098333	1010	560 (46 indels)	-	-	481	798 (1 'N')	1839 bp
Bolitoglossa sp. 30	JMP 1833	Peru: Departamento Loreto: Requena, track between Requena and el Lago Avispa	-5.057	-73.854	55	560 (43 indels)	587	782	481	817 (1 'N')	3227 bp
Bolitoglossa sp. 31	SCF 2078	Brazil: Estado Amazonas: Municipio Japura: Comunidade de Barreirinha	-1.63679	-67.70495	83	560 (45 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 32	ANDES-A 826	Colombia: Departamento Amazonas: Leticia, Km 13	-4.112	-69.961	87	560 (45 indels)	587	782	481	817	3227 bp
Bolitoglossa sp. 32	ANDES-A 827	Colombia: Departamento Amazonas: Leticia, Km 13	-4.112	-69.961	87	560 (45 indels)	-	782	442	775	2559 bp
Bolitoglossa sp. 32	ANDES-A 914	Colombia: Departamento Amazonas: Leticia, Km 9-10	-4.124	-69.941	100	560 (45 indels)	587	782	481	817	3227 bp
Bolitoglossa sp. 32	ANDES-A 959	Colombia: Departamento Amazonas: Leticia, Huallar ka ka stream near to Tanimboca	-4.119	-69.951	83	560 (45 indels)	-	782	481	817	2640 bp
Bolitoglossa sp. 32	ANDES-A 960	Colombia: Departamento Amazonas: Leticia, Huallar ka ka stream near to Tanimboca	-4.119	-69.951	83	560 (45 indels)	-	782 (1 indels)	481	817 (1 'N')	2640 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 32	ANDES-A 961	Colombia: Departamento Amazonas: Leticia, Huallar ka ka stream near to Tanimboca	-4.119	-69.951	83	560 (45 indels)	-	782	481	817 (1 'N')	2640 bp
Bolitoglossa sp. 32	ANDES-A 962	Colombia: Departamento Amazonas: Leticia, Huallar ka ka stream near to Tanimboca	-4.119	-69.951	83	560 (45 indels)	-	782	481	817 (1 'N')	2640 bp
Bolitoglossa sp. 33	CORBIDI 12079	Peru: Departamento Loreto: Provincia Requena: Rio Tapiche	-5.635498333	-73.92371056	121	560 (47 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 33	MUBI 10081	Peru: Departamento Loreto: Provincia Loreto: Distrito Parinari: PV 6 Hamburgo, Rio Samiria	-5.224027778	-75.11933333	103	560 (46 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 33	MUBI 10099	Peru: Departamento Loreto: Provincia Loreto: Distrito Parinari: PV 4 Pithecia, Rio Samiria, EEBB Pithecia	-4.674722222	-74.31527778	91	560 (46 indels)	-	782	481	817	2640 bp
Bolitoglossa sp. 34	MZUTI 2665	Ecuador: Provincia Morona Santiago: Cantón Santiago de Méndez: Patuca, Centro Shuar Nunkandai	-2.73390	-78.23525	657	560 (47 indels)	-	782	-	817	2159 bp
Bolitoglossa sp. 34	MZUTI 2666	Ecuador: Provincia Morona Santiago: Cantón Santiago de Méndez: Patuca, Centro Shuar Nunkandai	-2.73390	-78.23525	657	560 (47 indels)	-	762	-	-	1322 bp
Bolitoglossa sp. 34	MZUTI 2673	Ecuador: Provincia Morona Santiago: Cantón Santiago de Méndez: San Simón	-2.85832	-78.23395	938	560 (47 indels)	-	768 (1 'N')	-	817	2145 bp
Bolitoglossa sp. 34	MZUTI 2674	Ecuador: Provincia Morona Santiago: Cantón Santiago de Méndez: Chupiantza, Nuevo Triunfo	-2.75488	-78.36751	1070	560 (47 indels)	-	782	-	817	2159 bp
Bolitoglossa sp. 34	MZUTI 2965	Ecuador: Provincia Morona Santiago: Cantón Santiago de Méndez: Patuca, Centro Shuar Nunkandai	-2.73593	-78.23349	740	560 (47 indels)	-	782	-	817	2159 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 34	**MZUTI 3309**	**Ecuador: Provincia Morona Santiago: Cantón Limón: Parroquia Yukianza: Cuenca Zamora, Quemado stream**	**-3.05611**	**-78.36137**	**674**	**560 (47 indels)**	**-**	**768 (1 'N')**	**-**	**817**	**2145 bp**
Bolitoglossa sp. 34	**MZUTI 3992**	**Ecuador: Provincia Tungurahua: La Candelaria**	**-1.430506**	**-78.312463**	**1920**	**560 (47 indels)**	**-**	**782**	**-**	**817**	**2159 bp**
Bolitoglossa sp. 34	**MZUTI 3994**	**Ecuador: Provincia Tungurahua: Río Zuñac**	**-1.37621**	**-78.154859**	**1500**	**526 (38 indels)**	**-**	**782**	**-**	**817**	**2125 bp**
Bolitoglossa sp. 35	**MUBI 8043**	**Peru: Departamento Loreto: Tambo Este, T05**	**-2.892937**	**-76.346987**	**232**	**560 (45 indels)**	**-**	**-**	**-**	**817**	**1377 bp**
Bolitoglossa sp. 36	INPA 3098	Brazil: Estado Amazonas: Rio Jurua	-6.466667	-68.766667	117	AY526127	-	AY526166	-	-	1205 bp
Bolitoglossa sp. 4	**CORBIDI 14387**	**Peru: Departamento Huanuco: Provincia Puerto Inca: Distrito llullapichis: Cordillera del Sira hospital camp**	**-9.478677778**	**-74.77815556**	**768**	**560 (47 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa sp. 4	**CORBIDI 14436**	**Peru: Departamento Huanuco: Provincia Puerto Inca: Distrito llullapichis: Cordillera del Sira hospital camp**	**-9.426172222**	**-74.73516667**	**526**	**560 (47 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 4	**CORBIDI 14456**	**Peru: Departamento Huanuco: Provincia Puerto Inca: Distrito llullapichis: Cordillera del Sira hospital camp**	**-9.502211111**	**-74.80425278**	**545**	**560 (47 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 4	**CORBIDI 8733**	**Peru: Departamento Loreto: Provincia Datem: Situche norte**	**-3.035610833**	**-77.37243417**	**230**	**526 (38 indels)**	**-**	**782 (3 'N')**	**481**	**817**	**2606 bp**
Bolitoglossa sp. 4	**FGZC 4837**	**Peru: Departamento Huánuco: Estación Biológica Panguana, lower Rio Llullapichis, ca. 140 km SSW Pucallpa**	**-9.617**	**-74.933**	**237**	**560 (47 indels)**	**587**	**782 (1 indels)**	**481**	**817**	**3227 bp**
Bolitoglossa sp. 4	**MUBI 9392**	**Peru: Departamento Ucayali: Provincia Coronel Portillo: Río Abujao**	**-8.412267377**	**-73.69093817**	**236**	**526 (46 indels)**	**-**	**-**	**443 (1 'N')**	**817**	**1786 bp**
Bolitoglossa sp. 5	**IIAP 2405**	**Peru: Departamento San Martin: Provincia Mariscal Caceres: Distrito Bellavista: Incaico**	**-7.337038**	**-76.422846**	**420**	**560 (44 indels)**	**-**	**-**	**-**	**-**	**560 bp**

