CERTIFICACIÓN

Yo, Ph.D. Hugo Navarrete, certifico que la disertación de Licenciatura en Ciencias Biológicas del candidato Alejandro Federico Arteaga Navarro, "*Comparative phylogeography reveals cryptic diversity and repeated patterns of cladogenesis for amphibians and reptiles in northwestern Ecuador*", ha sido concluida en conformidad con las normas establecidas, por lo tanto, puede ser presentada para la calificación correspondiente.

Ph.D. Hugo Navarrete

Director de la disertación

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COMPARATIVE PHYLOGEOGRAPHY REVEALS CRYPTIC DIVERSITY AND REPEATED PATTERNS OF CLADOGENESIS FOR AMPHIBIANS AND REPTILES IN NORTHWESTERN ECUADOR

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Alejandro Federico Arteaga Navarro

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Comparative phylogeography reveals cryptic diversity and repeated patterns of cladogenesis for amphibians and reptiles in northwestern Ecuador

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Short title: Comparative phylogeography of amphibians and reptiles in northwestern Ecuador.

Abstract

Comparative phylogeography is now a common approach to understand how historical processes have shaped the formation of lineages in a broad spectrum of codistributed populations of different taxa. However, these types of studies are scarce in the Neotropics, a region that is characterized by speciose assemblages, complex geological history, and poorly understood historical biogeography. To cope with this lack of knowledge, in this study, we apply a broad comparative approach to investigate the diversification patterns, if any, of five lineages of amphibians and reptiles codistributed at the biogeographic boundaries of the Choco and Andes ecoregions in northwester Ecuador. Mitochondrial sequences were used to determine the degree of diversification within species. Our results highlight congruent patterns of parapatric speciation and common geographical barriers for distantly related taxa. These comparisons indicate similar biological and demographic characteristics for the included clades, and reveal the existence of two new species of *Pristimantis* previously subsumed under *P. walkeri*. Our data supports the hypothesis that widely distributed Chocoan taxa may generally experience their greatest opportunities for isolation and parapatric speciation across elevational thermal gradients in the adjacent montane forests. Finally, our study provides critical information to predict which unstudied lineages may harbor cryptic diversity, and how geology and climate are likely to have shaped their evolutionary history.

Introduction

Northwestern South America is privileged for being located in an area where two of the most biodiverse terrestrial ecoregions of the planet meet, the Andes and the Chocoan lowlands. Together, these to ecoregions harbor nearly 18.5% of the world's total diversity of terrestrial vertebrates (Mittermeier *et al* 2011; Jenkins *et al* 2013; Kluge 2008). For example, in Mindo, Ecuador, a transitional valley of only 268 km^2 located where the Choco meets the Andes (ca 1000 m), 101 species of amphibians and reptiles have been registered (Arteaga, Bustamante & Guayasamin 2013). Another locality in NW Ecuador, Bilsa Biological Station, harbors 109 species of herpetofauna in only 33 km^2 (Ortega-Andrade *et al* 2010). The reason why this transitional area is so diverse in relation to other tropical areas could be explained by a history of biological interchanges (Elmer *et al* 2013; Pinto Sánchez *et al* 2012; Pyron & Wiens 2013), lower rates of extinction (Pyron & Wiens 2013; Rolland *et al* 2014), or greater rates of speciation than other regions (Pyron & Wiens 2013; Rolland *et al* 2014). This latter cause explained by the interaction between the geographic and climatic complexity of tropical mountainous areas (Weir & Price 2011; Kozak & Wiens 2007), the evolutionary conservatism of climatic niches (Cadena *et al* 2012; Hutter, Guayasamin & Wiens 2013), and the time the lineages have persisted in the region (Smith *et al* 2014; Rolland *et al* 2014; Hutter, Guayasamin & Wiens 2013).

From the scenarios outlined above, one that we can evaluate with our data is that of speciation in complex watersheds and montane ecosystems through simple models of vicariance (Wiens 2004). Several authors (Lynch & Duellman 1997; Arteaga, Bustamante & Guayasamin 2013; Torres-Carvajal & Lobos 2014; Guayasamin *et al* 2015) have already suggested that both the valleys and the large river systems of this region have effectively limited dispersion among herpetofaunal populations. However, no studies have been made to

determine if these elements of the landscape have affected distantly related lineages of herpetofauna in the same way. Barriers and ecological gradients might be common to all lineages, but ultimately what determines the pattern of speciation in an area is the measure by which those elements of the landscape affect the ability of the organisms to disperse (García *et al* 2012; Daza, Castoe & Parkinson 2010). Evidence for allopatric speciation driven by geographical barriers is abundant (Vences & Wake 2007), but evidence for parapatric speciation along ecological gradients remains scarce (Coyne & Orr 2004; Price 2008), although this latter pattern has been suggested to have play an important role in the speciation of amphibians in the Andes (Lynch & Duellman 1997).

One way to study the effect that geographical barriers have on the diversification of distinct groups of organisms is comparative phylogeography (Avise 2000; Ree & Smith 2008; Ree & Sanmartín 2009). These studies at the molecular and geographical level make it possible to infer patterns of species diversification from the current geographic distribution of genetic diversity (Feldman & Spicer 2006; Leaché, Crews & Hickerson 2007), and to evaluate the impact of historical events on the genetic composition and structure of biotic assemblages (Rocha *et al* 2002; Zink 2002; Pastorini *et al* 2003). In addition, this information allows us to create hypotheses about current patterns of species distributions and to infer which lineages that have not been studied at the molecular level may harbor cryptic diversity.

Recent studies in Ecuador addressing geographic patterns of diversification have been focused on groups of closely related amphibians (Elmer *et al* 2013; Hutter, Guayasamin & Wiens 2013) and reptiles (Torres *et al* 2014; Torres-Carvajal & Mafla-Endara 2013). None of these studies have used a comparative phylogeographic approach across reptiles and amphibians. Studies from other regions (Daza *et al* 2010; Feldman & Spicer 2006) that contain a diverse sample of taxonomic and ecological groupings have been able to answer

different questions and provide a wider perspective of the co-diversification and speciation of their target area. In this study, we use two pairs of species of reptiles belonging to the families Gymnopthalmidae and Viperidae; and three pairs of species of amphibians belonging to the family Craugastoridae, to describe geographic patterns of diversification. The sister-species pairs were chosen for i) being co-distributed in northwestern Ecuador and ii) having been considered conspecific in the past.

Materials and methods

Ethics statement

This study was carried out in strict accordance with the guidelines for use of live amphibians and reptiles in field research compiled by the American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists' League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR). Research and collection were done under permits of the Ecuadorian Ministry of the Environment: N°14-2011-IC-FAU-DPAP-MA, N°05-2013-IC-FAU-DPAP-MA and N°01-2014-AD-RIC-FAU-DPAP-MA, granted to Juan M. Guayasamin through Universidad Tecnológica Indoamérica, and permit N°012-IC-FAN-DPEO-MAE, granted to Mario Yánez-Muñoz through the Museo Ecuatoriano de Ciencias Naturales. Specimens were euthanized with 20% benzocaine, fixed in 10% formalin and stored in 70% ethanol. Museum vouchers were deposited at the Museo de Zoología of the Universidad Tecnológica Indoamérica (MZUTI).

Sampling

A total of 103 samples representing 13 species (including two yet undescribed species) were obtained from 24 localities throughout their distributions in western Ecuador

(Table 1). Our study focuses on five lineages. Each lineage contains two species known to be the closest morphological relative of each other. One species is relatively widely distributed on evergreen lowland and foothill forests of western Ecuador (Fig. 1), and the other species is mostly restricted to evergreen lower-montane forests in northwestern Ecuador (Fig. 1). The five lineages share similar patterns of distribution, but have totally different dispersal characteristics and life history traits. Samples from genera other than *Bothrops*, *Alopoglossus* and *Pristimantis* were used as outgroups for phylogenetic analyses (S1 Table). 199 sequences from GenBank were included in the analyses as well (S1 Table).

Figure 1. Main vegetation zones and rivers in the Ecuadorian northwest. The map is a simplified version of the main vegetation zones of Sierra (1999).

Table 1. Sampling locations.

Province	Locality	Latitude	Longitude	Elev.
Azuay	Flor y Selva	-2.65706	-79.53111	136
Cañar	Huatacón	-2.49018	-79.18223	1048
El Oro	Buenaventura	-3.66598	-79.73933	1042
El Oro	California	-3.37146	-79.73430	328
Esmeraldas	Bilsa	0.34910	-79.70967	555
Esmeraldas	Canandé	0.52645	-79.20937	360
Esmeraldas	Itapoa	0.51306	-79.13396	341
Esmeraldas	Mache Chindul	0.51032	-79.72552	175
Esmeraldas	Tundaloma	1.18317	-78.75245	74
Imbabura	Los Cedros	0.31842	-78.78373	1764
Pichincha	Cascadas de Mindo	-0.07837	-78.76429	1438
Pichincha	Chontilla	0.11187	-78.90275	1191
Pichincha	El Abrazo	-0.00916	-78.81133	1086

Pichincha	Las Gralarias	-0.00158	-78.73858	1793
Pichincha	Mashpi lodge	0.16352	-78.87274	1060
Pichincha	Milpe	0.03489	-78.86713	1070
Pichincha	Sachatamia	-0.02470	-78.75909	1704
Pichincha	Selva Virgen	0.10673	-78.18542	355
Pichincha	Séptimo Paraíso	-0.02808	-78.76667	1537
Pichincha	Silanche	0.14577	-79.14338	418
Pichincha	Sueños de Bambú	-0.06655	-78.77158	1391
Pichincha	Tandayapa Lodge	0.00249	-78.68083	1730
Pichincha	Yellow House	-0.04505	-78.75938	1498
Santo Domingo	Otongachi	-0.32145	-78.95094	661

All specimens included in the genetic analyses came from the localities listed on Table 1 and were morphologically identified according to Lynch & Duellman (1997), Arteaga *et al* (2013), Campbell & Lamar (2004) and Torres-Carvajal & Lobos (2014). Novel sequences are marked with an asterisk under S1 Table, which includes museum vouchers at the Museo de Zoología of the Universidad Tecnológica Indoamérica (MZUTI) and the División de Herpetología del Museo Ecuatoriano de Ciencias Naturales (MECN), along with individuals released after sampling (ANF and AA). Among the newly sequenced specimens, there are: 4 *Bothrops osbornei*, 5 *B. punctatus*, 8 *Alopoglossus festae*, 2 *A. viridiceps*, 12 *Pristimantis crenunguis*, 14 *P. labiosus*, 31 *P. luteolateralis*, 3 *Pristimantis mindo*, 2 *P. parvillus*, 12 *P. subsigillatus*, 4 *P. walkeri*, and six specimens belonging to the two new species described here (S1 Table).

Laboratory techniques

Genomic DNA was extracted from 96% ethanol-preserved tissue samples (liver, muscle tissue or scales) using a modified salt precipitation method based on the Puregene DNA purification kit (Gentra Systems). For amphibians, we amplified the mitochondrial 12S gene using the primers t-Phe-frog and Val-frog developed by Wiens *et al* (2005) and 12L29E-F and 12H46E-R developed by Heinicke *et al* (2007). For the 16S gene we used the primers 16SC and 16Sbr-H developed by Darst & Cannatella (2004) and Palumbi *et al* (1991), respectively. For reptiles, we amplified the 12S gene using the primers 12Sa and 12Sb developed by Kocher *et al* (1989), and the 16S gene using the primers 16Sar and 16Sbr developed by Simon *et al* (1994). Additionally, the cytb gene was obtained with the primers L14910 and H16064 developed by Burbrink *et al* (2000), whereas the subunit 4 of the NADH dehydrogenase mitochondrial gene was amplified with using the primers ND4_F and ND4_R developed by Arévalo *et al* (1994). The DNA amplification reactions of gene fragments contained 1 μL of extracted DNA, 0.5 μL of dNTPs, 0.5 μL of forward and reverse primers, 1.5 μL of MgCl2, 0.25 μL of Taq DNA polymerase, 2.5 μL of ThermoPol buffer, and 18.25 μL H2O. PCR products were visualized in 1% agarose gel, and unincorporated primers and dNTPs were removed from PCR products by ExoI/SAP digestion. Cycle sequencing reactions were performed by Macrogen Labs (Macrogen Inc., Korea). All fragments were sequenced in both forward and reverse directions. The sequences were deposited in GenBank (S1 Table).

DNA sequence analyses

Sequences obtained during this work were edited and assembled using the program

Geneious ProTM 5.4.7 (Drummond *et al* 2010). The resulting sequences and those already available from Genbank (S1 Table) were aligned using MAFFT v.7 (Katoh & Standley 2013), under the default parameters in Geneious ProTM 5.4.7. Genes were combined into a single matrix with eight partitions, three per protein coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 1.1.1 (Lanfear *et al* 2012) and jModeltest (Darriba *et al* 2012) under the Bayesian information criterion. In the mitochondrial matrix, we defined eight *a priori* partitions (12S, 16S and one partition for each codon position of ND4 and cytb). Phylogenetic relationships were assessed under a Bayesian approach in MrBayes 3.2.0 (Ronquist & Huelsenbeck 2003). Four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 6.7 million generations and four Markov chains with default heating settings. GenBank accession numbers are listed in S1 Table. Trees were sampled every 1,000 generations, resulting in 5,000 saved trees per analysis after 25% of those were arbitrarily discarded as ‘‘burn-in.’’ Stationarity was confirmed by plotting the –ln L per generation in the program Tracer 1.2 (Rambaut and Drummond 2003). Genetic distances were calculated using the uncorrected distance matrix in PAUP 4.0 (Swofford 2004).

Morphological data

Generic and family names used in this study follow Pyron & Wiens (2011) and Guayasamin (2004) for amphibians, Hendry *et al* (2014) for vipers and Pellegrino *et al* (2001) for lizards. To examine species boundaries within *Pristimantis*, our diagnoses and descriptions generally follow Duellman & Lehr (2009). We examined comparative alcohol-preserved specimens from the herpetology collections at the MZUTI, MECN and Fundación Herpetológica Gustavo Orcés (FHGO) (S2 Table). When providing the standard deviation,

we use the ± symbol. Morphological measurements were taken with digital calipers to the nearest 0.1 mm, as described by Lehr and Coloma (2008). These are as follows: (1) snout–vent length (SVL), (2) tibia length, (3) foot length, (4) head length, (5) head width, (6) eye diameter, (7) interorbital distance, (8) upper eyelid width, (9) internarial distance, (10) eye–nostril distance. Sexual maturity was determined by the presence of testis or vocal slits in males and by the presence of eggs or convoluted oviducts in females.