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 5	IIAP 2407	Peru: Departamento San Martin: Provincia Mariscal Caceres: Distrito Bellavista: Incaico	-7.337038	-76.422846	420	560 (44 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 5	IIAP 2481	Peru: Departamento San Martin: Provincia Mariscal Caceres: Distrito Bellavista: ABOFOA	-6.848102	-76.46531	392	560 (46 indels)	-	782 (3 'N')	431	817	2590 bp
Bolitoglossa sp. 5	MUBI 6724	Peru: Departamento San Martin: Provincia Mariscal Caceres: Distrito Juanjui: Cueva de los Franceses, PN. Rio Abiseo	-7.362472222	-76.83716667	600	560 (46 indels)	-	742 (1 'N')	-	817	2119 bp
Bolitoglossa sp. 5	RGP 12	Peru: Departamento Cusco: Provincia Echarate: Comunidad Nativa de Sababantiari	-12.53683	-73.180817	1028	560 (47 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 5	RGP 1908	Peru: Departamento Ucayali: Provincia Contamana: Santa Rosa 1	-10.705316	-73.863597	363	560 (47 indels)	-	782	-	817	2159 bp
Bolitoglossa sp. 5	MUSA 6965	Peru: Departamento Junin: Bueno Aires, Mazamari	-11.241031	-74.384294	1056	560 (46 indels)	-	-	-	-	560 bp
Bolitoglossa sp. 6	MUBI 5435	Peru: Departamento HUANUCO: Provincia Leoncio Prado: Distrito Mariano Damazo Veraun: Parque Nacional Tingo Maria, PV 3 de mayo	-9.419555556	-75.97083333	740	560 (47 indels)	-	782	-	817	2159 bp
Bolitoglossa sp. 7	CORBIDI 10709	Peru: Departamento Cusco: Provincia La convención: KP 55, Bajo Puyantimari	-12.21281135	-73.00831003	1103	526 (37 indels)	-	782	481	817	2606 bp
Bolitoglossa sp. 7	GC 100	Peru: Departamento Cusco: Near to las malvinas camp of Pluspetrol, Valle del Bajo Urubamba	-11.8458	-72.9472	410	560 (46 indels)	-	761 (9 indels)	481	817	2619 bp
Bolitoglossa sp. 7	MUBI 7878	Peru: Departamento Puno: near to Limapampa	-13.30981	-70.29147	526	526 (37 indels)	-	-	-	-	526 bp
Bolitoglossa sp. 7	MUBI 10897	Peru: Departamento Madre de Dios: Provincia Manu: Distrito Manu: Near to Rio Azul, Rio Azul	-12.972249	-70.941475	577	560 (46 indels)	-	-	-	-	560 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 7	**MUBI 14470**	**Peru: Departamento Madre de Dios: Provincia Manu: Distrito Huepetuhe: Lote 76, PAD A, Reserva Comunal Amarakaeri**	**-12.980097**	**-71.020543**	**605**	**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 7	**MUBI 5569**	**Peru: Departamento Cusco: Provincia La Convención: Distrito Echarate: Qda Yanari, RCM, Bajo Urubamba, Kiñancaroni**	**-11.58227778**	**-73.36363889**	**484**	**560 (46 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa sp. 7	**MUBI 7312**	**Peru: Departamento Cusco: Provincia Quispicanchis: Distrito Camanti: Nusinuscato, T1**	**-13.13697222**	**-70.85161111**	**685**	**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 7	**MUBI 7524**	**Peru: Departamento Cusco: Provincia Quispicanchis: Distrito Camanti: San Lorenzo, C1**	**-13.20155556**	**-70.20155556**	**520**	**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 7	**MUBI 7986**	**Peru: Departamento Madre de Dios: Provincia Manu: Distrito Huaypetue: Puente Inambari**	**-13.181334**	**-70.843494**	**452**	**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa sp. 8	QCAZ 25784	Ecuador: Provincia Sucumbios: Parroquia Lumbaqui: Comunidad Asociación Chonta Yacu	0.1115	-77.37433	629	-	-	DQ353813	-	-	725 bp
Bolitoglossa sp. 8	QCAZ 25793	Ecuador: Provincia Sucumbios: Parroquia Lumbaqui: Comunidad Asociación Chonta Yacu	0.1115	-77.37433	629	-	-	DQ353814	-	-	725 bp
Bolitoglossa sp. 8	QCAZ 25794	Ecuador: Provincia Sucumbios: Parroquia Lumbaqui: Comunidad Asociación Chonta Yacu	0.1115	-77.37433	629	-	-	DQ353815	-	KC614443	1529 bp
Bolitoglossa sp. 8	QCAZ 25795	Ecuador: Provincia Sucumbios: Lumbaqui: Comunidad Asociación Chonta Yacu	0.1115	-77.37433	629	-	-	DQ353812	-	-	712 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa sp. 9	**CORBIDI 4685**	**Peru: Departamento Loreto: Provincia Loreto: Andoas**	**-2.351111111**	**-75.81622222**	**187**	**560 (47 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa tapajonica	**MPEG 31688**	**Brazil: Estado Para: Juruti**	**-2.163370**	**-56.095255**	**30**	**560 (46 indels)**	**-**	**782 (1 'N')**	**481**	**817**	**2640 bp**
Bolitoglossa tapajonica	**MPEG 31695**	**Brazil: Estado Para: Lorena**	**-4.704388889**	**-56.38333333**	**74**	**560 (45 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Aquiloeurycea cephalica	IBH 22603					KP886863	KP886919	KP900066	KP900108	KP900152	3227 bp
Aquiloeurycea galeanae	IBH 24595					KP886847	KP886904	KP900051	KP900093	KP900137	2791 bp
Aquiloeurycea quetzalanensis	MZFC 19301					KP886851	-	KP900055	KP900097	KP900141	2657 bp
Chiropterotriton arboreus	IBH 28191					KP886890	KP886946	KP900083	KP900124	KP900170	2581 bp
Chiropterotriton magnipes	IBH 28176					KP886892	-	KP900085	KP900126	KP900172	2616 bp
Bolitoglossa sp.	**DQ 175**					**560 (46 indels)**	**-**	**750**	**-**	**-**	**1310 bp**
Bolitoglossa sp.	**DQ 177**					**526 (37 indels)**	**-**	**-**	**481**	**798**	**1805 bp**
Bolitoglossa sp.	**GGD 111**	**Colombia: Departamento Caldas: Municipio Salamina: Finca tribunas, El canelo**	**5.365575**	**-75.42758611**	**2600**	**560 (46 indels)**	**-**	**742 (2 'N')**	**481**	**817**	**2600 bp**
Bolitoglossa sp.	**GGD 640**	**Colombia: Departamento Caldas: Municipio Pensilvania: Pensilvania, road to arboleda**	**5.408684**	**-75.141566**	**2246**	**560 (45 indels)**	**-**	**732**	**481**	**817**	**2590 bp**
Ixalotriton niger	IBH 29715					KP886874	KP886930	KP900077	KP900118	KP900163	3016 bp
Bolitoglossa sp.	**MOE 1**	**Venezuela: Estado Lara: Sanare, Estacion El Blanquito, Parque Nacional Yacambu**	**9.71**	**-69.58**	**1580**	**560 (48 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Parvimolge townsendi	CARIE 1174					KP886876	KP886932	KP900078	KP900119	KP900165	2935 bp
Pseudoeurycea cochranae	IBH 23064					KP886864	KP886920	KP900067	KP900109	KP900153	3210 bp
Pseudoeurycea leprosa	IBH 22406					KP886866	KP886922	KP900069	-	KP900155	2746 bp
Pseudoeurycea longicauda	IBH 22247					KP886849	KP886906	KP900053	KP900095	KP900139	2451 bp
Pseudoeurycea obesa	MVZ 241574					KP886870	KP886926	KP900073	KP900114	KP900159	3252 bp
Pseudoeurycea rex	MVZ 263590					KP886852	KP886908	KP900056	KP900098	KP900142	3024 bp
Pseudoeurycea ruficauda	IBH 21646					KP886871	KP886927	KP900074	KP900115	KP900160	3252 bp
Thorius munificus	IBH 29716					KP886888	KP886944	KP900081	KP900122	KP900168	2967 bp
Thorius troglodytes	IBH 22597					KP886889	KP886945	KP900082	KP900123	KP900169	1947 bp
Bolitoglossa adspersa	MVZ 158485					AF218492	-	AF212984	-	-	1205 bp
Bolitoglossa alberchi	MVZ 264191					KP886843	KP886900	KP735278	KP735288	KP735306	2959 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa alvaradoi	MVZ 215735					AY526157	-	AY526194	-	-	917 bp
Bolitoglossa aurae	UCR 22842					KX779527	-	KX779528.	-	-	1087 bp
Bolitoglossa aureogularis	UCR 19858					JQ899151.1	-	JQ899182.1	-	-	1342 bp
Bolitoglossa sp.	**AFJ 6**	**Colombia: Departamento Valle del Cauca: Municipio Buenaventura: Corregimiento Cisneros: Los Tubos**	**3.849346**	**-76.786954**	**947**	**560 (48 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa sp.	**AFJ 10**	**Colombia: Departamento Valle del Cauca: Municipio Buenaventura: Corregimiento Cisneros: Los Tubos**	**3.849346**	**-76.786954**	**947**	**560 (48 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa biseriata	MHCH 2658					KM527322	KM527307	-	-	-	1021 bp
Bolitoglossa biseriata	MHCH 2659					KM527330	-	-	-	-	453 bp
Bolitoglossa biseriata	MHCH 2668					KM527334	KM527317	-	-	-	1027 bp
Bolitoglossa biseriata	MVZ 232943					AY526118	-	AY526161	-	KC614436	1915 bp
Bolitoglossa biseriata	S 13236					AY526118	-	-	-	-	560 bp
Bolitoglossa biseriata	SMF 97135					KM527339	-	-	-	-	441 bp
Bolitoglossa bramei	UCR 20851					JQ899142	-	JQ899172.1	-	-	1342 bp
Bolitoglossa carri	USNM 523277					AY526139	-	AY526176	-	KC614458	1948 bp
Bolitoglossa cataguana	JHT2114					KJ628089.1	-	KJ628090.1	-	-	1145 bp
Bolitoglossa celaque	LDW11093					AY526140	-	AY526177	-	-	1160 bp
Bolitoglossa cerroensis	MVZ 181276					-	-	AF212096	-	KC614459	1449 bp
Bolitoglossa cerroensis	MVZ-S 12921					AF199233	-	AF199195	-	-	943 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa chinanteca	IBH 24708					KC287994.1	-	KC288086.1	KC288054.1	-	1823 bp
Bolitoglossa chucantiensis	MHCH 2665					KM527324	KM527308	-	-	-	1125 bp
Bolitoglossa colonnea	CH 6526					FJ766578	FJ766578.1	-	-	-	1147 bp
Bolitoglossa colonnea	SMF 97136 + No voucher					KM527326	KM527310	AY526162	-	-	1657 bp
Bolitoglossa colonnea	SMF 94460					JX434644	-	-	-	-	518 bp
Bolitoglossa compacta	UCR 20532					JQ899163	-	JQ899193.1	-	-	1342 bp
Bolitoglossa conanti	MVZ 225843					AY526142	-	AY526179	-	KC699924	2009 bp
Bolitoglossa cuchumatana	MVZ 251541					GU725454.1	-	GU725467.1	-	-	1316 bp
Bolitoglossa decora	USNM 497533					AY526143	-	AY526180	-	-	1142 bp
Bolitoglossa diaphora	MVZ 263440					GU725447	-	GU725460	-	-	1342 bp
Bolitoglossa dofleini	MVZ 263450					-	-	KP900047	KP900089	KP900133	2603 bp
Bolitoglossa dunni	USNM 523280					AY526145	-	AY526182	-	KC614438	1925 bp
Bolitoglossa engelhardti	MVZ 251495 + No voucher					GU725448	-	GU725461	-	KC699925	2146 bp
Bolitoglossa epimela	MVZ 181260					AY526120	-	AF212097	-	-	1110 bp
Bolitoglossa eremia	UTA 58387					-	-	HQ009988	-	-	608 bp
Bolitoglossa eremia	UTA 58429					-	-	HQ009992	-	-	576 bp
Bolitoglossa eremia	UTA 58430					-	-	HQ009998	-	-	653 bp
Bolitoglossa eremia	UTA 58552					-	-	HQ010005	-	-	639 bp
Bolitoglossa flavimembris	MVZ 143698					AY526183	-	AY526146	-	-	875 bp
Bolitoglossa flavimembris	MVZ 177786					KP886840	-	GU725462	KP900087	KP900132	2631 bp
Bolitoglossa flavimembris	UTA 58686					-	-	HQ010013	-	-	524 bp
Bolitoglossa flaviventris	MVZ 194288					AF218489	-	AF212983	-	-	1205 bp
Bolitoglossa franklini	MVZ 185991					AY526184	-	AY526147	-	KC614439	1958 bp
Bolitoglossa gomezi	UCR 20849					JQ899141	-	JQ899171	-	-	1342 bp
Bolitoglossa gracilis	MVZ 229170					AF212067	-	AY526121	-	-	1205 bp
Bolitoglossa guaneae	PAG 926					KC257105	-	-	-	-	560 bp
Bolitoglossa guaneae	UIS-A 5275					KU985264	-	KX458162	-	-	1331 bp
Bolitoglossa guaneae	UIS-A 5276					KU985265	-	KX458163	-	-	1342 bp
Bolitoglossa hartwegi	MVZ 177790					AF218494	-	AF212985	-	-	875 bp
Bolitoglossa hartwegi	MVZ 263458					KP886839	KP886897	KC288103	KC288103	KP900131	3010 bp
Bolitoglossa hartwegi	UTA 54817					-	-	HQ009996	-	-	634 bp
Bolitoglossa heiroreias	MVZ 200535					AY526155	-	AY526192	-	-	1196 bp
Bolitoglossa helmrichi	UTA 51457 + MVZ 257804					GU725450	-	AY691755	-	AY650124	2159 bp
Bolitoglossa hermosa	MVZ 163690					AF416686	-	AF416678	-	-	1160 bp
Bolitoglossa hypraca	**SAS 446**					**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa hypraca	**SAS 447**					**560 (46 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa jugivagans	SMF 94467					KC428634	-	-	-	-	442 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa kamuk	UCR 20852					JQ899143	-	JQ899173	-	-	1342 bp
Bolitoglossa kaqchikelorum	UTA 58685					-	-	HQ010020	-	-	530 bp
Bolitoglossa leandrae	**ANDES-A 1886**	**Colombia: Departamento Boyaca: Santa Maria**	**4.857988**	**-73.262355**	**827**	**560 (47 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa leandrae	**ANDES-A 1887**	**Colombia: Departamento Boyaca: Santa Maria**	**4.857988**	**-73.262355**	**827**	**560 (47 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa leandrae	**VVO 837**	**Colombia: Departamento Meta: Municipio Villaviciencio: Jardin Botanico de Villaviciencio**	**4.1525**	**-73.654167**	**645**	**560 (47 indels)**	**-**	**782 (3 'N')**	**481**	**817**	**2640 bp**
Bolitoglossa leandrae	MCNUP 63					KC257102	-	-	-	-	560 bp
Bolitoglossa leandrae	MCNUP 64					KC257103	-	-	-	-	560 bp
Bolitoglossa leandrae	MCNUP 65					KC257104	-	-	-	-	560 bp
Bolitoglossa lignicolor	MHCH 2602					JX434638	-	-	-	-	560 bp
Bolitoglossa lignicolor	MVZ-S 11132					AF218484	-	-	-	-	560 bp
Bolitoglossa lincolni	MVZ 143564					AY526148	-	AY526185	-	KC614440	1958 bp
Bolitoglossa longissima	USNM 523285					AY526149	-	AY526186	-	KC614441	1990 bp
Bolitoglossa lozanoi	H 3					KU985266	-	KX458164	-	-	1284 bp
Bolitoglossa lozanoi	UIS-A 5269					KU985267	-	KX458165	-	-	1316 bp
Bolitoglossa macrinii	13800					AF416680	-	AF416689	-	-	1205 bp
Bolitoglossa marmorea	MVZ 210286					AF218493	-	U89631	-	-	1116 bp
Bolitoglossa medemi	S 13237					AY526123	-	AY526163	-	KC614437	2009 bp
Bolitoglossa medemi	MHCH 2660					KM527325	KM527309	-	-	-	1018 bp
Bolitoglossa medemi	SMF 97131					KM527327	KM527311	-	-	-	1018 bp
Bolitoglossa medemi	SMF 97133					KM527328	KM527312	-	-	-	1006 bp
Bolitoglossa meliana	MVZ 265621					KJ175100	-	KJ175105	-	-	934 bp
Bolitoglossa mexicana	1032BolMex					EF017950	-	-	-	EF018055	852 bp
Bolitoglossa mexicana	MGPBo71					AF218470	-	AF212976	-	-	1160 bp
Bolitoglossa mexicana	MVZ 176838					GU725457	-	GU725470	-	-	1316 bp
Bolitoglossa mexicana	MVZ 191635					AF177588	-	AF212099	-	-	1205 bp
Bolitoglossa mexicana	MVZ 263477					KC288005	-	KC288104	KC288058	-	1823 bp
Bolitoglossa mexicana	UTA 54810					-	-	HQ009994	-	-	620 bp
Bolitoglossa minutula	MVZ 225870					AY526124	-	AF212098	-	KC614434	1939 bp
Bolitoglossa mombachoensis	SMF 78718					AY133488	-	AY133485	-	-	1205 bp
Bolitoglossa morio	MVZ 143677					AF218495	-	AF212986	-	-	1160 bp
Bolitoglossa morio	MVZ 251466					KJ175098	-	KJ175106	-	-	1333 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa morio	MVZ 257825					GU725452	-	GU725465	-	-	1316 bp
Bolitoglossa morio	MVZ 232970					AY526150	-	AY526187	-	-	1101 bp
Bolitoglossa morio	USAC 1546					KJ787708	-	KJ787752	-	-	1342 bp
Bolitoglossa morio	USAC 1568					KJ787729	-	KJ787754	-	-	943 bp
Bolitoglossa morio	UTA 53286					-	-	HQ009989	-	-	608 bp
Bolitoglossa morio	UTA 58521					-	-	HQ009991	-	-	576 bp
Bolitoglossa morio	UTA 58523					-	-	HQ010000	-	-	653 bp
Bolitoglossa morio	UTA 58527					-	-	HQ009987	-	-	645 bp
Bolitoglossa mucuyensis	CVULA 7100					JN635335	JQ665278	JQ665282	-	-	1718 bp
Bolitoglossa mulleri	UTA 50475					-	-	HQ010012	-	-	559 bp
Bolitoglossa nicefori	UIS-A 5270					KX458176	-	KX458166	-	-	1299 bp
Bolitoglossa nicefori	UIS-A 5271					KX458177	-	KX458167	-	-	1291 bp
Bolitoglossa nigrescens	CH 7478					JQ899165	-	JQ899168	-	-	1277 bp
Bolitoglossa nigrescens	UCR 20539					JQ899164	-	JQ899194	-	-	1333 bp
Bolitoglossa nympha	MVZ 257812					KP886838	KP886896	KC288068	KC288021	KP900130	3015 bp
Bolitoglossa oaxacensis	IBH 13374					-	-	AF416681	KP900088	-	1686 bp
Bolitoglossa occidentalis	AMA 2507					KC287914	-	-	-	-	560 bp
Bolitoglossa occidentalis	IBH 22546					KC287978	-	-	KC288039	-	1041 bp
Bolitoglossa occidentalis	MVZ 160875 + USCG 1867					KC287949	-	-	KC288008.1	-	1041 bp
Bolitoglossa occidentalis	MVZ 194214					KC287942	-	-	-	-	560 bp
Bolitoglossa occidentalis	MVZ 194238					KC287944	-	KC288088	-	-	943 bp
Bolitoglossa occidentalis	MVZ 194248					KC287939	-	-	-	-	560 bp
Bolitoglossa occidentalis	MVZ 263811 + MVZ 194251					KC287911	-	KC288087	KC288006	-	1424 bp
Bolitoglossa occidentalis	MVZ 263814					KC287912	-	KC288059	KC288007	-	1424 bp
Bolitoglossa occidentalis	MVZ 264208					KC287936	-	-	KC288033	-	1041 bp
Bolitoglossa odonnelli	MVZ 229068					AF218476	-	AF212977	-	KC699922	1915 bp
Bolitoglossa oresbia	USNM 579667					KJ175101	-	KJ175108	-	-	1333 bp
Bolitoglossa orestes	CVULA 7107 + SJ00					JN635340	JQ665277	JQ665280	-	-	1718 bp
Bolitoglossa pacaya	USAC 1545					KJ787707	-	KJ787751	-	-	943 bp
Bolitoglossa pacaya	USAC 1562					KJ787724	-	KJ787753	-	-	943 bp
Bolitoglossa pacaya	USAC 1574					KJ787734	-	-	-	-	560 bp
Bolitoglossa pacaya	USAC 1575					KJ787735	-	-	-	-	560 bp
Bolitoglossa pacaya	USAC 1586					KJ787738	-	-	-	-	560 bp
Bolitoglossa pacaya	USAC 1588					KJ787740	-	KJ787755	-	-	909 bp
Bolitoglossa pacaya	USAC 1597					KJ787744	-	-	-	-	560 bp
Bolitoglossa pacaya	USAC 1598					KJ787745	-	-	-	-	560 bp
Bolitoglossa pacaya	USAC 1599					KJ787746	-	-	-	-	560 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa pacaya	USAC 1600					KJ787747	-	KJ787756	-	-	943 bp
Bolitoglossa pesrubra	MVZ 190923					EU448104	-	AF212074	-	-	1205 bp
Bolitoglossa pesrubra	UCR 12068					AY526132	-	AF212069	-	-	1205 bp
Bolitoglossa platydactyla	GP 108					AF218487	-	AF212981	-	KC699923	1944 bp
Bolitoglossa porrasorum	MVZ 225852					AY526151	-	AY526188	-	-	1205 bp
Bolitoglossa riletti	MVZ 194328					AF416696	-	AF416682	-	-	1191 bp
Bolitoglossa robinsoni	UCR 20489					JQ899161	-	JQ899191	-	-	1342 bp
Bolitoglossa robusta	MVZ 190830					EU448109	-	EU448110	-	-	907 bp
Bolitoglossa rostrata	MVZ 251521					KJ175099	-	KJ175107	-	-	1333 bp
Bolitoglossa rostrata	MVZ 163683					AY526152	-	AY526189	-	-	911 bp
Bolitoglossa rufescens	IBH 22529 + MVZ 163834					KC287971	-	KC288095	KC288036	-	1424 bp
Bolitoglossa rufescens	MVZ 163805					KC287919	-	KC288063	-	-	1333 bp
Bolitoglossa rufescens	MVZ 231317					KC287921	-	KC288065	KC288019	KF911887	2605 bp
Bolitoglossa rufescens	MVZ 263969					KC287990	-	KC288082	KC288051	-	1823 bp
Bolitoglossa rufescens	MVZ 194254					AY526115	-	AY526158	-	KC614435	2009 bp
Bolitoglossa rufescens	UF 144902					-	-	KU670954	-	-	719 bp
Bolitoglossa schizodactyla	USNM 572791					AY526133	FJ766579	AY526171	-	-	1772 bp
Bolitoglossa sima	MVZ 163575					AY526134	-	AY526172	-	-	860 bp
Bolitoglossa sombra	MVZ 225871					AY526136	-	AY526174	-	-	1185 bp
Bolitoglossa sombra	MVZ 225875					AY728235	AY728235	AY728235	EU275851	EU275810	3227 bp
Bolitoglossa sooyorum	MVZ 190847					EU448108	-	-	-	-	560 bp
Bolitoglossa sp	QCAZ 39981					-	-	KC614431	-	KC614456	1471 bp
Bolitoglossa sp	MGP 1/MVZ 167947					AY526135	-	AY526173	-	-	1205 bp
Bolitoglossa sp	SMF 97138					KM527329	KM527313	-	-	-	1062 bp
Bolitoglossa splendida	UCR 19835					JQ899150	-	JQ899181	-	-	1342 bp
Bolitoglossa striatula	MVZ 181280					AF218488	-	AF212982	-	-	769 bp
Bolitoglossa stuarti	UTA 58145					-	-	HQ010009	-	-	603 bp
Bolitoglossa subpalmata	MVZ 229172					AF416697	-	AF212094	-	-	1205 bp
Bolitoglossa suchitanensis	UTA 58149					-	-	HQ009986	-	-	591 bp
Bolitoglossa suchitanensis	UTA 58150					-	-	HQ009997	-	-	653 bp
Bolitoglossa suchitanensis	UTA 58422					-	-	HQ010001	-	-	653 bp
Bolitoglossa suchitanensis	UTA 58423					-	-	HQ009999	-	-	653 bp
Bolitoglossa synoria	SMF 78084					AY526156	-	AY526193	-	-	1160 bp
Bolitoglossa tamaense	MCNUP 51					KC257100	-	-	-	-	497 bp
Bolitoglossa tamaense	MCNUP 53					KC257101	-	-	-	-	560 bp
Bolitoglossa tamaense	MCNUP 56					KC257098	-	-	-	-	497 bp
Bolitoglossa tamaense	MCNUP 57					KC257099	-	-	-	-	477 bp
Bolitoglossa taylori	MHCH 2663					KM527331	KM527314	-	-	-	1062 bp