Distribution maps

We present ranges of occurrence graphically in the form of spatially distributed dots on a colored representation of Ecuador's relief. Each dot indicates a locality where the species has been observed. This includes published records, photographic vouchers and museum specimens deposited at MZUTI, MECN, FHGO and (The University of Kansas) KU. For all species in the study, a distribution model accompanies the dot maps. These models estimate potential areas of distribution, on the basis of observed presences and a set of environmental predictors (Elith & Leathwick 2009). To create the models, we used presence localities listed on S2 Table, along with the 19 bioclimatic variables from Worldclim 1.4 (Hijmans *et al* 2005) and Maxent 3.3.3e, an algorithm based on the principle of maximum entropy (Phillips *et al* 2006; Elith *et al* 2011; Renner & Warton 2013). The convergence threshold was set to 10^{-5}, maximum iterations to 500, and the regularization parameter to "auto".

Results

Bothrops punctatus and *B. osbornei*

Molecular analyses.

Including the outgroups, we used 129 mtDNA sequences to build a molecular phylogeny of the genus *Bothrops* (Fig. 2). The resulting topology and support is similar to numerous recent studies (Hendry *et al* 2014; Fenker *et al* 2014; Carrasco *et al* 2012). In agreement with previous results (Hendry *et al* 2014; Fenker *et al* 2014), *B. punctatus* is recovered as the sister species of *B. osbornei*. Comparisons of an 759 bp fragment of the mitochondrial NADH dehydrogenase subunit 4 gene between the two species shows a genetic distance of 7.6%, whereas sequence variation within each of the two species is 0%.

Figure 2. Maximum likelihood phylogram depicting relationships within bothropoid pitvipers. The phylogram was derived from analysis of 2908 bp of mitochondrial DNA (gene fragments 12S, 16S, cytb and ND4). Posterior probabilities and voucher numbers are shown.

Distribution maps.

Using a database of museum (S2 Table) and literature (Amaral 1923; Freire-Lascano 1991; Schätti & Kramer 1991; Campbell & Lamar 2002; Morales 2004; Yánez-Muñoz *et al* 2009) records corresponding to 17 localities for *Bothrops punctatus* and 17 for *B. osbornei*, we modeled the habitat suitability for each of the species (Fig. 3). The resulting distribution map is similar to previous works (Campbell & Lamar 2004; Arteaga *et al* 2013), but nearly doubles the number of known localities and shows a distinct geographical separation between the two vipers. The predicted area of potential distribution for *B. punctatus* is related with evergreen lowland and foothill forests in Ecuador (Sierra 1999) (Figs. 1 and 3), whereas for *B. osbornei*, the predicted area of potential distribution is mostly related with evergreen lower-montane forests, cloudforests and foothill forests (Sierra 1999) (Figs. 1 and 3). From our database of known localities, we estimated altitude limits of distributions: 15–864 m for *B. punctatus* and 775–1657 for *B. osbornei*. We found no localities of sympatry between the

two species.

Figure 3. Distribution of *Bothrops osbornei* and *B. punctatus* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Fig. 2.

Systematics.

Several authors (Campbell & Lamar 1992; McDiarmid *et al* 1999; Arteaga *et al* 2013) have considered *Bothrops punctatus* and *B. osbornei* to be conspecific. Our results at the molecular and ecological level now support the view of other authors (Freire-Lascano 1991; Schätti & Kramer 1991; Schätti & Kramer 1993; Campbell & Lamar 2004) who have used morphological data to support the validity of *Bothrops osbornei*. Although similar in outer morphology and scale counts (Campbell & Lamar 2004) our sampled individuals show indeed subtle but consistent differences in coloration (Fig. 4). *B. osbornei* has a dorsal pattern of dark trapezoidal blotches, whereas *B. punctatus* has a pattern of spots arranged in in the form of squares.

Figure 4. Morphological variation within sampled *Bothrops* species. (a) Juvenile of *B. punctatus* (ANF 1575). (b) Adult (ANF 2101) of *B. punctatus*. (c) Juvenile of *B. osbornei* (ANF 2005). (d) Adult of *B. osbornei* (ANF 2767).

Phylogeography.

Despite uncertainties in the higher-level relationships of *Bothrops*, both the species distribution modeling (Fig. 3) and the mtDNA phylogeny suggest that *B. osbornei* is an upland vicariant of *B. punctatus*. The fact that they were previously considered conspecific and that they share numerous morphological traits (Campbell & Lamar 1992; McDiarmid *et*

al 1999; Wüster *et al* 2002) further supports this view.

Alopoglossus festae and *A. viridiceps*

Molecular analyses.

Including the outgroups, we used 26 mtDNA sequences to build a molecular phylogeny of the genus *Alopoglossus* (Fig. 5). The resulting topology and support is similar to the most recent study (Torres-Carvajal & Lobos 2014), and *A. festae* is recovered as the sister species of *A. viridiceps*. Comparisons of a 596 bp fragment of the mitochondrial NADH dehydrogenase subunit 4 gene between the two species shows a genetic distance of 12.4–13.4%, whereas sequence variation within *A. viridiceps* is 0%, and within *A. festae* is 2.2–6.4%.

Figure 5. Maximum likelihood phylogram depicting relationships within the genus *Alopoglossus*. The phylogram was derived from analysis of 1221 bp of mitochondrial DNA (gene fragments 12S, 16S, cytb and ND4). Posterior probabilities and voucher numbers are shown.

Distribution maps.

Using a database of museum (S2 Table) and literature (Peracca 1904; Miyata 1976; Yánez-Muñoz 2005; Savid 2006; Almendariz & Carr 2007; Köhler *et al* 2012; Lynch *et al* 2014; Torres-Carvajal & Lobos 2014) records corresponding to 88 localities for *Alopoglossus festae* and 9 for *A. viridiceps*, we modeled the habitat suitability for each of the species (Fig. 6). The resulting distribution map of *A. festae* is similar to previous works (Köhler *et al* 2012; Arteaga *et al* 2013; Torres-Carvajal & Lobos 2014), but increases the number of localities. The know distribution of *A. viridiceps* is expanded from two localities reported in the

province of Pichincha by Torres-Carvajal & Lobos (2014) to nine localities, including the provinces of Imbabura and Esmeraldas. The predicted area of potential distribution for *A. festae* is related with evergreen and semideciduous lowland and foothill forests in Ecuador (Sierra 1999) (Figs. 1 and 6), whereas for *A. viridiceps*, the predicted area of potential distribution is mostly related with evergreen lower-montane forests and cloudforests (Sierra 1999) (Figs. 1 and 6). From our database of known localities, we estimated altitude limits of distributions: 3–1377 m for *A. festae* and 1165–1879 for *A. viridiceps*. We found no localities of sympatry between the two species.

Figure 6. Distribution of *Alopoglossus festae* and *A. viridiceps* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Fig. 5.

Systematics.

Based on morphological characters, *Alopoglossus viridiceps* is most closely related to *A. festae* (Torres-Carvajal & Lobos 2014) (Fig. 7). This similarity might explain why several specimens of *A. viridiceps* housed at MZUTI and MECN, were previously identified as *A. festae*. Collections of the latter species from the highlands of Pichincha and Imbabura housed at the AMNH might actually represent *A. viridiceps.*

Figure 7. Morphological variation within sampled *Alopoglossus* species. (a) Adult of *A. viridiceps* (MZUTI 3552). (b) Juvenile of *A. festae* (MZUTI 2630). (c) Adult of *A. festae* (MZUTI 2994). (d) Adult female of *A. festae* (MZUTI 3370).

Phylogeography.

Given the morphological similarities between *A. festae* and *A. viridiceps*, and their

close, but non-overlapping ranges of distribution, Torres-Carvajal & Lobos (2014) suggested that one of the species originated from the other by allopatric or parapatric speciation. Our species distribution models, the mtDNA phylogeny, and the fact that both species were previously confused with each other in museum collections further supports this view. Published (Savit 2006; Torres-Carvajal & Lobos 2014) and museum (S2 Table) distribution records of *A. viridiceps* are located south of the Lita river and north of the Toachi river (Figs. 1, 6). These two rivers might have acted as effective barriers for latitudinal dispersal of *A. viridiceps*. Our phylogeny (Fig. 5) shows a deep genetic split between populations of *A. festae* north of Jubones river and populations of that species south of thar river, suggesting that this geographical has effectively prevented migration between populations

Pristimantis labiosus and *P. crenunguis*

Molecular analyses.

Including the outgroups, we used 47 mtDNA sequences to build a molecular phylogeny of the *Pristimantis* (*Hypodictyon*) *rubicundus* species series (Lynch & Miyata 1980; Hedges *et al* 2008) (Fig. 8). The resulting topology and support is similar to recent studies (Pyron & Wiens 2011; Pinto-Sánchez *et al* 2011; Padial *et al* 2014), and *P. labiosus* is recovered as the sister species of *P. crenunguis*. A comparison of a 495 bp fragment of the mitochondrial 16S gene between the two species shows a genetic distance of 5.9–8.1%, whereas sequence variation within *P. crenunguis* is 0–0.6%. Within *P. labiosus*, however, sequence variation ranged from 0% to 6.5%. Based on these results, we suggest that *P. labiosus* is composed of at least two cryptic species. The clade formed by MECN 9527, 9528 and MZUTI 3018 is the most genetically distinct and is shown to be sister to all other sampled populations of *P. labiosus* (Fig. 8).

Figure 8. Maximum likelihood phylogram depicting relationships within the *Pristimantis* (*Hypodictyon*) *rubicundus* species series. The phylogram was derived from analysis of 1032 bp of mitochondrial DNA (gene fragments 12S and 16S). Posterior probabilities and voucher numbers are shown.

Distribution maps.

Using a database of museum (S2 Table) and literature (Lynch 1976; Lynch *et al* 1994; Lynch & Duellman 1997; Morales 2004; Yánez-Muñoz 2005) records corresponding to 46 known localities for *Pristimantis labiosus* and 22 for *P. crenunguis*, we modeled the habitat suitability for each of the species (Fig. 9). The resulting distribution maps are similar to those most recently published (Arteaga *et al* 2013). The predicted area of potential distribution for *P. labiosus* is related with evergreen lowland and foothill forests in Ecuador (Sierra 1999) (Figs. 1 and 9), whereas for *P. crenunguis*, the predicted area of potential distribution is almost exclusively related with evergreen lower-montane forests (Sierra 1999) (Figs. 1 and 9). From our database of known localities, we estimated altitude limits of distributions: 63–1161 m for *P. labiosus* and 1165–1793 for *P. crenunguis*. We found no localities of sympatry between the two species.

Figure 9. Distribution of *Pristimantis labiosus* and *P. crenunguis* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Fig. 8.

Systematics.

Based on morphological characters, *Pristimantis labiosus* is most closely related to *P.*

crenunguis (Lynch *et al* 1994; Lynch & Duellman 1997; Arteaga *et al* 2013). Literature (López *et* al 1998; Reyes 2008) and museum records of *P. labiosus* above 1200 m likely correspond to *P. crenunguis*.

Figure 10. Morphological variation within sampled species of the *Pristimantis* (*Hypodyction*) *rubicundus* series. (a) Juvenile of *Pristimantis crenunguis* (Not vouchered). (b) Adult of *P. crenunguis* (Not vouchered). (c) Juvenile of *P. labiosus* (MZUTI 3511). (d) Adult of *P. labiosus* (Not vouchered).

Phylogeography.

Lynch and Duellman (1997) and Arteaga *et al* (2013) suggested that *Pristimantis labiosus* and *P. crenunguis* are altitudinal replacements of each other. The assumption was made based on the similarities in size, structure, microhabitat utilization, and the adjacent ranges of distribution of the two species. Our species distribution models (Fig. 9) and the mtDNA phylogeny (Fig. 8) support this view. The close, but non-overlapping ranges of distribution suggest an allopatric or parapatric pattern of speciation. However, the existence of at least two genetically structured lineages within *P. labiosus* suggest a more complex scenario of speciation than just one event of vicarance between *P. labiosus* and *P. crenunguis*. Samples of *P. labiosus* of evergreen lowland forest north of the Esmeraldas river (MZUTI 3000, 3051) form a clade distinct from samples of the same species inhabiting evergreen foothill forests south of the Esmeraldas river (Figs. 1, 8, 9). Although most samples of *P. crenunguis* show a degree of geographical structure (north and south of the Guayllabamba river), some samples from south of the Guayllabamba river (MZUTI 1398, 2987) are nested within the samples north of that river (Fig. 8). All published (Lynch 1976; Lynch and Duellman 1997; Yánez-Muñoz *et al* 2009; Yánez-Muñoz and Bejarano-Muñoz 2013) distribution records of *P. crenunguis* are located south of the Lita river and north of the

Toachi river (Figs. 1, 9). These two rivers might have acted as effective barriers for latitudinal dispersal of *P. crenunguis*.

Pristimantis subsigillatus and *P. mindo*

Molecular analyses.

Including the outgroups, we used 53 mtDNA sequences to build a molecular phylogeny of the *Pristimantis lacrimosus* species group (Lynch & Duellman 1980; Hedges *et al* 2008) (Fig. 11), which includes *P. subsigillatus* and *P. mindo* (Arteaga *et al* 2013). The resulting topology and support is similar to numerous recent studies (Pyron & Wiens 2011; Pinto-Sánchez *et al* 2011; Arteaga *et al* 2013; Padial *et al* 2014; Rivera-Prieto *et al* 2014), and *P. subsigillatus* is recovered as the sister species of *P. mindo*. Comparisons of a 695 bp fragment of the mitochondrial 16S gene between the two species shows a genetic distance of 10.4–10.9%, whereas sequence variation within *P. mindo* is 0–0.4%, and within *P. subsigillatus* is 0–2.0%.

Figure 11. Maximum likelihood phylogram depicting relationships within the *Pristimantis lacrimosus* species group. The phylogram was derived from analysis of 2598 bp of mitochondrial DNA (gene fragments 12S and 16S). Posterior probabilities and voucher numbers are shown.

Distribution maps.

Using a database of museum (S2 Table) and literature (Lynch & Duellman 1997; Ortega-Andrade & Altamirano 2004; Yánez-Muñoz 2005; Almendariz & Carr 2007; Yánez-Muñoz *et al* 2009; Arteaga *et al* 2013) records corresponding to 45 localities for *Pristimantis subsigillatus* and 12 for *P. mindo*, we modeled the habitat suitability for each of the species

(Fig. 12). The resulting distribution maps are similar to the most recent revision of the species (Arteaga *et al* 2013). For *P. mindo*, the seven localities of occurrence reported at the time of description (Arteaga *et al* 2013) are now expanded to 14 localities, including the current upper and lower limits of the altitudinal distribution of the species. The localities were added based on museum vouchers (S2 Table) or photographic or acoustic vouchers from Curipogio (00.13112 N 78.67632 S; 1171 m), Cascadas de Mindo (00.08002 S 78.76251 W; 1381 m), Milpe (00.03905 N 78.87054 W; 1055 m), Mashpi Lodge (00.16537 N 78.87244 W; 1060 m), Estación La Favorita (00.22833 S 78.76503 W; 1810 m) and Saragoza Río Cinto (00.12891 S 78.75437 W 1522 m). The predicted area of potential distribution for *P. subsigillatus* is related with evergreen lowland and foothill forests in Ecuador (Sierra 1999) (Figs. 1 and 12), whereas for *P. mindo*, the predicted area of potential distribution is mostly related with evergreen lower-montane forests and cloudforests (Sierra 1999) (Figs. 1 and 12). From our database of known localities, we estimated altitude limits of distributions: 27–1092 m for *P. subsigillatus* and 1056–1810 for *P. mindo*. We found two localities of sympatry between the two species (i.e. Mashpi and Milpe).