Supplementary 1. Continuation

Taxa	Voucher	Locality	Latitude	Longitude	Altitude	16S	COI	CYTB	POMC	RAG1	Total length
Bolitoglossa taylori	MHCH 2664					KM527333	KM527316	-	-	-	1044 bp
Bolitoglossa taylori	MHCH 2666					KM527340	KM527321	-	-	-	1062 bp
Bolitoglossa taylori	SMF 97128					KM527336	KM527319	-	-	-	1062 bp
Bolitoglossa taylori	SMF 97130					KM527337	KM527320	-	-	-	1062 bp
Bolitoglossa taylori	SMF 97140					KM527323	-	-	-	-	497 bp
Bolitoglossa taylori	SMF 97141					KM527335	KM527318	-	-	-	1062 bp
Bolitoglossa tenebrosa	MVZ 264289					KJ175103	-	KJ175110	-	-	1310 bp
Bolitoglossa tica	UCR 20514					JQ899162	-	JQ899192	-	-	1314 bp
Bolitoglossa vallecula	**AFJ 48**	**Colombia: Departamento Valle del Cauca: Municipio El Cairo: El Cerro del Ingles**	**4.740728**	**-76.299616**	**2186**	**560 (48 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa walkeri	**AFJ 2**	**Colombia: Departamento Valle del Cauca: Municipio Cali: San Antonio**	**3.499985**	**-76.623155**	**2186**	**560 (43 indels)**	**-**	**782**	**481**	**817**	**2640 bp**
Bolitoglossa walkeri	**AFJ 3**	**Colombia: Departamento Valle del Cauca: Municipio Cali: San Antonio**	**3.499985**	**-76.623155**	**2107**	**560 (43 indels)**	**-**	**-**	**-**	**-**	**560 bp**
Bolitoglossa yariguiensis	H 151					KU985272	-	KX458170	-	-	1265 bp
Bolitoglossa yariguiensis	UIS-A 5265					KU985270	-	KX458168	-	-	1296 bp
Bolitoglossa yariguiensis	UIS-A 5266					KU985271	-	KX458169	-	-	1316 bp
Bolitoglossa yariguiensis	UIS-A 5278					KU985273	-	KX458171	-	-	1342 bp
Bolitoglossa yariguiensis	UIS-A 5279					KU985274	-	KX458172	-	-	1342 bp
Bolitoglossa yariguiensis	UIS-A 5280					KU985275	-	KX458173	-	-	1284 bp
Bolitoglossa yariguiensis	UIS-A 5281					KU985276	-	KX458174	-	-	1342 bp
Bolitoglossa yariguiensis	UIS-A 5282					KU985277	-	KX458175	-	-	1342 bp
Bolitoglossa yucatana	MVZ 197507					AF218485	-	AF212980	-	-	1205 bp
Bolitoglossa zacapensis	MVZ 257805					GU725456	-	GU725469	-	-	1322 bp
Bolitoglossa zapoteca	IBH 13375					AF416698	-	AF416683	-	-	1205 bp

Supplementary 2. Primers and PCR conditions used

Gene and Primers	Sequence (5’ - 3’)	PCR condition	Reference
Mitochondrial			
1. 16S			
16Sar	CGCCTGTTTATCAAAAACAT	1 cycle: 15' 95°C 35 cycles: 30'' 94 °C; 30'' 50 °C; 1' 72 °C 1 cycle: 10' 72°C	Palumbi et al. (1991)
16Sbr	CCGGTCTGAACTCAGATCACGT	1 cycle: 10' 4°C	
2. Cytb			
MVZ15	GAACTAATGGCCCACACWWTACGNAA	1 cycle: 15'' 95°C 35 cycles: 18'' 95 °C; 18'' 48 °C; 1' 72 °C 1 cycle: 10' 72°C	Moritz et al (1992)
MVZ16	AAATAGGAARTATCAYTCTGGTTTRAT	1 cycle: 10' 4°C	
3. COI			
VR1-d	TAGACTTCTGGGTGGCCRAARAAYCA	1 cycle: 2' 94°C 35 cycles: 36'' 94 °C; 36''51 °C; 1' 72 °C 1 cycle: 10' 72°C	Ivanova et al. (2006)
VF1-d	TTCTCAACCAACCACAARGAYATYGG	1 cycle: 10' 4°C	
Nuclear			
4. POMC			
POMC_DRV_F1	ATATGTCATGASCCAYTTYCGCTGGAA	1 cycle: 15'' 95°C 35 cycles: 18'' 95 °C; 18'' 48 °C; 1' 72 °C 1 cycle: 10' 72°C	Vieites et al. (2007)
POMC_DRV_R1	GGCRTTYTTGAAWAGAGTCATTAGWGG	1 cycle: 10' 4°C	
5. Rag1			
Rag1BolitoF	CTTGAACTAGGGGGCATACTCAGAAC	1 cycle: 15' 95°C 35 cycles: 30'' 94 °C; 30'' 54 °C; 1' 72 °C 1 cycle: 10' 72°C	Elmer et al. (2013)
Rag1BolitoR	TGCCTGGCATTCATTTTCCGGAAACG	1 cycle: 10' 4°C	

Supplementary 3. New terminals identification according to literature

New identification	Original name and identification
Bolitoglossa alberchi	*Bolitoglossa mexicana* (Clade 2) of García-Paris et al. (2000b) is *B. alberchi* according to García-Parra et al. (2002)
Bolitoglossa caldwellae	The terminal *Bolitoglossa paraensis* LSUMZH 13735 of Parra-Olea et al. (2004), correspond to *B. caldwellae* MPEG 12881 according to Brcko et al. 2013
Bolitoglossa equatoriana	The terminal *B. peruviana* LSUMZ 12838 of Parra-Olea et al. (2004), correspond to *B. equatoriana* QCAZ 5930 according to Elmer et al. 2013
Bolitoglossa nympha	In the GenBank database was identified as *B. rufescens* MVZ 194333, but in Rovito et al. (2012) was determined as *B. nympha* MVZ 194333, probably was an error when upload de data
Bolitoglossa odonnelli	García-Paris et al. 2000b identified as *Bolitoglossa mexicana* MVZ 229068 (Clade 3). But in the same work the terminals MVZ 163793-95, MVZ 163797, MVZ 229068, UTA(MEA 446), UTA(ENS7862) are more closely related to *B. odonnelli* MVZ 161046 than *B. mexicana*
Bolitoglossa rufescens	In GenBank database was identified as *B. occidentalis* MVZ 194254. But in Rovito et al. (2012) was determined as *B. rufescens* MVZ 194254, probably was an error when upload de data
Bolitoglossa sombra	In GenBank databe was identified as *B. nigrescens* CH 7478. But in Rovito et al. (2012) was determined as*B. sombra* CH 7478, probably was an error when upload de data
Bolitoglossa heiroreias	The exemplar *Bolitoglossa* sp. 3 MVZ 200535 of Parra-Olea et al. (2004) correspond to the paratype of *B. heiroreias,* Greenbaum (2004)