Figure 12. Distribution of *Pristimantis mindo* and *B. subsigillatus* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Figure 11.

Systematics.

Based on morphological characters, *Pristimantis subsigillatus* is most closely related to *P. mindo* (Arteaga *et al* 2013) (Fig. 13). This similarity might explain why several specimens of *P. mindo* housed at MZUTI and MECN, were previously identified as *P. subsigillatus*. Literature and museum records of *P. subsigillatus* above 1200 m likely correspond to *P. mindo*. Although not seen by us, it appears that one *Pristimantis*

subsigillatus (KU 218147) from a previous study (Heinicke *et al* 2007) may have been misidentified, and is actually a *P. nyctophylax*.

Figure 13. Morphological variation within sampled species of the *Pristimantis lacrimosus* species group. (a) Adult male of *Pristimantis subsigillatus* (MZUTI 2228). (b) Adult female of *P. subsigillatus* (MZUTI 2653). (c) Adult male of *P. mindo* (MZUTI 1382). (d) Adult female of *P. mindo* (MZUTI 1766).

Phylogeography.

The morphological similarities between *P. subsigillatus* and *P. mindo*, and their close, and slightly overlapping ranges of distribution, lead Arteaga *et al* (2013) to suggest that one of the species originated from the other through a parapatric process of speciation. Our species distribution models (Fig. 12), the mtDNA phylogeny (Fig. 11), the discovery of localities of sympatry, and the fact that both species were previously confused with each other in museum collections further supports this view. Each of the two known populations of *P. mindo* are reciprocally monophyletic and exhibit greater genetic distance from each other (0.4%) than within populations (0%). This pattern is best explained by the presence of the Guayllabamba river (Fig. 1), which seems to be acting as a dispersal barrier. On the contrary, the pattern of cladogenesis within *P. subsigillatus* is not geographically structured (Fig. 11), with samples north and south of the different river systems not clustering together.

Pristimantis walkeri and *P. luteolateralis*

Molecular analyses.

Including the outgroups, we used 78 mtDNA sequences to build a molecular phylogeny of the Ecuadorian yellow-groined rainfrogs of the *Pristimantis unistrigatus*

species group (Lynch & Duellman 1997; Hedges *et al* 2008) (Fig. 14). Based on the topology recovered in previous studies (Hedges *et al* 2008; Pyron and Wiens 2011; Pinto-Sánchez *et al* 2011; Padial *et al* 2014), we decided to include three members of the *Pristimantis* (*Hypodiction*) *ridens* series (Hedges *et al* 2008) as outgroups, along novel sequences for *P. luteolateralis*, *P. parvillus*, *P. walkeri,* and two other species previously subsumed under *P. walkeri*. With similar support values as in recent studies (Hedges *et al* 2008; Pyron & Wiens 2011; Pinto-Sánchez *et al* 2011), we recover a sister relationship between *P. walkeri* and *P. luteolateralis* (Fig. 14). However, as currently circumscribed (Lynch and Duellman 1997; Arteaga *et al* 2013), *P. walkeri* is paraphyletic, with *P. luteolateralis* and *P. parvillus* nested right within *P. walkeri*. To cope with this problem and to accurately reflect their distinct evolutionary histories, we treat each of the three clades of *P. walkeri* in our phylogeny as distinct species: *P. aff. walkeri* N, *P. walkeri sensu stricto and P. aff. walkeri* S (together referred to as *P. walkeri sensu lato*). As well as in other studies, (Hedges *et al* 2008; Pyron & Wiens 2011; Pinto-Sánchez *et al* 2011) the yellow-groined rainfrogs *Pristimantis luteolateralis*, *P. parvillus* and *P. walkeri* form a strongly supported clade. Our study shows, however, that *P. aff. walkeri* N belongs to the assemblage, but *P. chalceus*, *P. esmeraldas* and *P. aff. walkeri* S do not. A comparison of a 731 bp fragment of the mitochondrial 12S gene between *P. walkeri sensu stricto* and *P. luteolateralis* shows a genetic distance of 2.9–4.5%, whereas sequence variation within *P. walkeri* is 0–0.1%, and within *P. luteolateralis* is 0–0.7%. For the same fragment, *P. walkeri sensu stricto* and *P. aff. walkeri* N show a genetic distance of 5.2–5.5%, whereas sequence variation within *P. aff. walkeri* N is 0–0.1%.

Figure 14. Maximum likelihood phylogram depicting relationships of the yellow-groined Trans-Andean *Pristimantis* of Ecuador. The phylogram was derived from analysis of 1905 bp of mitochondrial DNA (gene fragments 12S and 16S). Posterior probabilities and

voucher numbers are shown.

Distribution maps.

Using a database of museum (S2 Table) and literature (Lynch & Duellman 1997; Morales 2004; Ortega-Andrade & Altamirano 2004; Almendariz & Carr 2007; Yánez-Muñoz *et al* 2009; Valencia & Garzón 2013; Lynch *et al* 2014) records corresponding to 53 localities for *Pristimantis walkeri,* 9 for *P. aff. walkeri* N, 6 for *P. aff. walkeri* S, and 39 for *P. luteolateralis*, we modeled the habitat suitability for each of the species (Fig. 15). For *P. luteolateralis*, the resulting distribution map greatly expands that of Lynch & Duellman (1997), and closely resembles that of Arteaga *et al* (2013). Unlike previous works (Lynch & Duellman 1997; Arteaga *et al* 2013), our distribution map of *P. walkeri sensu stricto* shows that it is endemic to the evergreen lowland and foothill forests of central Ecuador (Sierra 1999), whereas the northern and southern portion of its previously reported range now corresponds to that of *P. aff. walkeri* N and *P. aff. walkeri* S, respectively. For *P. luteolateralis*, the predicted area of potential distribution is almost exclusively related with evergreen lower-montane forests (Sierra 1999). From our database of known localities, we estimated altitude limits of distributions: 27–1155 m for *P. walkeri* and 905–1879 for *P. luteolateralis*. We found no localities of sympatry between any of the four species.

Figure 15. Distribution of *P. aff. walkeri* S, *P. luteolateralis*, *P. aff. walkeri* N and *P. walkeri* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Figure 14.

Systematics.

Based on morphological characters, *Pristimantis walkeri* is most closely related to *P. luteolateralis* (Lynch 1976; Lynch & Duellman 1997; Arteaga *et al* 2013) (Fig. 16). Some

authors (Yánez-Muñoz *et al* 2009; Arteaga *et al* 2013) have confused intermediate elevation populations of *P. luteolateralis* with *P. walkeri sensu stricto*. Our genetic analyses of *P. walkeri sensu lato* demonstrate the existence of at least three highly distinct lineages that deserve full-species status. By examining specimens of *P. walkeri sensu lato*, we found consistent morphological differences among the three genetic lineages. One of these, *P. walkeri sensu stricto*, is herein restricted to the populations in the central Pacific lowlands of Ecuador, where the type locality of *P. walkeri* lies (Las Palmas). Populations of the type locality were included in the molecular analyses, and they are nested within the clade herein defined as *P. walkeri sensu stricto*.

Figure 16. Ecuadorian Trans-Andean *Pristimantis* characterized by their yellow to orange pigmentation in the hidden surfaces of the hind limbs. (a) Adult male of *P. aff. walkeri* S (MZUTI 3270). (b) Adult male holotype of *P. aff. walkeri* S (MZUTI 3480). (c) Adult female paratype of *P. aff. walkeri* S (MZUTI 3356). (d) Adult male of *P. aff. walkeri* N (MZUTI 3913). (e) Adult male of *P. aff. walkeri* N (MZUTI 3914). (f) Adult male of *P. aff. walkeri* N (MZUTI 3915). (g) Adult male of *P. luteolateralis* (MZUTI 3092). (h) Adult male of *P. luteolateralis* (MZUTI 3904). (i) Adult female of *P. luteolateralis* (Not vouchered). (j) Adult male of *P. parvillus* (Not vouchered). (k) Adult male of *P. walkeri* (MZUTI 1768). (l) Adult female of *P. walkeri* (MZUTI 1769). (m) Adult female of *P. scolodiscus* (Not vouchered). (n) Adult male of *P. esmeraldas* (MZUTI 3545). (o) Adult female of *P. esmeraldas* (MZUTI 3375).

Phylogeography.

The topology of our mtDNA phylogeny (Fig. 14) suggests that the clade containing *P. walkeri sensu lato* and *P. luteolateralis* originated North of the Esmeraldas river, where *P. aff. walkeri* N is currently extant. These populations presumably gave rise to the populations

of *P. walkeri sensu stricto* currently present south of the Esmeraldas river and throughout the Chocoan lowlands of Central Ecuador. Lynch and Duellman (1997) and Arteaga *et al* (2013) suggested that *Pristimantis walkeri* and *P. luteolateralis* are altitudinal replacements of each other. The assumption was made based on the similarities in size, structure, microhabitat utilization, and the adjacent ranges of distribution of the two species. Our species distribution models and the mtDNA phylogeny support this relationship when the name *P. walkeri* is restricted to the populations south of the Esmeraldas river. We found populations of *P. aff. walkeri* S to be more closely related to *P. unistrigatus* than to *P. walkeri*, suggesting no direct common ancestry between *P. aff. walkeri* S and *P. walkeri.*

Table 2. Character states in the Ecuadorian Trans-Andean *Pristimantis* with yellow to orange pigmentation in the hidden surfaces of the hind limbs.

Species	Heel tubercles	Groin pattern	Oblique lateral stripe
P. aff walkeri S	Present, low	Orange spots outlined in black	Absent
P. aff walkeri N	Present, low	Yellow blotches outlined in black	Present, faint
P. esmeraldas	Absent	Yellow blotches, sometimes absent	Absent
P. luteolateralis	Present, subconical	Yellow blotches outlined in black	Present, distinct
P. parvillus	Present, low	Large yellow oval spot	Absent
P. scolodiscus	Present, low	Large yellow oval spot	Absent
P. walkeri	Present, low	Yellow blotches outlined in black	Absent

Discussion

When analyzed together, our five mtDNA molecular phylogenies and 14 species distribution models reveal a pattern of cladogenesis that is common for at least five pairs of codistributed sister taxa in northwestern Ecuador. The pattern can be described as a

parapatric speciation event in which a widely distributed Chocoan taxon gives rise to a more restricted montane-forest vicariant. A parapatric model is suggested because populations of the sister species are not separated by a geographical barrier, but by changes in vegetation zones.

The common phylogeographic pattern involves a Chocoan ancestor whose geographical range included a portion of the adjacent foothill and lower-montane forests in the area between the Mira and Toachi river valleys, the area in Ecuador where these two vegetation zones are wider (Fig. 1) and closest to the Equatorial line. Under this suggested scenario, divergence may have occurred because of reduced gene flow between Chocoan and montane populations. Closer to the Equator, elevation gradients have a stronger effect on the dispersal of organisms than a similar gradient on temperate regions (Janzen 1967). This greater climatic stratification of tropical mountains is hypothesized to increase the likelihood of parapatric speciation along elevational climatic gradients (Kozak & Wiens 2007; Moritz *et al* 2000), and may in part explain why our sampled Chocoan lineages (some of which range into Colombia and Panama) have upland vicariants only in the montane forests closer to the Equatorial line. Several studies (Huey 1978; Wake & Lynch 1976; Ghalambor *et al* 2006; Deutsch *et al* 2008; Huey *et al* 2009; McCain 2009; Buckley & Jetz 2008) confirm that closer to the Equator, species occupy more restricted elevational ranges and have narrower thermal tolerances.

From our results, the strongest evidence to support the scenario described above comes from the phylogeographic pattern of *Pristimantis walkeri* and *P. luteolateralis* (Fig. 14). The phylogeny shows that a Chocoan distribution is the ancestral trait, whereas the Andean distribution is the derived trait. The second strongest evidence comes from the phylogenies of *Alopoglossus festae*, *A. viridiceps*, *P. crenunugis*, *P. labiosus*, *P. mindo* and

P. subsigillatus. In all cases, mtDNA sequence variation is greater among populations of the Chocoan species than among populations of the Andean species, suggesting that Chocoan lineages are older and have had greater time to accumulate non-synonymous substitutions. The third strongest evidence to support the parapatic pattern of speciation comes from the existence of at least two localities of sympatry between *P. mindo* and *P. subsigillatus* (the most genetically distinct pair of species included in this study), suggesting secondary contact after a process of speciation.

Besides the deep and geographically structured split between the sister taxa included in this study, our results also show geographically structured mitochondrial subdivisions within taxa. In Ecuador, we can identify at least two barriers where the taxa share a major break in genetic composition. The Guayllabamba river (Fig. 1) is likely responsible for the majority of the genetic heterogeneity observed in *Pristimantis crenunguis* (Fig. 8), *P. mindo* (Fig. 11), *P. luteolateralis* (Fig. 14). The Guayllabamba river has also been recognized as a genetic boundary in other cloudforest taxa (Guayasamin *et al* 2015). The Esmeraldas river (Fig. 1) is likely responsible for the majority of the genetic heterogeneity observed in *P. labiosus*, and is the main barrier separating populations of *P. aff. walkeri* N and *P. walkeri sesu stricto*. Two other rivers (Mira and Toachi) have presumably acted as effective barriers of dispersal for *Alopoglossus viridiceps*, *P. crenunguis*, *P. mindo* and *P. luteolateralis*, since none of these species has been found either north of Mira river or south of Toachi river. From our list of sampled species, the only one known to be distributed south of the Toachi river is *Bothrops osbornei*, wich occurs as far south as Sacramento, Chimborazo province (Freire-Lascano 1991). The barrier that has most likely prevented this species from colonizing montane forests further south is the dry valley of the Chimbo river (Fig. 1).