Supplementary 4. The top 50 wildcards terminals with the optimal topology ditances after removed

No.	Teminal name	Average distances
1	*B. pacaya* USAC_1598	314,7
2	*B. pacaya*_USAC_1575	314,8
3	*B. pacaya*_USAC_1574	314,8
4	*B. pacaya*_USAC_1597	314,9
5	*B. pacaya*_USAC_1586	314,9
6	*B. pacaya*_USAC_1599	315,0
7	*B*. sp. 11 MZUTI 3526 Wildsumaco, Napo, Ecu	315,1
8	*B. equatoriana* QCAZ 37304 Tiputini, Orellana, Ecu	316,1
9	*B*. sp. 10 MZUTI 1603 Canton Tena, Napo, Ecu	316,5
10	*B. pacaya*_USAC_1588	317,5
11	*B. morio*_USAC_1568	317,6
12	*B. pacaya*_USAC_1562	317,6
13	*B. pacaya*_USAC_1545	317,6
14	*B. morio*_MVZ_257825	317,6
15	*B. pacaya*_USAC_1600	317,6
16	*B*. sp. 10 MZUTI 1650 Canton Tena, Napo, Ecu	318,9
17	*B. yariguiensis*_UIS_A_5282	319,2
18	*B. yariguiensis*_UIS_A_5279	319,2
19	*B. yariguiensis*_UIS_A_5281	319,2
20	*B. yariguiensis*_UIS_A_5278	319,3
21	*B. yariguiensis*_UIS_A_5266	319,3
22	*B. yariguiensis*_UIS_A_5280	319,3
23	*B. yariguiensis*_UIS_A_5265	319,3
24	*B. yariguiensis*_H151	319,3
25	*B*. sp. 11 QCAZ 25420 La Selva, Orellana, Ecu	320,0
26	*B*. sp. 11 QCAZ 25425 La Selva, Orellana, Ecu	320,0
27	*B*. sp. 7 MUBI 7524 San Lorenzo, Cusco, Per	320,0
28	*B*. sp. 11 QCAZ 25426 La Selva, Orellana, Ecu	320,0
29	*B*. sp. 7 MUBI 7312 Nusinuscato, Cusco, Per	320,0
30	*B*. sp. 11 QCAZ 25422 La Selva, Orellana, Ecu	320,0
31	*B*. sp. 7 MUBI 7878 Limapampa, Puno, Per	320,0
32	*B*. sp. 10 QCAZ 25753 Jatun Sacha, Napo, Ecu	320,1
33	*B*. sp. 10 QCAZ 25771 Jatun Sacha, Napo, Ecu	320,1
34	*B*. sp. 10 QCAZ 25289 Jatun Sacha, Napo, Ecu	320,1
35	*B*. sp. 10 QCAZ 25758 Jatun Sacha, Napo, Ecu	320,1
36	*B. equatoriana* DFCH 2730 Jatun Sacha, Napo, Ecu	320,1
37	*B*. sp. 10 KU217421 Jatun Sacha, Napo, Ecu	320,1
38	*B*. sp. 10 QCAZ 25455 Jatun Sacha, Napo, Ecu	320,1
39	*B. equatoriana* QCAZ 25450 Jatun Sacha, Napo, Ecu	320,1
40	*B. equatoriana* QCAZ 25777 Jatun Sacha, Napo, Ecu	320,1
41	*B. equatoriana* QCAZ 25448 Jatun Sacha, Napo, Ecu	320,1
42	*B. equatoriana* QCAZ 25449 Jatun Sacha, Napo, Ecu	320,1
43	*B. equatoriana* QCAZ 25443 Jatun Sacha, Napo, Ecu	320,1
44	*B*. sp. 6 MUBI 5435 Tingo Maria, Huanco, Per	320,1
45	*B*. sp. 11 QCAZ 25387 La Selva, Orellana, Ecu	320,2
46	*B*. sp. 11 QCAZ 25385 La Selva, Orellana, Ecu	320,2
47	*B*. sp. 32 ANDES-A 960 Leticia, Amazonas, Col	320,2
48	*B. suchitanensis*_UTA_58422	320,4
49	*B*. sp. 4 CORBIDI 14436 Cordillera Sira, Huanuco, Per	320,4
50	*B. suchitanensis*_UTA_58149	320,5

Supplementary 5. Uncorrected genetic distances for 16S (bottom left) and cytb (top right), indicating the sample size for each terminal

16S (N= 130)\cytb (N= 115)	B. sp. 33 (n= 1)	B. sp. 15 (n= 1)	B. sp. 25 (n= 2)	B. caldwellae (n= 1)	B. sp. 20 (n= 1)	B. madeira (n= 2)	B. sp. 21 (n= 1)	B. sp. 22 (n= 4)	B. sp. 36 (n= 1)	B. sp. 19 (n= 1)	B. sp. 23 (n= 1)	B. sp. 17 (n= 1)
B. sp. 33 (n= 3)	0.7–1.6\0.0	11	12.3–12.3	11	9.3	10.6	10	10.4	9.7	11.2	9.8	11.9
B. sp. 15 (n= 1)	6.1–6.5	0.0\0.0	11.4	12.7	10.6	12.9	12.5	11.2	13.1	12.1	12.7	12.5
B. sp. 25 (n= 5)	5.4–7.4	5.0–5.4	0.0–2.0\0.8	12.5–12.9	11	12.3–13.3	13.3	11.0–13.4	12.7	11.2	9.7	13.3
B. caldwellae (n= 2)	6.8–7.0	3.8	3.6–4.1	0.5\0.0	11.2	13.6	11.4	10.4–13.1	12.7	11	11.7	11.7
B. sp. 20 (n= 1)	7.2–7.7	5.6	3.6–4.9	4.9–5.0	0	10.2	8.9	8.5	8.1	7.6	8.7	10.6
B. madeira (n= 3)	6.1–7.7	4.1–5.0	3.2–5.2	2.7–4.5	2.5–2.7	0.7–2.3\1.9	12.5–13.3	10.8–12.9	8.7	11.2	12.5–13.4	13.1–13.6
B. sp. 21 (n= 4)	6.3–7.0	4.1–4.3	3.1–4.0	2.9–3.2	3.1–3.6	1.8–3.4	0.0–0.4\0.0	8.0–10.2	9.8	10	12.1	12.9
B. sp. 22 (n= 11)	5.9–7.4	3.8–5.0	3.8–5.4	3.4–4.5	3.4–4.3	2.0–4.1	1.6–3.2	0.0–2.0\3.0–5.3	9.7	8.9–10.2	9.8–11.6	12.1–13.4
B. sp. 36 (n= 1)	6.3–6.5	4.5	3.1–3.8	2.9–3.4	2.9	1.8–2.7	1.8–2.0	2.5–3.6	0.0\0.0	8.7	11.2	12.3
B. sp. 19 (n= 2)	5.9–7.2	4.1–4.5	2.7–4.3	2.9–3.6	3.4–3.8	2.5–3.8	2.3–2.9	2.7–4.0	2.2–2.9	2.0\0.0	9.7	12.1
B. sp. 23 (n= 1)	6.3–6.8	4.3	3.1–3.6	4.3	4.9	3.8–5.2	3.6–3.8	4.0–4.9	3.6	3.8–4.0	0.0\0.0	12.1
B. sp. 17 (n= 1)	6.3–6.8	4.3	3.1–3.6	4.3	4.9	3.8–5.2	3.6–3.8	4.0–4.9	3.6	3.8–4.0	3.8	0.0\0.0
B. equatoriana (n= 4)	5.2–7.4	5.7–6.3	4.3–6.8	4.5–5.0	5.7–6.5	4.3–5.9	4.5–5.2	4.8–6.5	4.5–5.0	4.7–5.6	5.4–5.9	4.3–6.1
B. sp. 2 (n= 2)	6.1–7.7	5.9–6.8	5.2–6.3	5.6–6.5	5.9–6.5	5.2–6.1	4.5–5.4	5.4–6.8	5.2–5.9	5.4–6.5	5.2–6.1	6.1–7.2
B. sp. 6 (n= 1)	7.4–7.9	5.9	5.9–6.1	5.4	6.3	5.6–5.9	5.2–5.6	5.9–7.0	5.6	4.5–5.2	6.8	5.6
B. sp. 4 (n= 6)	6.1–6.5	5	5.6–6.1	5.9	5.6	4.8–5.2	4.7–5.2	5.2–6.1	5	4.7–5.2	5.2	5.2
B. sp. 7 (n= 9)	5.9–7.4	5.4–7.0	5.2–7.2	4.5–6.1	5.2–5.9	3.8–5.6	3.4–5.4	4.1–6.3	3.4–5.0	4.3–5.6	5.4–6.5	5.2–7.2
B. sp. 3 (n= 3)	6.1–6.5	4.5	5.0–5.4	5.2	5.6	4.5–4.8	4.1–4.5	4.5–5.9	4.5	4.5–4.7	5.2	4.7–4.8
B. sp. 5 (n= 7)	5.6–7.2	5.0–5.4	4.3–6.1	4.5–5.4	5.2–6.1	3.8–5.2	3.4–4.7	4.1–6.1	3.8–4.7	3.8–5.0	5.0–5.4	4.8–5.6
B. tapajonica (n= 2)	6.1–8.4	5.4–6.8	4.3–6.5	4.9–6.1	4.0–5.2	4.1–5.2	4.0–5.6	4.7–6.5	4.0–5.2	3.6–5.9	5.2–6.3	4.3–5.6
B. sp. 18 (n= 8)	7.2–7.4	5.6	5.0–5.9	5	4.1	3.6–4.3	3.8–4.3	4.5–5.2	3.8	4.3–4.5	4.5	5.4
B. palmata (n= 3)	5.6–6.5	5.2–5.4	5.0–6.1	5.0–5.2	5.6–5.9	4.3–5.6	4.3–5.0	4.5–5.9	4.7–5.0	4.3–4.7	4.1–4.3	5.2–5.4
B. sp. 12 (n= 5)	5.4–6.3	5.7–6.1	5.0–5.9	4.7–5.4	4.5–5.0	4.1–5.5	4.1–5.2	4.5–6.1	4.3–5.0	4.3–5.2	4.8–5.4	5.2–6.1
B. sp. 9 (n= 1)	5.2–5.9	5.4	4.7–5.4	4.7–5.2	5	4.5–5.2	4.1–4.5	4.5–5.4	3.8	4.3–4.7	4.5	4.3
B. sp. 13 (n= 1)	5.7–5.9	6.3	5.2–5.9	5.4	4.7	4.8–5.4	4.3–4.7	5.0–5.9	4.5	4.1–4.5	4.7	5.4
B. sp. 10 (n= 7)	5.7–6.5	6.3–6.8	5.4–6.5	5.2–5.9	3.8–4.1	4.1–4.8	4.3–5.2	4.7–6.1	4.1–4.5	4.5–5.4	5.2–5.6	5.4–5.9
B. sp. 34 (n= 8)	5.4–6.3	5.7–6.3	5.0–6.5	5.4–5.9	4.5–5.2	4.5–5.9	4.3–5.2	4.7–5.9	4.3–4.5	4.1–5.0	5.0–5.4	5.0–5.9
B. sp. 30 (n= 1)	8.6–9.0	6.3–6.8	6.3–7.6	7	6.5	6.1–7.2	6.5–7.0	6.7–7.2	6.5	5.8–6.1	5.8	5.9
B. sp. 24 (n= 1)	7.0–7.7	6.6	4.5–5.9	6.3–6.3	5.4	5.2–5.9	5.4–5.9	5.9–6.6	5	5.4–5.7	5.7	5.7
B. peruviana (n= 4)	7.0–7.9	6.1–6.3	3.6–5.2	4.9–5.2	4.5–4.7	3.8–5.2	4.5–5.2	4.9–5.8	4.0–4.3	4.5–5.4	4.7–4.9	5.4–5.6
B. sp. 31 (n= 1)	8.6–8.8	7	6.1–7.0	7.4	7	6.8–7.0	6.3–6.5	6.7–7.2	6.7	6.3–6.5	5.8	6.3
B. sp. 32 (n= 7)	8.6–8.8	6.5	4.7–5.6	6.3	5.2	5.2–5.9	4.5–4.9	5.6–6.3	4.7	4.5–5.2	4.7	5.2
B. paraensis (n= 2)	7.9–8.8	6.5–6.8	5.8–7.0	6.3–6.5	6.5–6.7	6.1–7.4	5.6–6.1	6.1–7.0	6.1	6.1–6.5	5.2–5.4	5.9–6.1
B. sp. 29 (n= 1)	7.7–7.9	6.3	4.7–6.1	6.3	5.2	5.2–5.9	4.9–5.4	5.4–5.8	4.9	4.5–4.7	4.9	5.2