The shared phylogenetic breaks at the species and population level across the

vegetation zones and river valleys in the Ecuadorian northwest suggests that allopatric speciation may have driven an important part of the current observed diversity of this region. Our study suggests that widely distributed Chocoan taxa may generally experience their greatest opportunities for isolation and parapatric speciation across elevational thermal gradients in the montane forests within 0.8 degrees latitude from the Equatorial line in Ecuador. We expect that our discovery of hidden species richness and their common patterns of speciation represent sound testable hypotheses for unstudied taxa or communities that range both in Chocoan lowlands and their adjacent Equatorial montane forests (e.g. *Anadia rhombifera, Tantilla melanocephala* and *Pristimantis parvillus*). These may in fact be species complexes, with populations inhabiting the montane forests representing distinct evolutionary units that deserve full-species status.

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Supporting Information

S1 Table. GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Specimens with novel sequence data are marked with an asterisk (*).

Species	Voucher	12S	16S	cytb	ND4
Craugastor longirostris	KU177803	EF493395	EF493395	–	–
Pristimanits latidiscus	KU218016	EF493698	EF493698	–	–
Pristimantis acerus	KU217786	EF493678	EF493678	–	–
Pristimantis actites	KU217830	EF493696	EF493696	–	–
Pristimantis acuminatus	QCAZ19664	–	EU130579	–	–
Pristimantis altae	AJC0398	JN991496	–	–	–
Pristimantis appendiculatus	KU177637	EF493524	EF493524	–	–
Pristimantis bromeliaceus	KU291702	EF493351	EF493351	–	–
Pristimantis aff. walkeri S*	MZUTI3270	–	–	–	–
Pristimantis aff. walkeri S *	MZUTI3356	–	–	–	–
Pristimantis aff. walkeri S *	MZUTI3480	–	–	–	–
Pristimantis calcarulatus	KU177658	EF493523	EF493523	–	–
Pristimantis caryophyllaceus	MVZ203810	EU186686	EU186686	–	–
Pristimantis cerasinus	AJC1142	JN991502	JN991438	–	–
Pristimantis chalceus	KU177638	EF493675	EF493675	–	–
Pristimantis cremnobates	KU177252	EF493528	EF493528	–	–
Pristimantis crenunguis	KU1777730	EF493693	EF493666	–	–
Pristimantis crenunguis*	MZUTI530	–	–	–	–
Pristimantis crenunguis*	MZUTI531	–	–	–	–
Pristimantis crenunguis*	MZUTI532	–	–	–	–
Pristimantis crenunguis*	MZUTI1398	–	–	–	–
Pristimantis crenunguis*	MZUTI1399	–	–	–	–
Pristimantis crenunguis*	MZUTI2987	–	–	–	–
Pristimantis crenunguis*	MZUTI3067	–	–	–	–
Pristimantis crenunguis*	MZUTI3068	–	–	–	–

Pristimantis crenunguis*	MZUTI3069	–	–	–	–
Pristimantis crenunguis*	MZUTI3292	–	–	–	–
Pristimantis crenunguis*	MZUTI3296	–	–	–	–
Pristimantis crenunguis*	MZUTI3304	–	–	–	–
Pristimantis crucifer	KU177733	EU186736	EU186718	–	–
Pristimantis dissimulatus	KU179090	EF493522	EF493522	–	–
Pristimantis erythropleura	UVC15886	–	JN371036	–	–
Pristimantis galdi	QCAZ32368	EU186670	EU186670	–	–
Pristimantis glandulosus	KU218002	EF493676	EF493676	–	–
Pristimantis inusitatus	KU218015	EF493677	EF493677	–	–
Pristimantis labiosus*	MECN9527	–	–	–	–
Pristimantis labiosus*	MECN9528	–	–	–	–
Pristimantis labiosus*	MZUTI573	–	–	–	–
Pristimantis labiosus*	MZUTI574	–	–	–	–
Pristimantis labiosus*	MZUTI577	–	–	–	–
Pristimantis labiosus*	MZUTI589	–	–	–	–
Pristimantis labiosus*	MZUTI594	–	–	–	–
Pristimantis labiosus*	MZUTI1759	–	–	–	–
Pristimantis labiosus*	MZUTI3000	–	–	–	–
Pristimantis labiosus*	MZUTI3018	–	–	–	–
Pristimantis labiosus*	MZUTI3051	–	–	–	–
Pristimantis labiosus*	MZUTI3078	–	–	–	–
Pristimantis labiosus*	MZUTI3079	–	–	–	–
Pristimantis labiosus*	MZUTI3080	–	–	–	–
Pristimantis labiosus	QCAZ19771	EF493694	EF493694	–	–
Pristimantis lanthanites	KU222001	EF493695	EF493695	–	–
Pristimantis latidiscus*	MZUTI2992	–	–	–	–
Pristimantis luteolateralis	KU177807	EF493517	EF493517	–	–
Pristimantis luteolateralis*	MZUTI327	–	–	–	–
Pristimantis luteolateralis*	MZUTI328	–	–	–	–
Pristimantis luteolateralis*	MZUTI329	–	–	–	–
Pristimantis luteolateralis*	MZUTI330	–	–	–	–

Pristimantis luteolateralis*	MZUTI528	–	–	–	–
Pristimantis luteolateralis*	MZUTI529	–	–	–	–
Pristimantis luteolateralis*	MZUTI654	–	–	–	–
Pristimantis luteolateralis*	MZUTI655	–	–	–	–
Pristimantis luteolateralis*	MZUTI656	–	–	–	–
Pristimantis luteolateralis*	MZUTI657	–	–	–	–
Pristimantis luteolateralis*	MZUTI659	–	–	–	–
Pristimantis luteolateralis*	MZUTI660	–	–	–	–
Pristimantis luteolateralis*	MZUTI661	–	–	–	–
Pristimantis luteolateralis*	MZUTI662	–	–	–	–
Pristimantis luteolateralis*	MZUTI663	–	–	–	–
Pristimantis luteolateralis*	MZUTI665	–	–	–	–
Pristimantis luteolateralis*	MZUTI703	–	–	–	–
Pristimantis luteolateralis*	MZUTI1404	–	–	–	–
Pristimantis luteolateralis*	MZUTI1405	–	–	–	–
Pristimantis luteolateralis*	MZUTI1406	–	–	–	–
Pristimantis luteolateralis*	MZUTI1734	–	–	–	–
Pristimantis luteolateralis*	MZUTI1742	–	–	–	–
Pristimantis luteolateralis*	MZUTI2110	–	–	–	–
Pristimantis luteolateralis*	MZUTI2115	–	–	–	–
Pristimantis luteolateralis*	MZUTI2196	–	–	–	–
Pristimantis luteolateralis*	MZUTI2988	–	–	–	–
Pristimantis luteolateralis*	MZUTI2989	–	–	–	–
Pristimantis luteolateralis*	MZUTI2990	–	–	–	–
Pristimantis luteolateralis*	MZUTI3092	–	–	–	–
Pristimantis luteolateralis*	MZUTI3093	–	–	–	–
Pristimantis luteolateralis*	MZUTI3182	–	–	–	–
Pristimantis mindo	MZUTI1381	–	KF801583	–	–
Pristimantis mindo	MZUTI1382	–	KF801584	–	–
Pristimantis mindo*	MZUTI1383	–	–	–	–
Pristimantis mindo	MZUTI1755	–	KF801582	–	–
Pristimantis mindo	MZUTI1756	–	KF801581	–	–

Pristimantis mindo*	MZUTI2109	–	–	–	–
Pristimantis mindo*	MZUTI2284	–	–	–	–
Pristimantis moro	AJC1753	JN991519	JN991453	–	–
Pristimantis museosus	KRL0739	–	FJ784354	–	–
Pristimantis aff. walkeri N *	MZUTI3001	–	–	–	–
Pristimantis aff. walkeri N *	MZUTI3049	–	–	–	–
Pristimantis aff. walkeri N *	MZUTI3050	–	–	–	–
Pristimantis nyctophylax	KU177812	EF493526	EF493526	–	–
Pristimantis orcesi	KU218021	EF493679	EF493679	–	–
Pristimantis paisa	AJC1344	JN991524	JN991459	–	–
Pristimantis pardalis	CH6284	JN991525	JN991460	–	–
Pristimantis parvillus	KU177821	EF493352	EF493352	–	–
Pristimantis parvillus*	MZUTI2121	–	–	–	–
Pristimantis parvillus*	MZUTI483	–	–	–	–
Pristimantis pirrensis	AJC0594	JN991528	JN991462	–	–
Pristimantis pycnodermis	KU218028	EF493680	EF493680	–	–
Pristimantis ridens	AMNHA124551	EF493355	EF493355	–	–
Pristimantis schultei	KU212220	EF493681	EF493681	–	–
Pristimantis subsigillatus	MECN10117	–	KF801580	–	–
Pristimantis subsigillatus*	MZUTI1999	–	–	–	–
Pristimantis subsigillatus*	MZUTI2228	–	–	–	–
Pristimantis subsigillatus*	MZUTI2243	–	–	–	–
Pristimantis subsigillatus*	MZUTI2653	–	–	–	–
Pristimantis subsigillatus*	MZUTI2995	–	–	–	–
Pristimantis subsigillatus*	MZUTI2996	–	–	–	–
Pristimantis subsigillatus*	MZUTI2997	–	–	–	–
Pristimantis subsigillatus*	MZUTI3087	–	–	–	–
Pristimantis subsigillatus*	MZUTI3088	–	–	–	–
Pristimantis subsigillatus*	MZUTI3196	–	–	–	–
Pristimantis subsigillatus*	MZUTI3198	–	–	–	–
Pristimantis subsigillatus*	MZUTI3433	–	–	–	–
Pristimantis unistrigatus	KU218057	EF493387	EF493387	–	–

Pristimantis viejas	EMM250	JN991546	JN991476	–	–
Pristimantis w-nigrum	WED53045	AY326004	AY326004	–	–
Pristimantis walkeri	KU218116	EF493518	EF493518	–	–
Pristimantis walkeri*	MZUTI1768	–	–	–	–
Pristimantis walkeri*	MZUTI1770	–	–	–	–
Pristimantis walkeri*	MZUTI2993	–	–	–	–
Pristimantis walkeri*	MZUTI3183	–	–	–	–
Alopoglossus angulatus	QCAZ8915	–	–	–	KJ705317
Alopoglossus atriventris	LSUMZH13856	–	AF420746	–	AF420908
Alopoglossus atriventris	QCAZ5622	–	–	–	KJ705319
Alopoglossus buckleyi	QCAZ9961	–	–	–	KJ705320
Alopoglossus carinicaudus	LG1026	–	AF420744	–	AF420909
Alopoglossus copii	QCAZ8314	–	–	–	KJ705318
Alopoglossus festae	MZUTI2994	–	–	–	–
Alopoglossus festae*	MZUTI3281	–	–	–	–
Alopoglossus festae*	MZUTI3370	–	–	–	–
Alopoglossus festae*	MZUTI3381	–	–	–	–
Alopoglossus festae*	MZUTI3393	–	–	–	–
Alopoglossus festae*	MZUTI3751	–	–	–	–
Alopoglossus festae	QCAZ9158	–	–	–	KJ705315
Alopoglossus viridiceps*	MZUTI3550	–	–	–	
Alopoglossus viridiceps	QCAZ10670	–	–	–	KJ705316
Ptychoglossus brevifrontalis	MHNSM	–	AY507884	–	AY507895
Atropoides mexicanus	USNM578906	KC847268	KC847255	KC847271	KC847289
Bothriopsis bilineata	FHGO983	–	–	AF292592	AF292630
Bothriopsis chloromelas	LSUMZ41037	DQ305430	DQ305453	DQ305471	DQ305488
Bothriopsis pulchra	FHGO2142	–	–	AF292593	AF292631
Bothriopsis taeniata	FHGO195	AF057215	AF057262	AF292591	AF292629
Bothrocophias campbelli	INMHT	–	–	AF292584	AF292622
Bothrocophias hyoprora	FHGO4005	–	–	AF292576	AF292614
Bothrocophias microphtalmus	FHGO2566	–	–	AF292577	AF292615
Bothropoides alcatraz	CBGM002	–	–	AY865821	–

Bothropoides diporus	PT3404	DQ305431	DQ305454	DQ305472	DQ305489
Bothropoides erythromelas	IB55541	–	–	AF292588	AF292626
Bothropoides insularis	WWWg	AF057216	AF057263	AY223596	AF188705
Bothropoides jararaca	MM196	EU867254	EU867266	EU867278	EU867290
Bothropoides lutzi	MTR14196	–	–	KF801131	KF801261
Bothropoides marmoratus	CEPB8171	–	–	KF801137	KF801265
Bothropoides matogrossensis	NORMAT113	–	–	KF801149	KF801277
Bothropoides neuwiedi	IBSP74565	–	–	KF801169	KF801294
Bothropoides pauloensis	CLP3	EU867260	EU867272	EU867284	EU867296
Bothropoides pubescens	NOPA3860	–	–	KF801227	KF801344
Bothrops asper	MZUCR11152	AF057218	GQ372868	EU624301	FJ985716
Bothrops atrox	WWW743	AY223659	AY223672	AY223598	AY223641
Bothrops brazili	USNM17831	EU867252	EU867264	EU867276	EU867288
Bothrops caribbaeus	–	–	–	AF292598	AF292636
Bothrops jararacussu	DPL104	AY223661	AY223674	AY223602	AY223643
Bothrops lanceolatus	NV	–	–	AF292599	AF292637
Bothrops leucurus	CLP195	EU867255	EU867267	EU867279	EU867291
Bothrops lojanus	QCAZ6018	–	FR691566	FR691566	FR691536
Bothrops marajoensis	–	–	–	AF292605	AF292643
Bothrops moojeni	ITS418	EU867257	EU867269	EU867281	EU867293
Bothrops osbornei	FHGO2166	–	–	AF292595	AF292633
Bothrops osbornei*	ANF2005	–	–	–	–
Bothrops osbornei*	ANF2107	–	–	–	–
Bothrops osbornei*	MZUTI3542	–	–	–	–
Bothrops osbornei*	MZUTI3865	–	–	–	–
Bothrops pictus	–	–	–	AF292583	AF292621
Bothrops punctatus*	AA002	–	–	–	–
Bothrops punctatus*	ANF1465	–	–	–	–
Bothrops punctatus*	ANF1575	–	–	–	–
Bothrops punctatus*	ANF1577	–	–	–	–
Bothrops punctatus*	ANF2101	–	–	–	–
Bothrops punctatus	FHGO2452	–	–	AF292594	AF292632

Lachesis acrochorda	CLP319	JN870187	JN870197	JN870204	JN870212
Rhinocerophis alternatus	ITS358	EU867251	EU867263	EU867275	EU867287
Rhinocerophis ammodytoides	MVZ223514	AY223658	AY223671	–	AY223639
Rhinocerophis cotiara	WWW	AF057217	AF057264	AY223597	AY223640
Rhinocerophis fonsecai	IB55543	–	–	AF292580	AF292618
Rhinocerophis itapetiningae	ITS427	EU867253	EU867265	EU867277	EU867289

S2 Table. Additional specimens examined.