Supplementary 5. Continuation

16S	B. sp. 33 (n= 1)	B. sp. 15 (n= 1)	B. sp. 25 (n= 2)	B. caldwellae (n= 1)	B. sp. 20 (n= 1)	B. madeira (n= 2)	B. sp. 21 (n= 1)	B. sp. 22 (n= 4)	B. sp. 36 (n= 1)	B. sp. 19 (n= 1)	B. sp. 23 (n= 1)	B. sp. 17 (n= 1)
B. altamazonica (n= 5)	7.0–7.7	5.4–5.6	4.3–5.9	5.4–5.6	4.5–4.7	4.5–5.0	4.1–4.7	4.7–5.6	4.5–4.7	4.3–4.7	4.7–5.0	5.0–5.2
B. sp. 35 (n= 1)	6.1–6.5	4.3	4.1–5.4	4.7	4.5	4.1–5.2	4.3–4.7	4.5–5.2	4.3	4.1	3.8	4.5
B. sp. 26 (n= 1)	6.5–7.0	5.2	3.6–5.0	5.2	5	4.5–5.2	4.3–4.7	5.0–5.6	4.3	4.1–5.0	4.3	5.2
B. sp. 27 (n= 1)	6.5–6.8	5.4	4.3–5.6	5.4	5.2	4.7–5.0	4.5–5.0	5.2–5.9	4.5	4.3–4.7	5.2	5.2
B. sp. 28 (n= 3)	6.1–6.3	4.7	4.1–5.4	4.7–4.8	4.7	4.1–4.7	3.8–4.3	4.5–5.2	4.3	4.1–4.5	4.5	5.2
B. sp. 1 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 11 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 14 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 16 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 8 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Supplementary 5. Continuation

16S \cytb	B. equatoriana (n= 10)	B. sp. 2 (n= 1)	B. sp. 6 (n= 1)	B. sp. 4 (n= 3)	B. sp. 7 (n= 3)	B. sp. 3 (n= 0)	B. sp. 5 (n= 3)	B. tapajonica (n= 2)	B. sp. 18 (n= 8)	B. palmata (n= 2)	B. sp. 12 (n= 4)
B. sp. 33 (n= 3)	11.9	13.1	12.7	11.4	12.3	NA	12.1	10.2	9.8	9.3	10.0–11.7
B. sp. 15 (n= 1)	12.1	14.2	11.7	13.6	12.5	NA	12.7	12.5	12.5	11.4	11.2–12.7
B. sp. 25 (n= 5)	14.8–15.9	15.9–16.1	12.7–13.1	12.9–13.8	14.2–15.7	NA	12.3–13.1	10.4–12.3	11.0–11.6	12.3	11.9–12.9
B. caldwellae (n= 2)	13.8	14.6	13.1	14	12.7–12.7	NA	13.3	13.3	12.5	11.2	10.6–11.7
B. sp. 20 (n= 1)	12.7	12.9	11	11.2	10.7	NA	10.6	10	9.3	9.7	10.4–10.6
B. madeira (n= 3)	12.9–15.4	14.6	14.0–14.6	13.3–14.4	13.8–15.7	NA	12.9–14.0	11.9–13.4	12.1–13.3	11.9	12.3–14.6
B. sp. 21 (n= 4)	14.2	15.7	13.3	14	13.6	NA	12.1	12.5	11.7	11.4	11.2–12.5
B. sp. 22 (n= 11)	11.6–15.0	13.3–14.4	11.6–13.3	12.7–14.4	11.1–12.9	NA	10.2–13.1	11.0–12.9	9.1–11.6	9.7–11.0	9.5–12.7
B. sp. 36 (n= 1)	12.3	13.8	13.3	12.5	13.4	NA	12.9	11.9	10.8	10.8	12.3–14.2
B. sp. 19 (n= 2)	13.6	11.9	11.6	12.7	11.3	NA	11.7	10.8	11.2	9.8	11.4–12.5
B. sp. 23 (n= 1)	15.2	13.4	11.9	12.1	12.3	NA	12.7	11	10.4	11.2	11.7–12.3
B. sp. 17 (n= 1)	13.4–15.0	14.6	12.9	13.8	14	NA	13.8	12.3–13.6	11.2–11.7	11.2	11.4–12.9
B. equatoriana (n= 4)	0.0–2.9\0.0–10.1	14.6–16.3	15.2–16.5	15.0–15.9	13.6–16.5	NA	14.2–16.3	13.3–16.7	13.7–14.4	8.9–12.1	11.7–14.8
B. sp. 2 (n= 2)	5.9–7.3	1.1\0.0	11	10.8–11.4	9.4–11.4	NA	9.8–11.2	14.0–14.6	12.9–13.4	13.1	13.3–15.2
B. sp. 6 (n= 1)	5.4–6.3	5.0–6.1	0.0\0.0	8.5	7.9–8.5	NA	9.5	12.5–12.7	13.3–13.8	11.4	12.3–13.4
B. sp. 4 (n= 6)	5.0–5.6	4.1–5.2	4.3	0.0\0.2–0.6	8.0–8.7	NA	7.6–8.9	12.1–12.5	13.4–14.6	12.9–13.1	12.7–14.4
B. sp. 7 (n= 9)	4.3–6.1	3.4–5.6	3.4–5.2	2.9–4.1	0.0–2.7\4.0–5.3	NA	8.7–9.8	12.5–14.0	12.7–14.0	11.2–12.9	11.2–13.8
B. sp. 3 (n= 3)	4.8–5.2	3.4–4.5	2.7	2.5	2.0–3.6	0.0\NA	NA	NA	NA	NA	NA
B. sp. 5 (n= 7)	4.3–5.6	3.4–4.5	2.5–3.2	3.2–3.6	1.6–3.6	1.4–1.8	0.0–1.1\2.1–5.9	11.0–13.4	11.4–12.1	11.0–11.9	11.2–13.6
B. tapajonica (n= 2)	4.3–6.8	5.4–6.3	6.3–6.5	5.4–6.3	5.0–6.5	5.2–5.4	4.3–5.9	2.9\5.7	9.7–11.2	11.0–11.9	11.2–13.6
B. sp. 18 (n= 8)	5.5–6.1	5.2–5.9	6.1	5.6	5.0–5.9	5.4	4.5–5.4	3.2–3.4	0.0\0.0–0.6	10.1–10.4	11.6–12.5
B. palmata (n= 3)	4.7–5.2	4.7–5.4	5.9–6.1	4.7–5.0	4.5–5.4	4.3–4.5	3.6–4.3	5.2–5.9	4.7–5.0	0.0–0.2\0.0	8.7–9.5
B. sp. 12 (n= 5)	3.9–5.4	4.7–5.9	5.4–5.9	3.4–4.1	3.6–5.0	3.8–5.0	3.8–4.8	4.1–5.2	3.6–4.5	3.4–4.3	0.0–1.8\0.2–7.4
B. sp. 9 (n= 1)	3.6–5.2	4.3–5.0	5.2	3.6	3.6–5.0	3.8	3.4–4.1	3.2–4.1	3.2	3.4–3.6	1.6–2.0
B. sp. 13 (n= 1)	4.3–5.4	5.0–5.6	5.6	4.5	4.1–4.7	5	4.1–4.7	4.3–5.4	3.8	2.7–2.9	1.6–2.3
B. sp. 10 (n= 7)	5.0–6.3	5.0–6.1	5.6–6.1	4.3–4.8	4.1–5.2	5.0–5.4	4.1–5.2	4.1–5.2	3.4–3.6	3.4–4.1	1.8–2.7
B. sp. 34 (n= 8)	5.0–6.1	4.7–6.1	5.4–6.1	4.3–5.0	4.1–5.0	4.7–5.4	3.6–5.2	3.6–5.4	3.2–3.8	3.4–4.1	1.1–2.9
B. sp. 30 (n= 1)	6.6–7.9	7.9–8.3	8.1–8.1	5.9	7.2–8.8	6.8	6.8–7.4	3.8–6.1	6.1	6.3–6.5	5.4–6.1
B. sp. 24 (n= 1)	6.2–7.3	7.0–7.7	6.6	5.9	5.9–7.0	6.3–6.4	5.2–5.9	4.8–5.9	5.2	6.6–6.8	5.0–5.9
B. peruviana (n= 4)	5.7–6.8	6.3–7.2	6.8–7.0	5.6–5.9	5.6–7.0	5.4–5.9	4.5–5.9	4.7–5.6	4.1–4.3	6.1–6.5	5.2–5.9
B. sp. 31 (n= 1)	6.6–8.1	7.7–8.3	8.3	6.8	7.4–9.0	7.4	7.0–7.7	5.4–7.4	5.9	6.8–7.0	5.9–6.8
B. sp. 32 (n= 7)	6.8–7.9	7.2–7.4	7.7	6.8	7.0–8.1	7	6.1–6.8	4.5–5.6	5.2	6.8–7.0	5.6–6.6
B. paraensis (n= 2)	6.8–8.8	7.7–8.3	7.9–8.1	6.8	7.0–8.8	7.0–7.2	6.3–7.4	4.3–6.1	5.2–5.4	7.0–7.4	6.3–6.8
B. sp. 29 (n= 1)	6.3–7.4	7.0–7.7	7	5.9	6.3–7.4	6.3	5.4–6.1	3.6–5.0	4.3	5.9–6.1	5.0–5.7