Species	Voucher	Locality	Latitude	Longitude	Elev.
Alopoglossus festae	MZUTI 2630	Esmeraldas, Bilsa	0.34910	-79.70967	555
Alopoglossus festae	MZUTI 2994	Pichincha, Selva Virgen	0.10673	-78.18542	355
Alopoglossus festae	MZUTI 3281	Pichincha, Milpe	0.03905	-78.87054	998
Alopoglossus festae	MZUTI 3370	Azuay, Flor y Selva	-2.65706	-79.53111	136
Alopoglossus festae	MZUTI 3381	El Oro, Buenaventura	-3.66598	-79.73933	1042
Alopoglossus festae	MZUTI 3393	El Oro, Buenaventura	-3.64797	-79.75507	947
Alopoglossus festae	MZUTI 3442	El Oro, California	-3.37146	-79.73430	328
Alopoglossus festae	MZUTI 3463	El Oro, California	-3.37146	-79.73430	328
Alopoglossus festae	MZUTI 3751	Pichincha, Milpe	0.03076	-78.86667	1162
Alopoglossus viridiceps	MZUTI 3550	Pichincha, Séptimo Paraíso	-0.02808	-78.76667	1537
Alopoglossus viridiceps	MZUTI 3551	Pichincha, Séptimo Paraíso	-0.02808	-78.76667	1537
Alopoglossus viridiceps	MZUTI 3552	Pichincha, Séptimo Paraíso	-0.02808	-78.76667	1537
Bothrops osbornei	MZUTI 3542	Pichincha, Mashpi lodge	0.16352	-78.87274	1060
Bothrops osbornei	MZUTI 3865	Pichincha, Mashpi lodge	0.16603	-78.87862	905
Pristimantis crenunguis	MZUTI 530	Pichincha, Séptimo Paraíso	-0.02885	-78.76599	1525
Pristimantis crenunguis	MZUTI 531	Pichincha, Séptimo Paraíso	-0.02885	-78.76599	1525
Pristimantis crenunguis	MZUTI 532	Pichincha, Séptimo Paraíso	-0.02885	-78.76599	1525
Pristimantis crenunguis	MZUTI 1398	Pichincha, Sachatamia	-0.02470	-78.75909	1704
Pristimantis crenunguis	MZUTI 1399	Pichincha, Sachatamia	-0.02513	-78.75896	1716
Pristimantis crenunguis	MZUTI 2987	Pichincha, Séptimo Paraíso	-0.02829	-78.76596	1541
Pristimantis crenunguis	MZUTI 3067	Pichincha, Séptimo Paraíso	-0.02891	-78.76586	1523

Pristimantis crenunguis	MZUTI 3068	Pichincha, Séptimo Paraíso	-0.02891	-78.76586	1523
Pristimantis crenunguis	MZUTI 3069	Pichincha, Séptimo Paraíso	-0.02891	-78.76586	1523
Pristimantis crenunguis	MZUTI 3292	Imbabura, Los Cedros	0.31842	-78.78373	1764
Pristimantis crenunguis	MZUTI 3296	Imbabura, Los Cedros	0.31842	-78.78373	1764
Pristimantis crenunguis	MZUTI 3304	Imbabura, Los Cedros	0.31842	-78.78373	1764
Pristimantis esmeraldas	MZUTI 2251	Esmeraldas, Bilsa	0.34910	-79.70967	555
Pristimantis esmeraldas	MZUTI 2232	Esmeraldas, Canandé	0.52645	-79.20937	360
Pristimantis esmeraldas	MZUTI 3375	Esmeraldas, Tundaloma	1.18317	-78.75245	74
Pristimantis esmeraldas	MZUTI 3554	Esmeraldas, Tundaloma	1.18317	-78.75245	74
Pristimantis esmeraldas	MZUTI 3545	Esmeraldas, Tundaloma	1.18317	-78.75245	74
Pristimantis esmeraldas	MZUTI 3540	Esmeraldas, Tundaloma	1.18317	-78.75245	74
Pristimantis esmeraldas	MZUTI 3819	Esmeraldas, Itapoa	0.51306	-79.13396	341
Pristimantis esmeraldas	MECN 3311	Esmeraldas, Canandé	0.52645	-79.20937	360
Pristimantis esmeraldas	MZUTI 3199	Pichincha, Silanche	0.14577	-79.14338	418
Pristimantis labiosus	MZUTI 573	Pichincha, San Francisco			
Pristimantis labiosus	MZUTI 574	Pichincha, Chontilla	0.11187	-78.90275	1191
Pristimantis labiosus	MZUTI 577	Pichincha, Chontilla	0.11187	-78.90275	1191
Pristimantis labiosus	MZUTI 589	Pichincha, Chontilla	0.11187	-78.90275	1191
Pristimantis labiosus	MZUTI 594	Pichincha, Chontilla	0.11187	-78.90275	1191
Pristimantis labiosus	MZUTI 1759	Pichincha, El Abrazo	-0.00916	-78.81133	1086
Pristimantis labiosus	MECN 9527	Esmeraldas, Canandé	0.52645	-79.20937	360
Pristimantis labiosus	MECN 9528	Esmeraldas, Canandé	0.52645	-79.20937	360
Pristimantis labiosus	MZUTI 3000	Esmeraldas, Itapoa	0.51306	-79.13396	341
Pristimantis labiosus	MZUTI 3018	Esmeraldas, Itapoa	0.51307	-79.13400	321
Pristimantis labiosus	MZUTI 3051	Esmeraldas, Itapoa	0.51307	-79.13400	321
Pristimantis labiosus	MZUTI 3078	Pichincha, Milpe	0.03125	-78.86621	1156
Pristimantis labiosus	MZUTI 3079	Pichincha, Milpe	0.03125	-78.86621	1156
Pristimantis labiosus	MZUTI 3080	Pichincha, Milpe	0.03125	-78.86621	1156
Pristimantis laticlavius	MZUTI 1728	Imbabura, Los Cedros	0.31125	-78.78095	1417
Pristimantis luteolateralis	MZUTI 2196	Pichincha, El Abrazo	-0.00913	-78.81321	1064
Pristimantis luteolateralis	MZUTI 2115	Pichincha, El Abrazo	-0.00913	-78.81321	1064

Pristimantis luteolateralis	MZUTI 3093	Pichincha, Milpe	0.03249	-78.86576	1113
Pristimantis luteolateralis	MZUTI 2988	Pichincha, Séptimo Paraíso	-0.02829	-78.76596	1541
Pristimantis luteolateralis	MZUTI 2110	Pichincha, El Abrazo	-0.00913	-78.81321	1064
Pristimantis luteolateralis	MZUTI 2989	Pichincha, Séptimo Paraíso	-0.02829	-78.76596	1541
Pristimantis luteolateralis	MZUTI 1767	Pichincha, El Abrazo	-0.00913	-78.81321	1064
Pristimantis luteolateralis	MZUTI 3183	Pichincha, Séptimo Paraíso	-0.02829	-78.76596	1541
Pristimantis luteolateralis	MZUTI 3092	Pichincha, Milpe	0.03249	-78.86576	1113
Pristimantis luteolateralis	MZUTI 3521	Pichincha, Mashpi lodge	0.16603	-78.87862	905
Pristimantis luteolateralis	MZUTI 1734	Imbabura, Los Cedros	0.32489	-78.78094	1621
Pristimantis luteolateralis	MZUTI 327	Pichincha, Yellow House	-0.04505	-78.75938	1498
Pristimantis luteolateralis	MZUTI 328	Pichincha, Yellow House	-0.04505	-78.75938	1498
Pristimantis luteolateralis	MZUTI 329	Pichincha, Yellow House	-0.04505	-78.75938	1498
Pristimantis luteolateralis	MZUTI 330	Pichincha, Yellow House	-0.04505	-78.75938	1498
Pristimantis luteolateralis	MZUTI 528	Pichincha, Cascadas de Mindo	-0.07837	-78.76429	1438
Pristimantis luteolateralis	MZUTI 529	Pichincha, Cascadas de Mindo	-0.07899	-78.76405	1409
Pristimantis luteolateralis	MZUTI 654	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 655	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 656	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 657	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 658	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 659	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 660	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 661	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 662	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 663	Pichincha, Chontilla	0.11187	-78.90275	1241
Pristimantis luteolateralis	MZUTI 703	Pichincha, Sueños de Bambú	-0.06655	-78.77158	1391
Pristimantis luteolateralis	MZUTI 1404	Pichincha, El Abrazo	-0.00914	-78.81284	1074
Pristimantis luteolateralis	MZUTI 1405	Pichincha, El Abrazo	-0.00914	-78.81284	1074
Pristimantis luteolateralis	MZUTI 1406	Pichincha, Yellow House	-0.04418	-78.75520	1492
Pristimantis luteolateralis	MZUTI 1742	Imbabura, Los Cedros	0.32489	-78.78094	1621
Pristimantis luteolateralis	MZUTI 2110	Pichincha, El Abrazo	-0.00914	-78.81284	1074

Pristimantis luteolateralis	MZUTI 2115	Pichincha, El Abrazo	-0.00914	-78.81284	1074
Pristimantis luteolateralis	MZUTI 2988	Pichincha, Séptimo Paraíso	-0.02829	-78.76596	1541
Pristimantis luteolateralis	MZUTI 2989	Pichincha, Séptimo Paraíso	-0.02829	-78.76596	1541
Pristimantis luteolateralis	MZUTI 2990	Pichincha, Séptimo Paraíso	-0.02829	-78.76596	1541
Pristimantis luteolateralis	MZUTI 3092	Pichincha, Milpe	0.03249	-78.86576	1113
Pristimantis luteolateralis	MZUTI 3093	Pichincha, Milpe	0.03249	-78.86576	1113
Pristimantis luteolateralis	MZUTI 3182	Pichincha, Séptimo Paraíso	-0.02886	-78.76598	1522
Pristimantis mindo	MZUTI 1381	Pichincha, Sachatamia	-0.02470	-78.75909	1704
Pristimantis mindo	MZUTI1382	Pichincha, Sachatamia	-0.02470	-78.75909	1704
Pristimantis mindo	MZUTI 1383	Pichincha, Sachatamia	-0.02064	-78.75928	1740
Pristimantis mindo	MZUTI 1755	Imbabura, Los Cedros	0.31840	-78.78370	1790
Pristimantis mindo	MZUTI 1756	Imbabura, Los Cedros	0.32328	-78.78111	1581
Pristimantis mindo	MZUTI 2109	Pichincha, Séptimo Paraíso	-0.02886	-78.76598	1522
Pristimantis mindo	MZUTI 2284	Pichincha, Yellow House	-0.04462	-78.75407	1511
Pristimantis parvillus	MZUTI 483	Pichincha, Las Gralarias	-0.00158	-78.73858	1793
Pristimantis parvillus	MZUTI 2121	Pichincha, Tandayapa Lodge	0.00249	-78.68083	1730
Pristimantis subsigillatus	MZUTI 1999	Pichincha, Selva Virgen	0.10712	-79.18007	250
Pristimantis subsigillatus	MZUTI 2228	Esmeraldas, Canandé	0.52615	-79.21282	361
Pristimantis subsigillatus	MZUTI 2243	Esmeraldas, Bilsa	0.34614	-79.71299	533
Pristimantis subsigillatus	MZUTI 2653	Pichincha, Selva Virgen	0.10547	-79.18734	345
Pristimantis subsigillatus	MZUTI 2995	Pichincha, Selva Virgen	0.10615	-79.18586	364
Pristimantis subsigillatus	MZUTI 2996	Pichincha, Selva Virgen	0.10615	-79.18586	364
Pristimantis subsigillatus	MZUTI 2997	Pichincha, Selva Virgen	0.10615	-79.18586	364
Pristimantis subsigillatus	MZUTI 3087	Pichincha, Milpe	0.03076	-78.86667	1162
Pristimantis subsigillatus	MZUTI 3088	Pichincha, Milpe	0.03076	-78.86667	1162
Pristimantis subsigillatus	MZUTI 3196	Pichincha, Silanche	0.14528	-79.14147	413
Pristimantis subsigillatus	MZUTI 3198	Pichincha, Silanche	0.14467	-79.14318	391
Pristimantis subsigillatus	MZUTI 3433	El Oro, California	-3.36799	-79.73551	225
Pristimantis walkeri	MECN 2762	Esmeraldas, Monte Saíno	0.69833	-80.02833	208
Pristimantis walkeri	MECN 2763	Esmeraldas, Monte Saíno	0.69833	-80.02833	208
Pristimantis walkeri	MZUTI 1770	Santo Domingo, Otongachi	-0.32145	-78.95094	661

Pristimantis walkeri	MZUTI 3781	Esmeraldas, Mache Chindul	0.51032	-79.72552	175
Pristimantis walkeri	MZUTI 3257	Cañar, Huatacón	-2.49018	-79.18223	1048
Pristimantis walkeri	MZUTI 3247	Cañar, Huatacón	-2.49018	-79.18223	1048
Pristimantis walkeri	MZUTI 3243	Cañar, Huatacón	-2.49018	-79.18223	1048
Pristimantis walkeri	MZUTI 3246	Cañar, Huatacón	-2.49018	-79.18223	1048
Pristimantis walkeri	MZUTI 3255	Cañar, Huatacón	-2.49018	-79.18223	1048
Pristimantis walkeri	MZUTI 3782	Esmeraldas, Mache Chindul	0.51032	-79.72552	175
Pristimantis walkeri	MZUTI 2990	Pichincha, Selva Virgen	0.10615	-79.18586	364
Pristimantis walkeri	MZUTI 1768	Santo Domingo, Otongachi	-0.32145	-78.95094	661
Pristimantis walkeri	MZUTI 1770	Santo Domingo, Otongachi	-0.32145	-78.95094	661
Pristimantis walkeri	MZUTI 2993	Pichincha, Selva Virgen	0.10547	-79.18734	345
Pristimantis walkeri	MZUTI 3183	Pichincha, Selva Virgen	0.10547	-79.18734	345

Image files

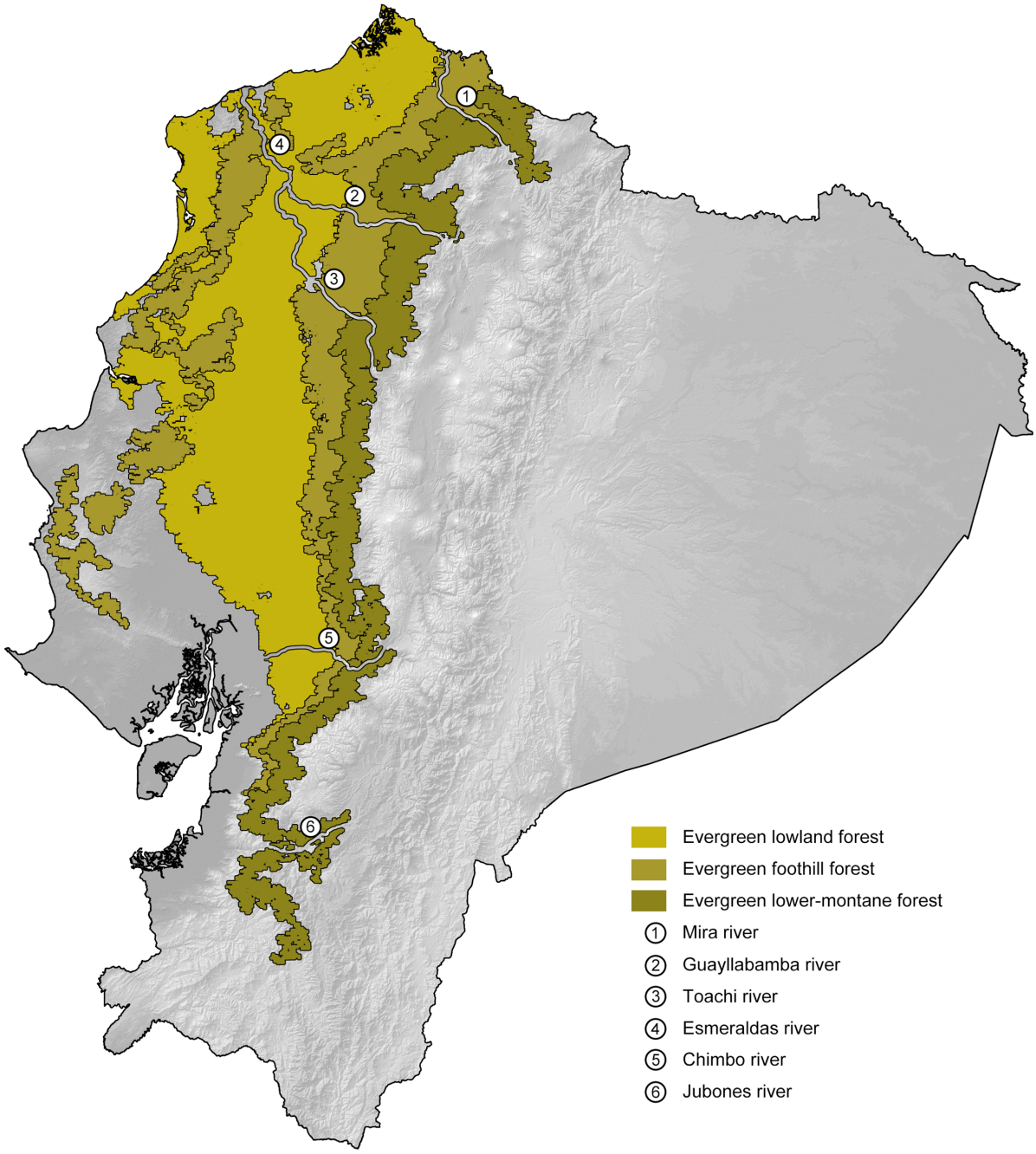

Figure 1. Main vegetation zones and rivers in the Ecuadorian northwest. The map is a simplified version of the main vegetation zones of Sierra (1999).

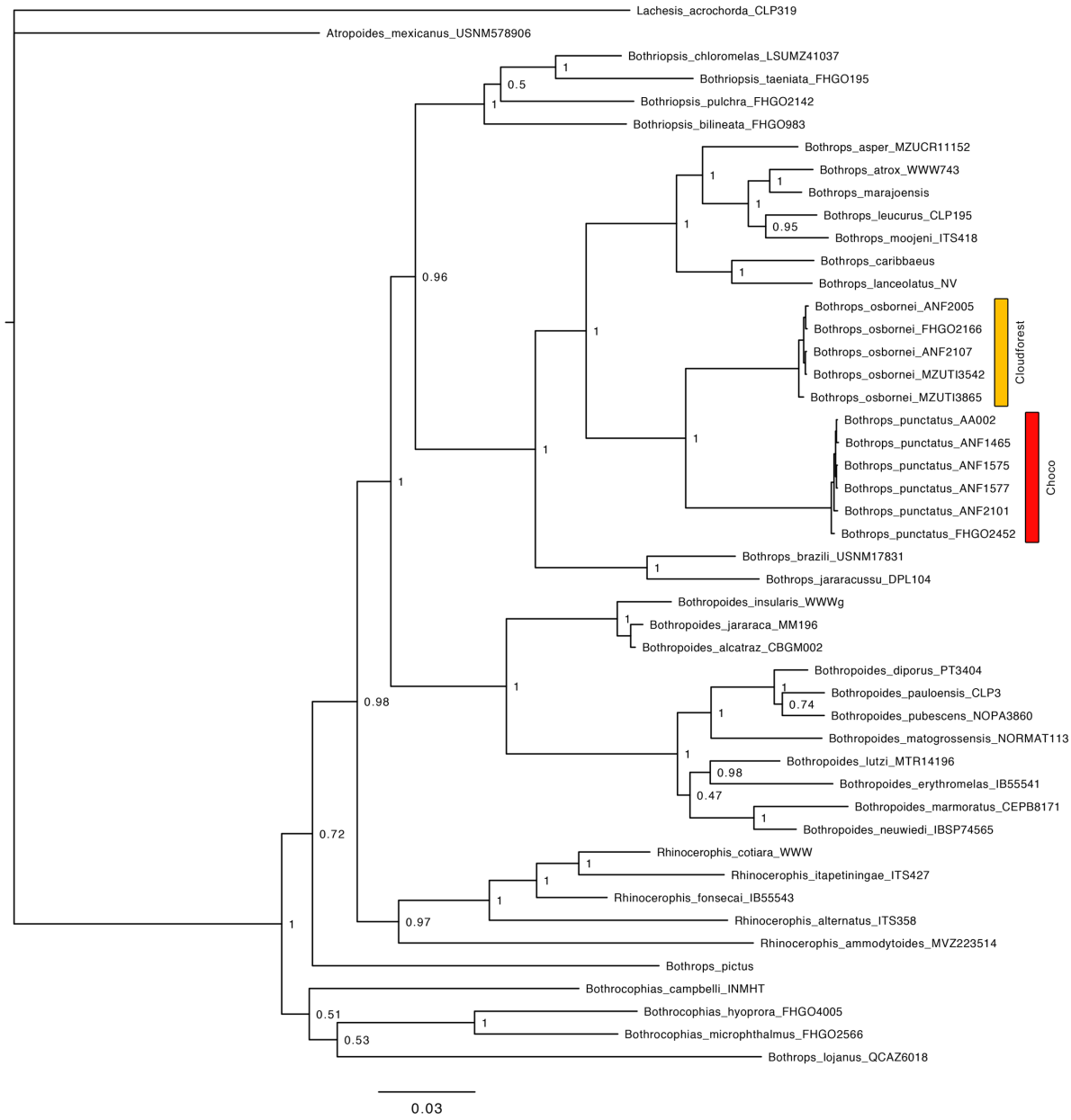

Figure 2. Maximum likelihood phylogram depicting relationships within bothropoid pitvipers. The phylogram was derived from analysis of 2908 bp of mitochondrial DNA (gene fragments 12S, 16S, cytb and ND4). Posterior probabilities and voucher numbers are shown.

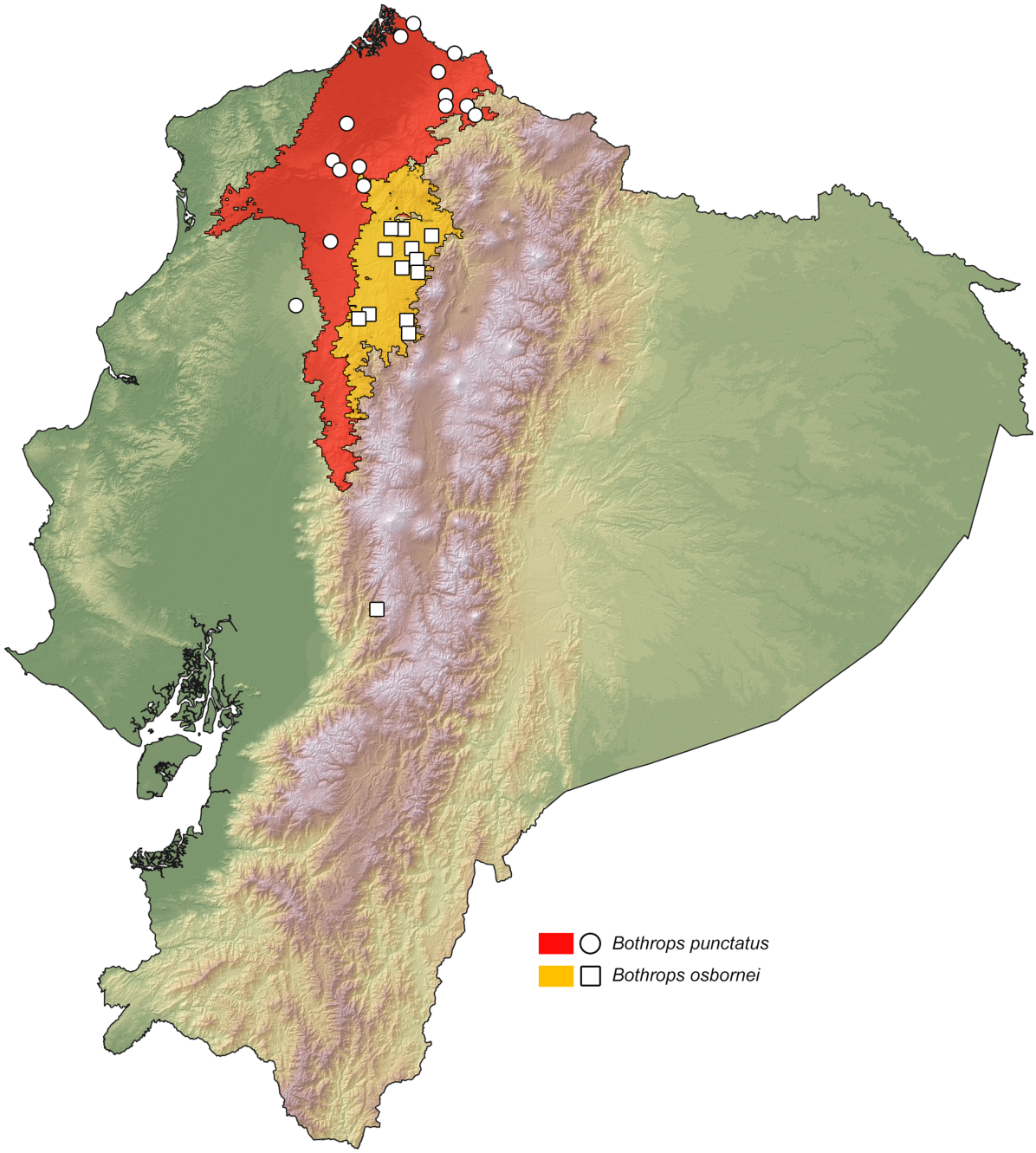

Figure 3. Distribution of *Bothrops osbornei* and *B. punctatus* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Fig. 2.

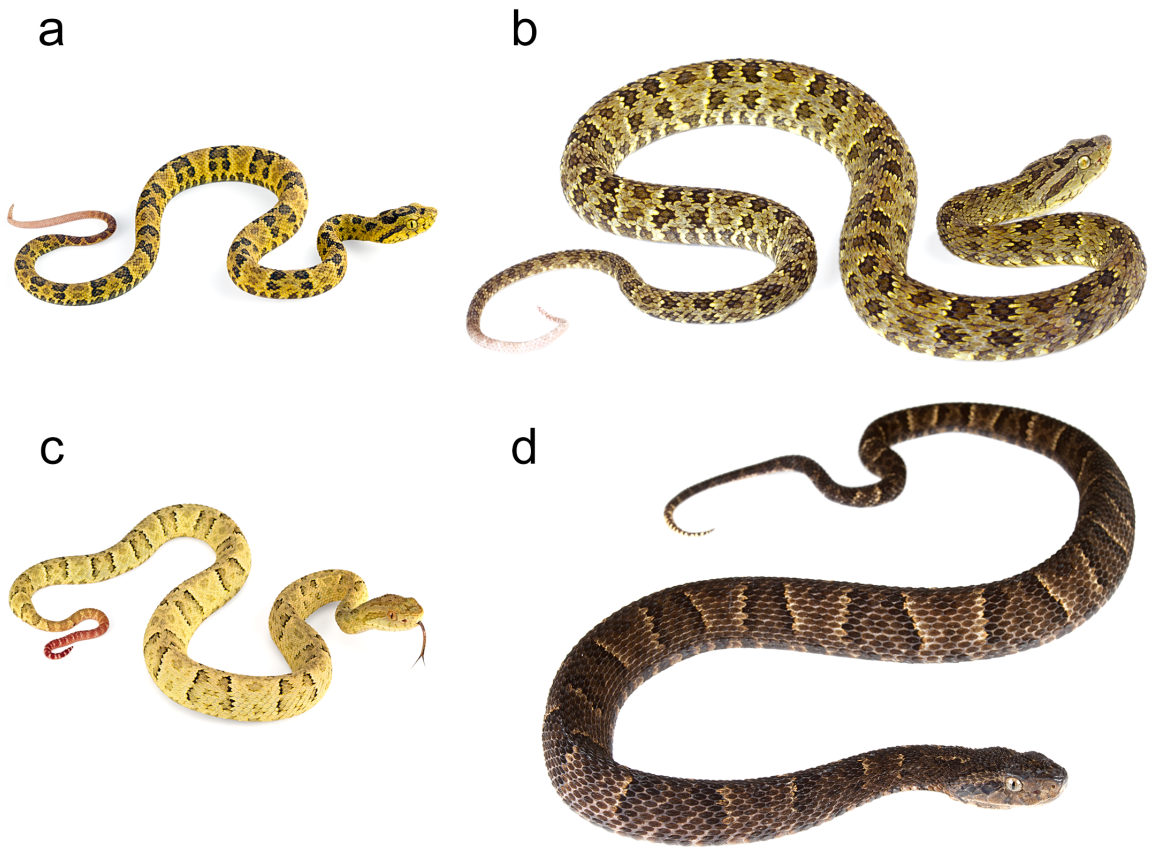

Figure 4. Morphological variation within sampled *Bothrops* species. (a) Juvenile of *B. punctatus* (ANF 1575). (b) Adult (ANF 2101) of *B. punctatus*. (c) Juvenile of *B. osbornei* (ANF 2005). (d) Adult of *B. osbornei* (ANF 2767).