Supplementary 5. Continuation

16S	B. equatoriana (n= 4)	B. sp. 2 (n= 2)	B. sp. 6 (n= 1)	B. sp. 4 (n= 6)	B. sp. 7 (n= 9)	B. sp. 3 (n= 3)	B. sp. 5 (n= 7)	B. tapajonica (n= 2)	B. sp. 18 (n= 8)	B. palmata (n= 3)	B. sp. 12 (n= 5)
B. altamazonica (n= 5)	5.5–6.8	6.3–7.2	5.9–6.1	5.2–5.4	5.0–6.5	5.0–5.2	4.5–5.4	3.4–5.0	3.8–4.1	5.4–5.9	4.3–5.0
B. sp. 35 (n= 1)	5.0–6.3	6.1–6.5	6.1	5	5.2–6.5	5.2	4.8–5.4	3.2–5.2	4.1	5.0–5.2	4.5–5.2
B. sp. 26 (n= 1)	5.2–5.9	6.1–6.8	6.8	5.4	5.6–6.5	5.6–5.7	4.8–5.4	3.8–5.4	4.5	4.7–5.0	4.8–5.7
B. sp. 27 (n= 1)	6.1–6.3	5.6–6.3	6.3	5	5.2–6.3	5.2	4.5–5.2	4.1–5.0	5	5.2–5.4	5.2–6.1
B. sp. 28 (n= 3)	5.7–5.9	5.7–6.8	6.3	4.5	5.2–5.9	5.2	4.8–5.7	4.3–5.4	4.5	5.0–5.2	5.2–6.1
B. sp. 1 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 11 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 14 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 16 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 8 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Supplementary 5. Continuation

16S\cytb	B. sp. 9 (n= 0)	B. sp. 13 (n= 4)	B. sp. 10 (n= 18)	B. sp. 34 (n= 8)	B. sp. 30 (n= 1)	B. sp. 24 (n= 1)	B. peruviana (n= 1)	B. sp. 31 (n= 0)	B. sp. 32 (n= 7)	B. paraensis (n= 1)	B. sp. 29 (n= 1)	B. altamazonica (n= 2)
B. sp. 33 (n= 3)	NA	9.8–12.1	11.2–12.3	9.5–10.2	11.2	12.1	10.4	NA	9.7	12.1	11.8	10.4
B. sp. 15 (n= 1)	NA	10.4–13.8	12.3–13.8	11.2–11.7	11	12.3	11.6	NA	10.2	12.5	11.8	10.8
B. sp. 25 (n= 5)	NA	10.8–14.0	12.7–14.0	12.1–12.9	9.1–9.5	12.3	10.8	NA	9.1–9.7	11.2–11.6	10.1–10.5	9.5–9.8
B. caldwellae (n= 2)	NA	10.4–12.1	10.4–11.6	11.6–11.9	12.1	11	11.7	NA	12.3–12.5	12.3	13.1	11.7
B. sp. 20 (n= 1)	NA	9.8–12.5	11.0–12.5	10.2–11.0	9.5	11.9	10.4	NA	9.1	11.4	10.7	10.2
B. madeira (n= 3)	NA	13.1–14.6	12.3–14.6	11.6–13.4	13.6–14.6	14.2–14.4	12.9–13.4	NA	11.7–12.9	13.8–14.6	13.3–14.3	12.9–14.2
B. sp. 21 (n= 4)	NA	11.4–13.1	12.3–13.1	10.4–11.4	12.5	11.6	11.7	NA	11.9	13.6	13.3	13.3
B. sp. 22 (n= 11)	NA	10.8–12.9	10.0–13.1	10.0–11.9	11.0–12.3	11.9–13.6	11.0–11.9	NA	10.4–11.6	11.4–11.9	11.2–13.3	11.2–12.3
B. sp. 36 (n= 1)	NA	12.1–14.2	12.9–14.2	11.0–11.9	11.7	12.5	11.9	NA	11.9	11.4	12.2	11.7
B. sp. 19 (n= 2)	NA	10.4–12.9	11.6–12.9	10.8–11.6	10.2	11.2	10.6	NA	9.3	10	10.5	10.4
B. sp. 23 (n= 1)	NA	11.2–12.5	11.4–12.5	10.8–11.4	8.9	10.4	9.7	NA	8.7	10.2	9.3	8.7
B. sp. 17 (n= 1)	NA	12.3–13.1	12.3–13.1	11.7–12.3	10.4	13.6	11.9	NA	11.4	12.3	10.9	12.3
B. equatoriana (n= 4)	NA	12.9–14.8	12.5–14.8	12.5–14.2	13.6	15.2	13.1	NA	12.9–15.4	13.8	14.5	13.3–15.9
B. sp. 2 (n= 2)	NA	13.8–15.3	14.4–15.3	13.4–14.4	13.3	15.5	13.3	NA	13.3	13.8	14.5	13.1
B. sp. 6 (n= 1)	NA	10.8–12.9	11.9–13.6	10.8–11.7	10.8	13.4	13.1	NA	12.1–12.3	14.2	12.4	12.7
B. sp. 4 (n= 6)	NA	13.1–14.4	12.7–14.4	11.9–13.3	11.7	12.9	13.3	NA	11.7–12.1	14.2	13	11.6–12.1
B. sp. 7 (n= 9)	NA	11.7–15.3	13.1–15.7	12.1–13.6	11.9	12.9	13.2	NA	12.5–13.6	14.4	14.3	12.9–13.1
B. sp. 3 (n= 3)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 5 (n= 7)	NA	11.9–15.2	12.1–15.2	12.3–13.4	11.9	14.4	14	NA	11.4–12.3	14	12.8	11.6–13.3
B. tapajonica (n= 2)	NA	10.8–14.6	11.9–15.0	10.4–12.5	11.7	12.1	11.7	NA	9.5–10.4	11.9	11.8	11.2–11.6
B. sp. 18 (n= 8)	NA	11.2–13.6	12.3–13.6	11.2–12.1	10.4	11.6	10.4	NA	9.3–9.8	11.2	10.7	10.2–10.8
B. palmata (n= 3)	NA	7.8–11.9	10.4–12.3	8.0–9.5	11.2	13.4	12.5	NA	10.4–10.6	11.9	12.2	11
B. sp. 12 (n= 5)	NA	6.1–8.3	8.1–10.2	6.1–7.8	11.4–12.1	11.7–12.7	11.7–12.1	NA	11.7–12.7	13.1–13.6	12.8–13.5	12.3–13.6
B. sp. 9 (n= 1)	0.0\NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 13 (n= 1)	2.5	0.0\0.0–3.0	8.0–10.0	4.4–5.5	9.8–12.9	10.4–13.1	11.4–11.9	NA	10.6–13.3	11.0–13.6	11.2–13.7	10.6–13.3
B. sp. 10 (n= 7)	1.8–2.0	2.3–2.7	0.0–0.9\0.0–3.6	7.0–9.1	11.2–12.9	12.3–13.1	11.2–11.9	NA	11.4–13.3	12.3–13.6	11.8–13.7	11.7–13.3
B. sp. 34 (n= 8)	2.0–2.5	1.8–2.5	1.6–2.3	0.0–1.1\0–2.8	10.4–10.6	11.0–11.6	11.0–11.7	NA	10.4–11.2	11.2–12.5	10.9–11.2	10.4–11.4
B. sp. 30 (n= 1)	5.4	6.5–6.5	6.3–6.8	5.9–6.5	0.0\0.0	10.2	8.9	NA	4.9–5.1	5.7	5	6.4
B. sp. 24 (n= 1)	5.5	6.1	5.7–6.4	5.2–5.9	5.9	0.0\0.0	8.7	NA	9.8	9.5	11.2	9.1
B. peruviana (n= 4)	5.4–5.6	5.9–6.1	5.9–6.3	5.4–6.5	6.1–6.3	2.9–3.2	0.0–0.2\0.0	NA	8.5	9.7	8	8.1
B. sp. 31 (n= 1)	5.9	6.1	6.3–7.0	5.9–6.5	3.8	6.1	6.5–6.7	0.0\NA	NA	NA	NA	NA
B. sp. 32 (n= 7)	5.6	6.3	5.9–6.3	5.6–6.3	3.4	4.8	4.9–5.2	4	0.0\0.0–0.2	5.3–5.5	5.3–5.5	5.1–5.3
B. paraensis (n= 2)	5.6–5.9	7.0–7.2	6.5–7.2	6.3–7.0	3.4–3.6	4.8–5.0	5.4–5.8	4.5–4.7	3.1–3.4	0.4\0.0	6.3	6.8
B. sp. 29 (n= 1)	5.2	5	5.0–5.4	4.3–5.0	3.4	4.3	4.3–4.5	4	2.7	2.9–3.1	0.0\0.0	6.3