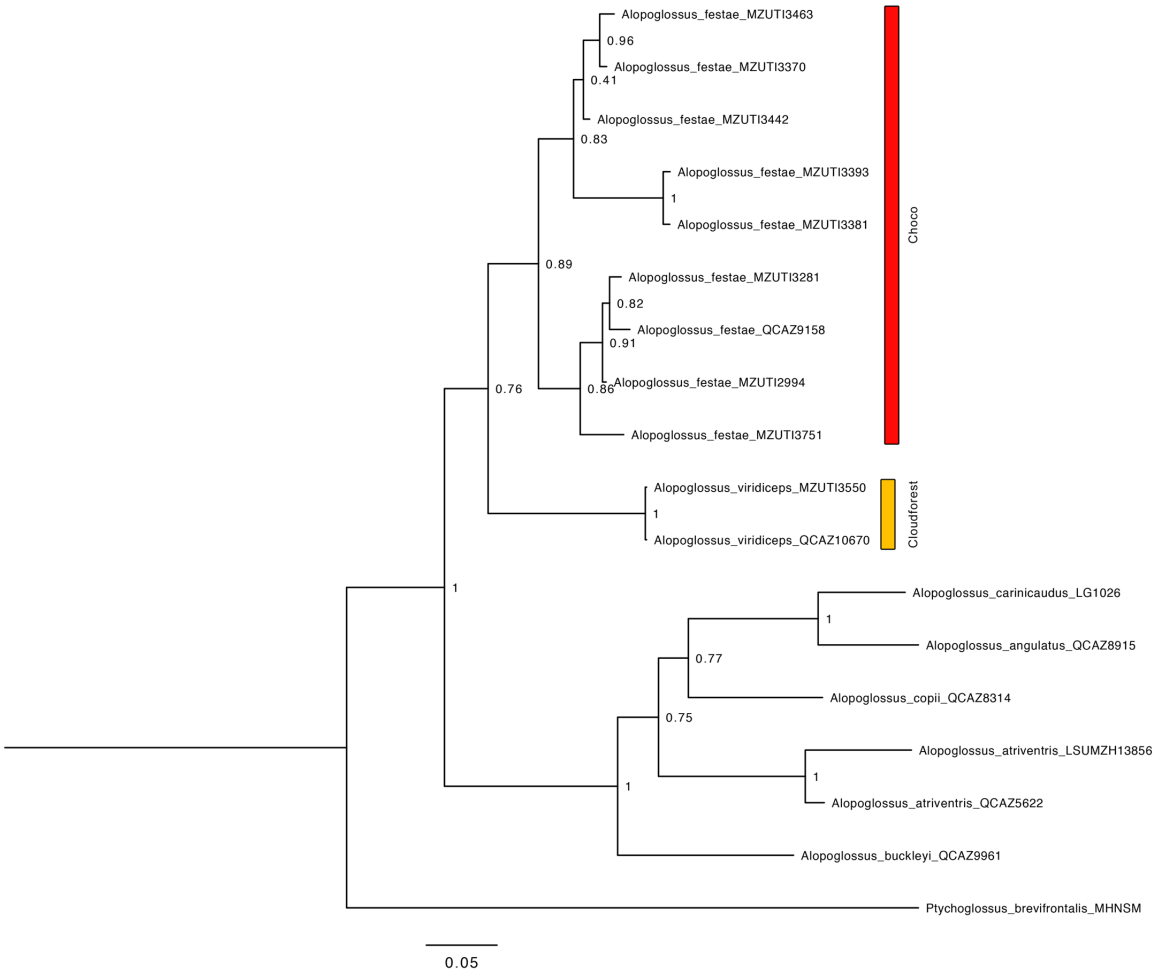

Figure 5. Maximum likelihood phylogram depicting relationships within the genus *Alopoglossus*. The phylogram was derived from analysis of 1221 bp of mitochondrial DNA (gene fragments 12S, 16S, cytb and ND4). Posterior probabilities and voucher numbers are shown.

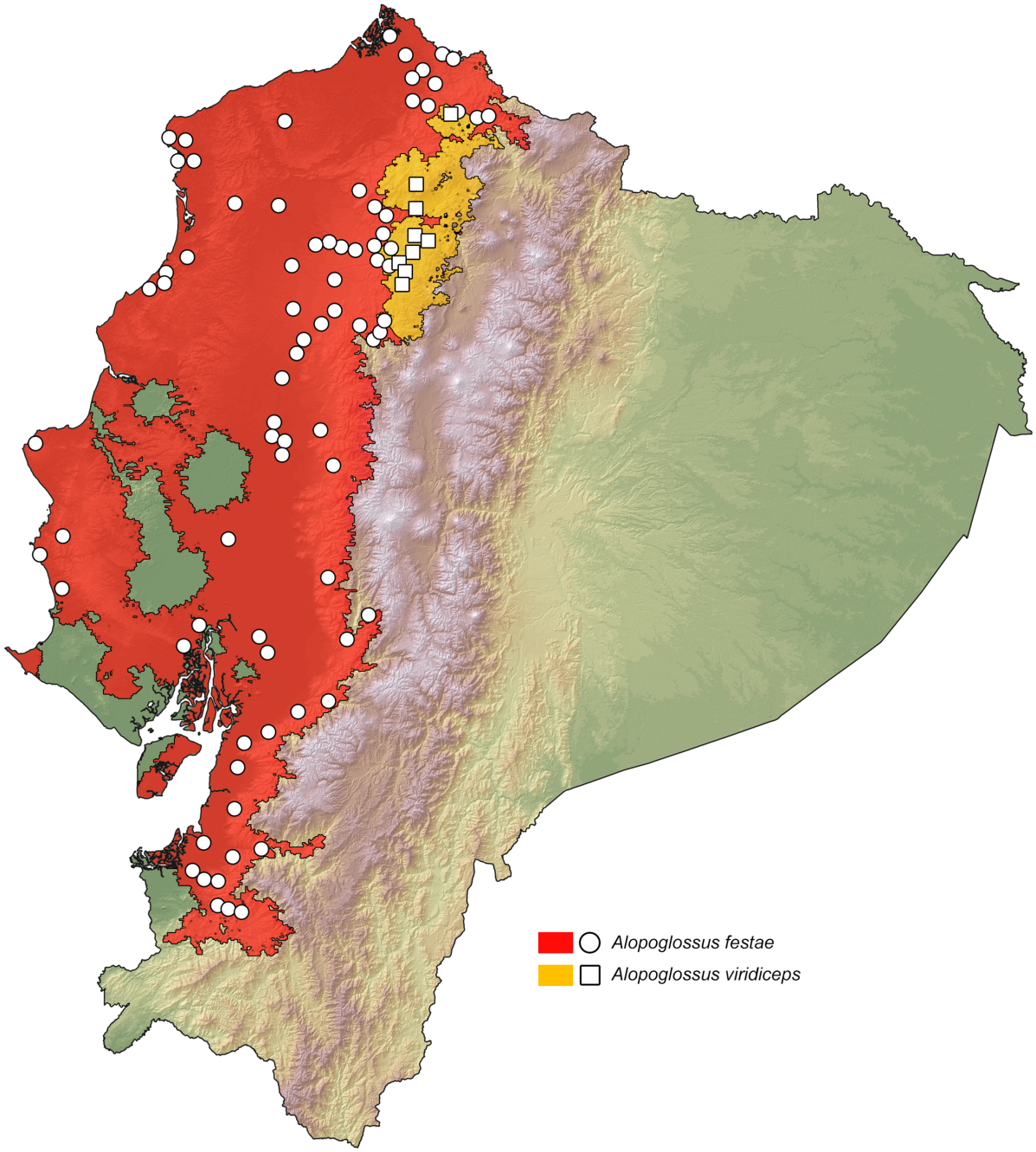

Figure 6. Distribution of *Alopoglossus festae* and *A. viridiceps* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Fig. 5.

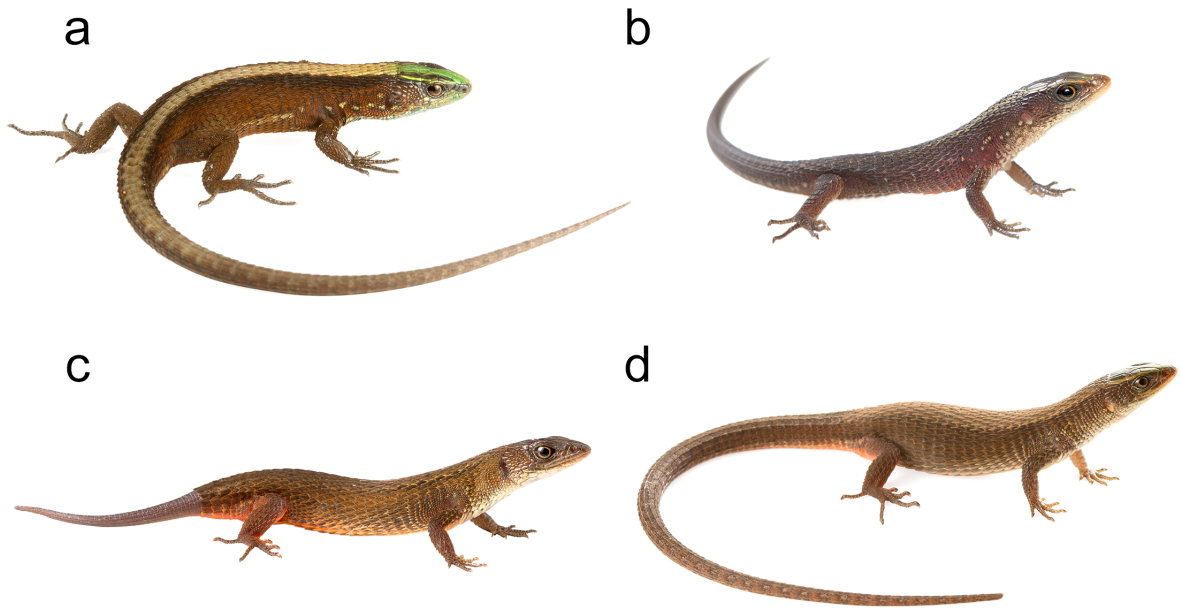

Figure 7. Morphological variation within sampled *Alopoglossus* species. (a) Adult of *A. viridiceps* (MZUTI 3552). (b) Juvenile of *A. festae* (MZUTI 2630). (c) Adult of *A. festae* (MZUTI 2994). (d) Adult female of *A. festae* (MZUTI 3370).

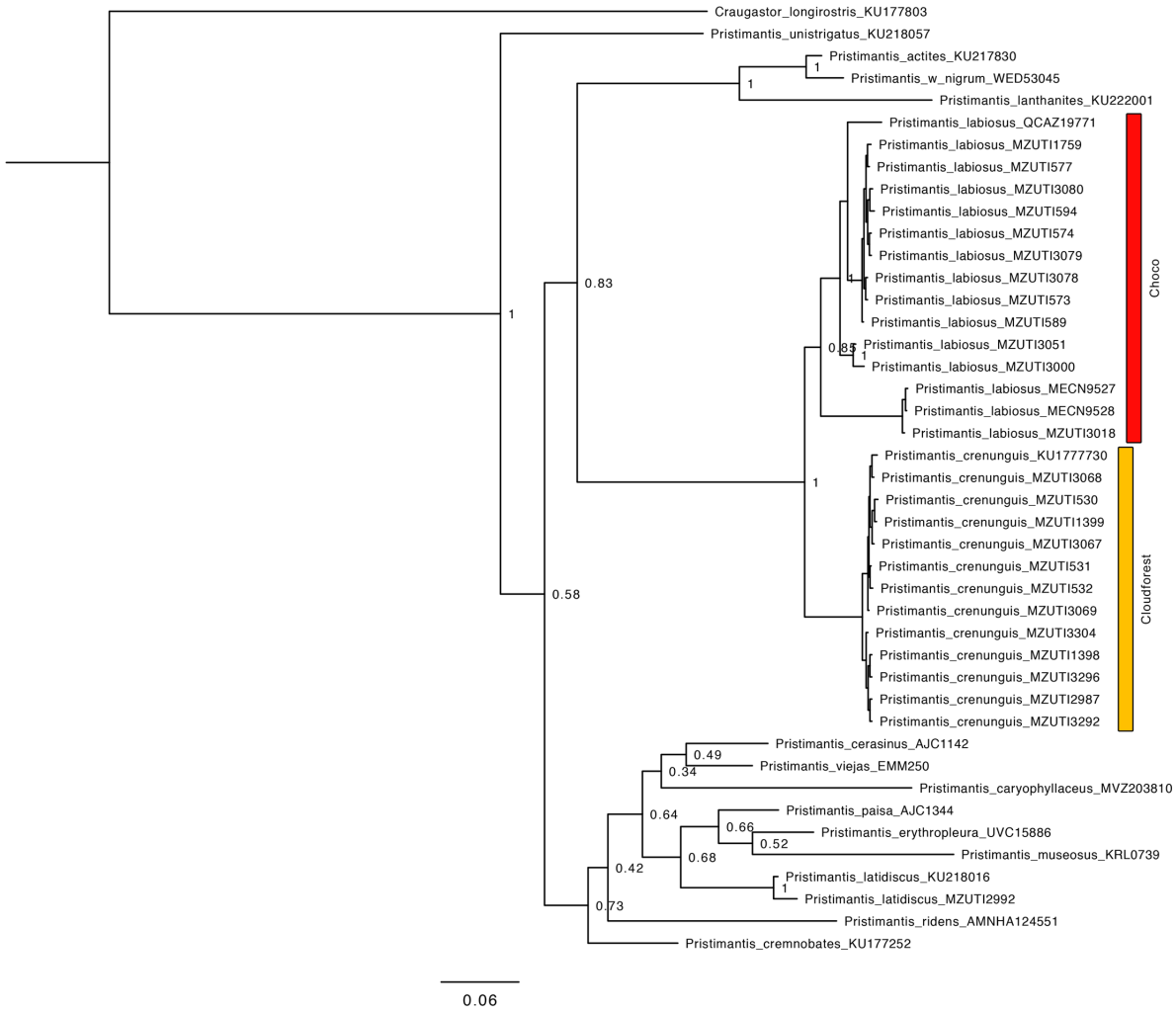

Figure 8. Maximum likelihood phylogram depicting relationships within the *Pristimantis* (*Hypodictyon*) *rubicundus* species series. The phylogram was derived from analysis of 1032 bp of mitochondrial DNA (gene fragments 12S and 16S). Posterior probabilities and voucher numbers are shown.