Supplementary 5. Continuation

16S	B. sp. 9 (n= 1)	B. sp. 13 (n= 1)	B. sp. 10 (n= 7)	B. sp. 34 (n= 8)	B. sp. 30 (n= 1)	B. sp. 24 (n= 1)	B. peruviana (n= 4)	B. sp. 31 (n= 1)	B. sp. 32 (n= 7)	B. paraensis (n= 2)	B. sp. 29 (n= 1)	B. altamazonica (n= 5)
B. altamazonica (n= 5)	4.1–4.3	5.2–5.4	4.5–5.2	4.5–5.4	3.2–3.4	4.1–4.3	4.1–4.5	3.4–3.6	2.5–2.7	2.5–2.9	1.6–1.8	0.0–0.2\0.0
B. sp. 35 (n= 1)	4.1	4.8	4.8–5.2	4.5–5.0	3.8	4.3	3.8–4.1	5.2	4.1	3.6–3.8	3.6	2.9–3.2
B. sp. 26 (n= 1)	5	4.8	5.2–5.7	4.5–5.4	5	4.3	3.8–4.1	5.6	4.3	4.7–5.0	3.8	3.6–3.8
B. sp. 27 (n= 1)	5.4	5.7	5.7–6.1	5.4–6.1	5.4	3.9	4.3–4.5	6.3	4.7	4.7–5.0	3.8	3.6–3.8
B. sp. 28 (n= 3)	5.2	5.4	5.4–5.9	5.0–5.9	5.6	4.3	4.3–4.5	6.1	5	5.4	4.5	3.8–4.1
B. sp. 1 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 11 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 14 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 16 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 8 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Supplementary 5. Continuation

cytb	B. sp. 35 (n= 0)	B. sp. 26 (n= 0)	B. sp. 27 (n= 0)	B. sp. 28 (n= 0)	B. sp. 1 (n= 1)	B. sp. 11 (n= 10)	B. sp. 14 (n= 1)	B. sp. 16 (n= 1)	B. sp. 8 (n= 4)
B. sp. 33 (n= 1)	NA	NA	NA	NA	14.6	10.6–11.4	11.7	10.2	11.4
B. sp. 15 (n= 1)	NA	NA	NA	NA	14.4	11.4–13.3	10.2	9.1	12.3
B. sp. 25 (n= 2)	NA	NA	NA	NA	15.0–15.3	10.6–12.5	9.3	11	12.9
B. caldwellae (n= 1)	NA	NA	NA	NA	15.5	11.6–12.5	11.7	11.7	11.4
B. sp. 20 (n= 1)	NA	NA	NA	NA	14.4	10.2–12.5	9.3	9.1	11.6
B. madeira (n= 3)	NA	NA	NA	NA	14.8	11.6–13.8	12.1	10.8	13.3–14.4
B. sp. 21 (n= 1)	NA	NA	NA	NA	16.1	12.1–13.3	12.9	11.9	12.7
B. sp. 22 (n= 4)	NA	NA	NA	NA	13.8–14.8	10.4–13.6	9.5	10	11.4–12.9
B. sp. 36 (n= 1)	NA	NA	NA	NA	14.6	12.5–13.1	12.3	10.2	13.6
B. sp. 19 (n= 1)	NA	NA	NA	NA	13.4	12.3–14.0	11	10.6	11.9
B. sp. 23 (n= 1)	NA	NA	NA	NA	15.3	11.4–12.5	9.1	9.5	11.9
B. sp. 17 (n= 1)	NA	NA	NA	NA	14.8	13.1–13.3	10.6	11.7	11.7
B. equatoriana (n= 10)	NA	NA	NA	NA	15.3–17.3	9.8–14.0	12.9	12.3	12.5–13.7
B. sp. 2 (n= 1)	NA	NA	NA	NA	11	14.2–16.3	13.3	13.3	14.6
B. sp. 6 (n= 1)	NA	NA	NA	NA	10.6	12.5–14.0	12.1	11.7	12.7
B. sp. 4 (n= 3)	NA	NA	NA	NA	11.6–12.1	13.3–15.0	12.1	12.7	11.9–12.3
B. sp. 7 (n= 3)	NA	NA	NA	NA	8.6–10.4	12.7–15.0	12.5	12.7	12.7–13.6
B. sp. 3 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 5 (n= 3)	NA	NA	NA	NA	11.4–12.5	11.7–13.8	10.8	11	12.3–13.1
B. tapajonica (n= 2)	NA	NA	NA	NA	15.3–15.5	11.4–14.6	11.7	11	12.9
B. sp. 18 (n= 8)	NA	NA	NA	NA	14.0–14.4	12.1–13.4	9.8	9.7	12.3
B. palmata (n= 2)	NA	NA	NA	NA	13.8–13.8	8.3–11.0	10	8.7	9.3
B. sp. 12 (n= 4)	NA	NA	NA	NA	13.6–16.9	7.4–11.0	8.7–10.6	8.1–10.0	7.4–8.0
B. sp. 9 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 13 (n= 4)	NA	NA	NA	NA	13.4–16.7	8.1–11.4	9.3–11.4	9.7–11.2	7.8–8.0
B. sp. 10 (n= 18)	NA	NA	NA	NA	15.3–16.7	8.7–12.1	9.8–11.4	10.2–11.2	7.6–8.5
B. sp. 34 (n= 8)	NA	NA	NA	NA	14.6–14.8	6.8–9.1	9.3–9.8	8.9–9.3	6.6–7.4
B. sp. 30 (n= 1)	NA	NA	NA	NA	12.1	10.4–11.9	8.7	9.8	11.4
B. sp. 24 (n= 1)	NA	NA	NA	NA	14.8	11.6–14.0	12.5	11.9	12.5
B. peruviana (n= 1)	NA	NA	NA	NA	14.8	11.4–12.7	10.6	11.2	11.2
B. sp. 31 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 32 (n= 7)	NA	NA	NA	NA	13.4–13.6	11.6–13.4	9.1	9.5	11.4–11.6
B. paraensis (n= 1)	NA	NA	NA	NA	15.5	12.1–13.4	10	10.4	12.9
B. sp. 29 (n= 1)	NA	NA	NA	NA	13.9	12.4–13.7	10.1	11	12.4

Supplementary 5. Continuation

16S\cytb	B. sp. 35 (n= 0)	B. sp. 26 (n= 0)	B. sp. 27 (n= 0)	B. sp. 28 (n= 0)	B. sp. 1 (n= 1)	B. sp. 11 (n= 10)	B. sp. 14 (n= 1)	B. sp. 16 (n= 1)	B. sp. 8 (n= 4)
B. altamazonica (n= 5)	NA	NA	NA	NA	14.2	10.8–12.3	9.7	10.6	11.4
B. sp. 35 (n= 1)	0.0\NA	NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 26 (n= 1)	2.3	0.0\NA	NA	NA	NA	NA	NA	NA	NA
B. sp. 27 (n= 1)	3.4	2.9	0.0\NA	NA	NA	NA	NA	NA	NA
B. sp. 28 (n= 3)	2.9	2.7	2.5	0.0\NA	NA	NA	NA	NA	NA
B. sp. 1 (n= 0)	NA	NA	NA	NA	NA\0.0	15.2–16.7	14.4	14.8	14
B. sp. 11 (n= 0)	NA	NA	NA	NA	NA	NA\0.0–5.5	9.8–11.0	8.7–10.4	8.7–9.8
B. sp. 14 (n= 0)	NA	NA	NA	NA	NA	NA	NA\0.0	7	9.7
B. sp. 16 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA\0.0	9.3
B. sp. 8 (n= 0)	NA	NA	NA	NA	NA	NA	NA	NA	NA\0.0

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