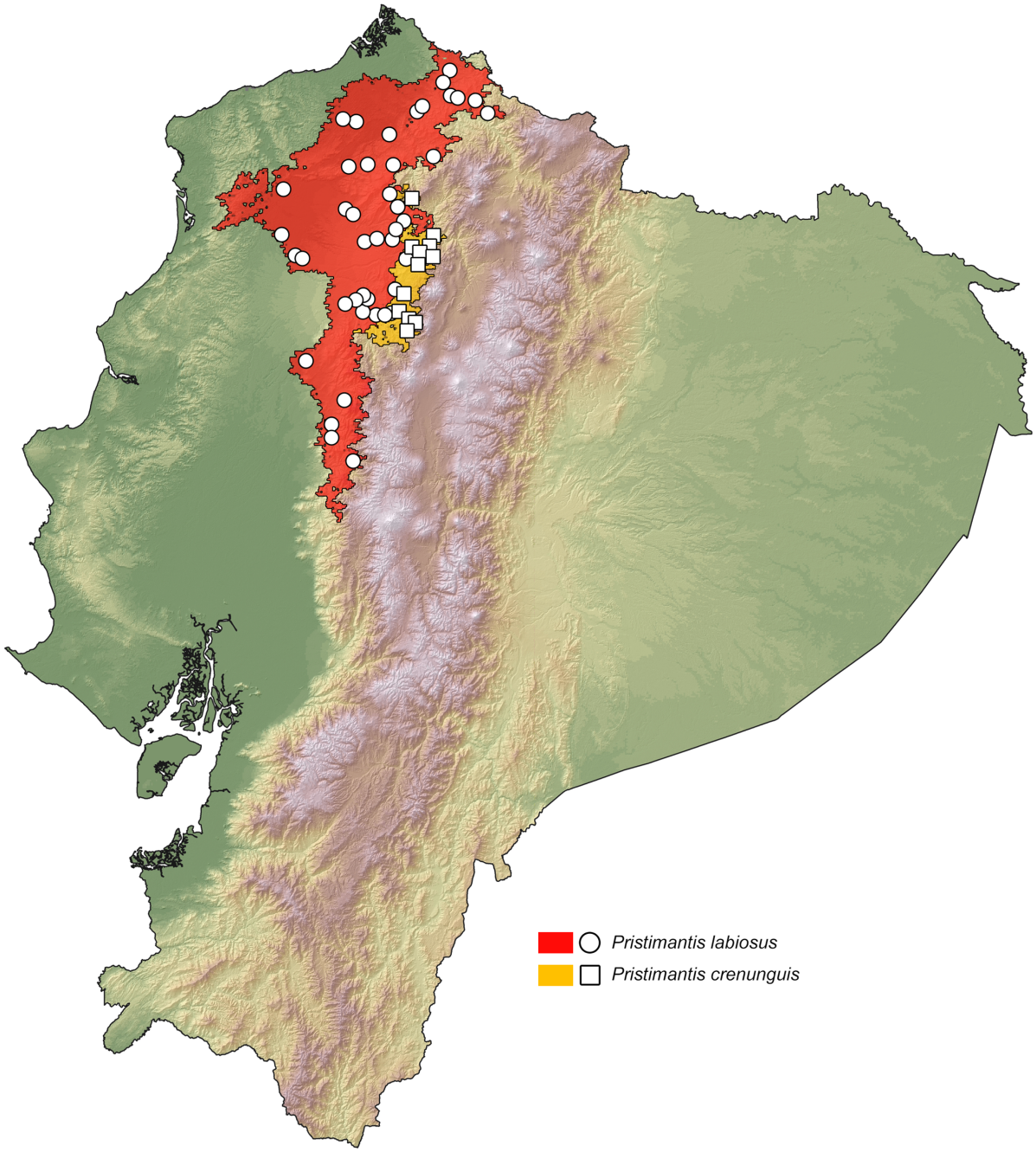

Figure 9. Distribution of *Pristimantis labiosus* and *P. crenunguis* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Fig. 8.

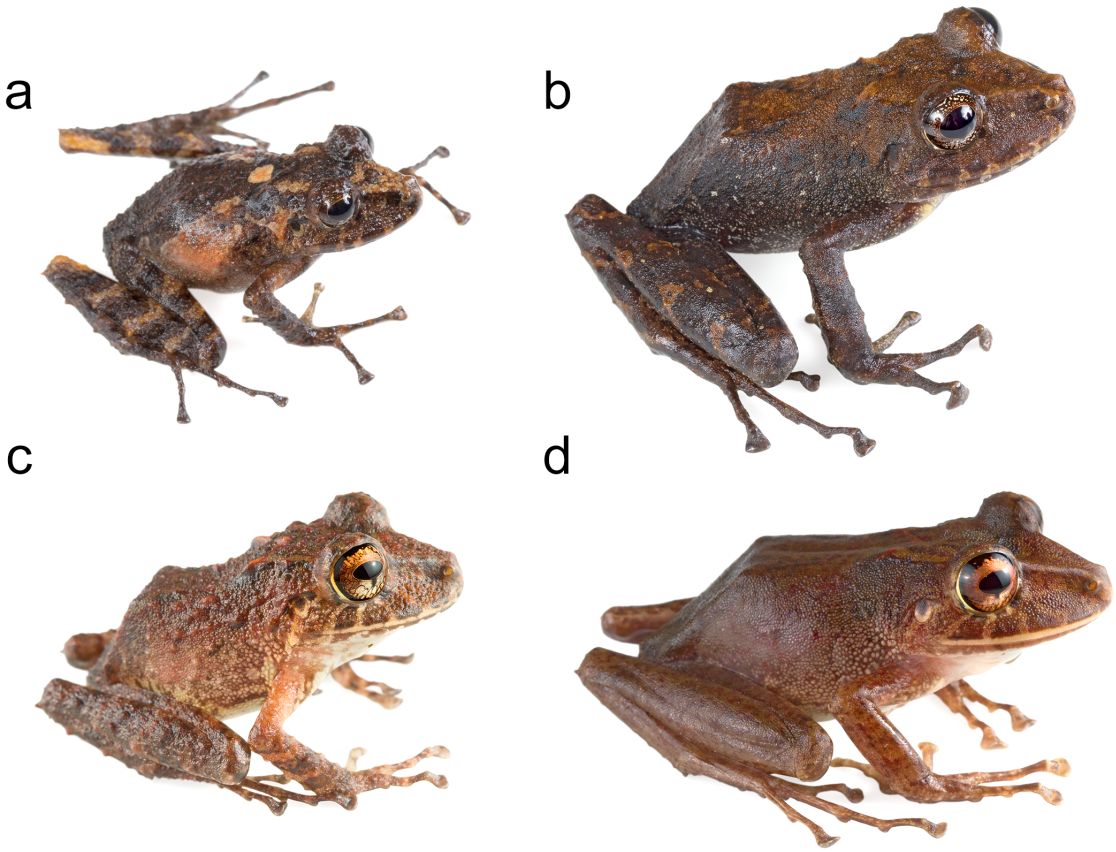

Figure 10. Morphological variation within sampled species of the *Pristimantis* (*Hypodyction*) *rubicundus* series. (a) Juvenile of *Pristimantis crenunguis* (Not vouchered). (b) Adult of *P. crenunguis* (Not vouchered). (c) Juvenile of *P. labiosus* (MZUTI 3511). (d) Adult of *P. labiosus* (Not vouchered).

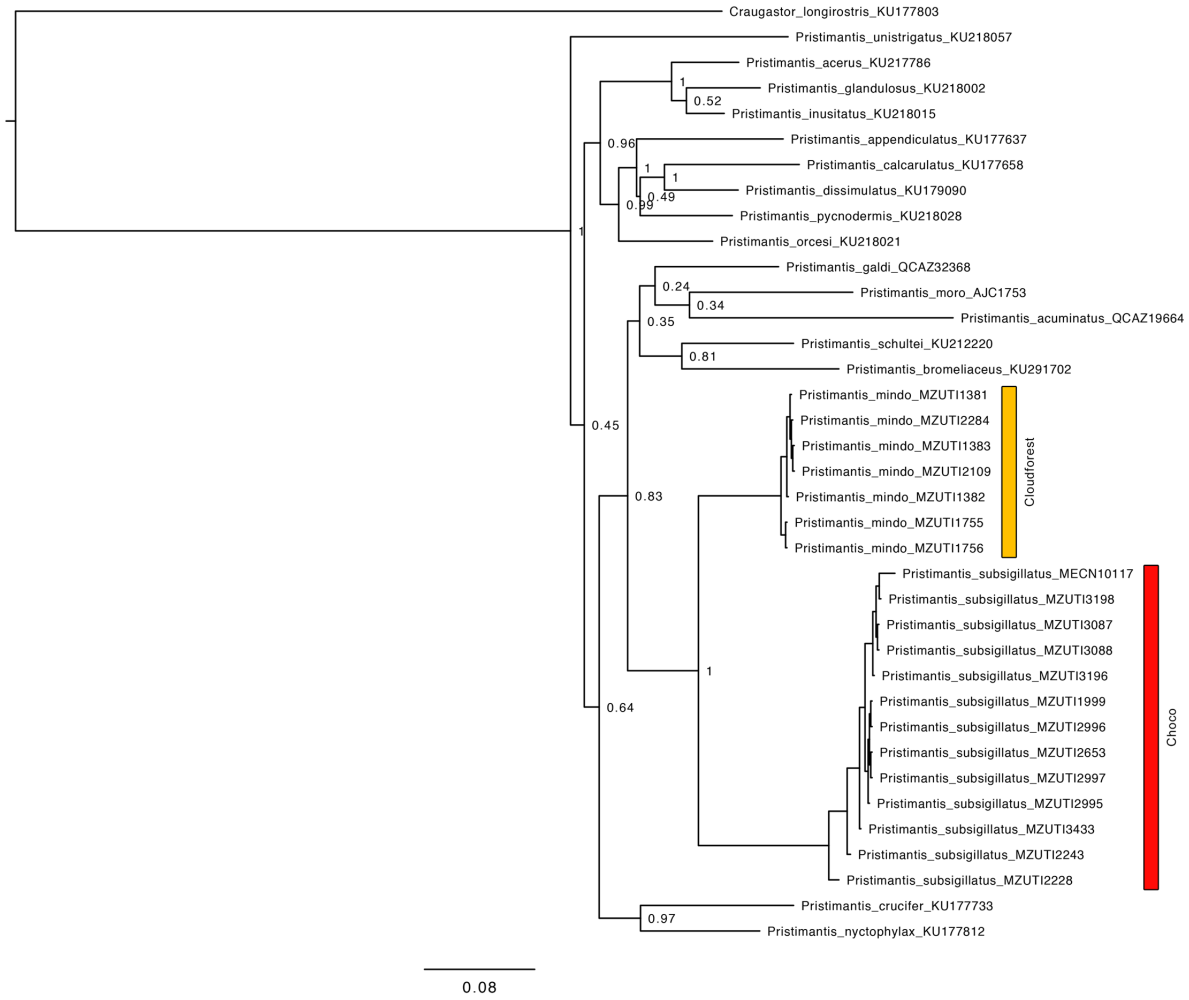

Figure 11. Maximum likelihood phylogram depicting relationships within the *Pristimantis lacrimosus* species group. The phylogram was derived from analysis of 2598 bp of mitochondrial DNA (gene fragments 12S and 16S). Posterior probabilities and voucher numbers are shown.

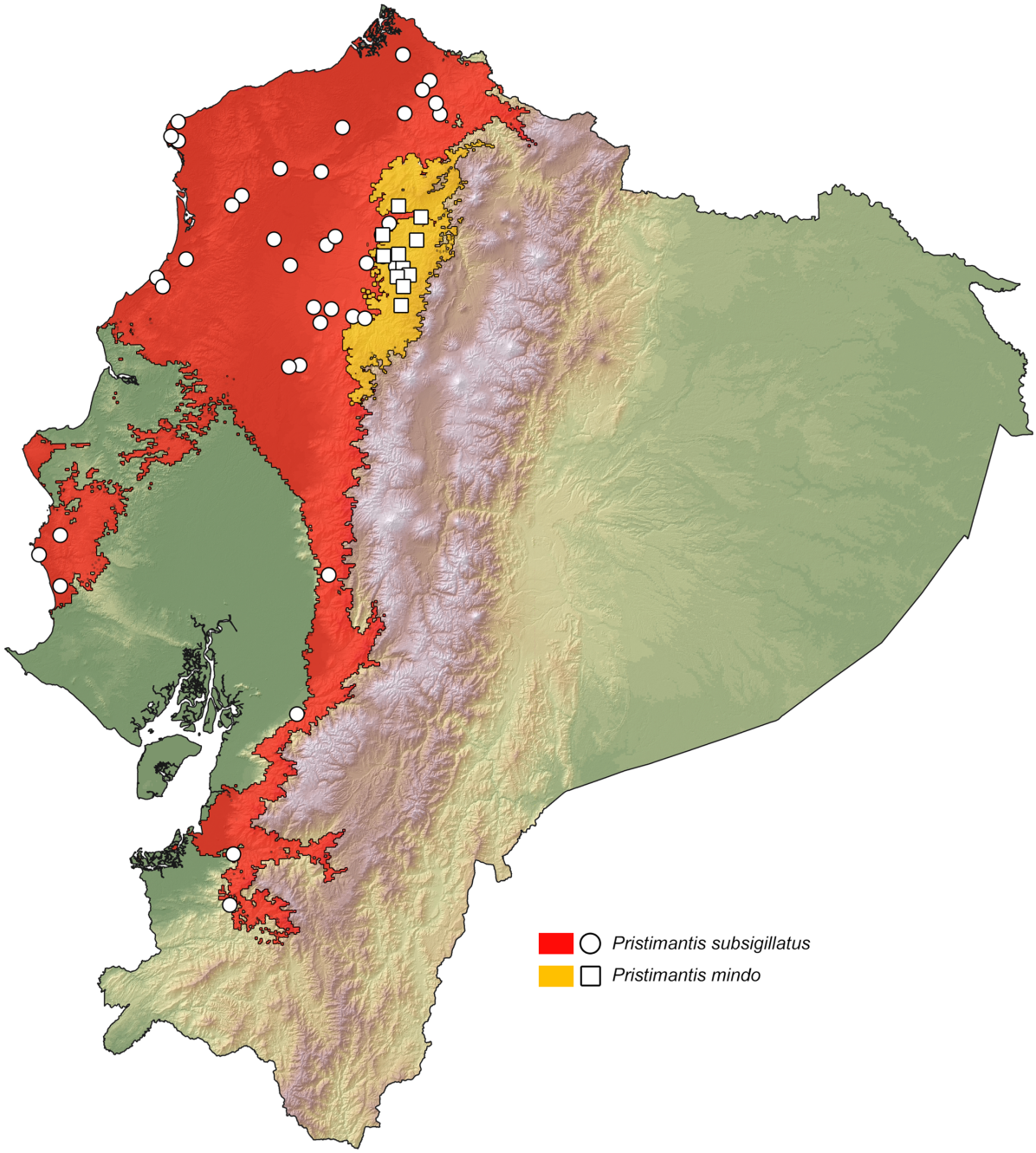

Figure 12. Distribution of *Pristimantis mindo* and *B. subsigillatus* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Figure 11.

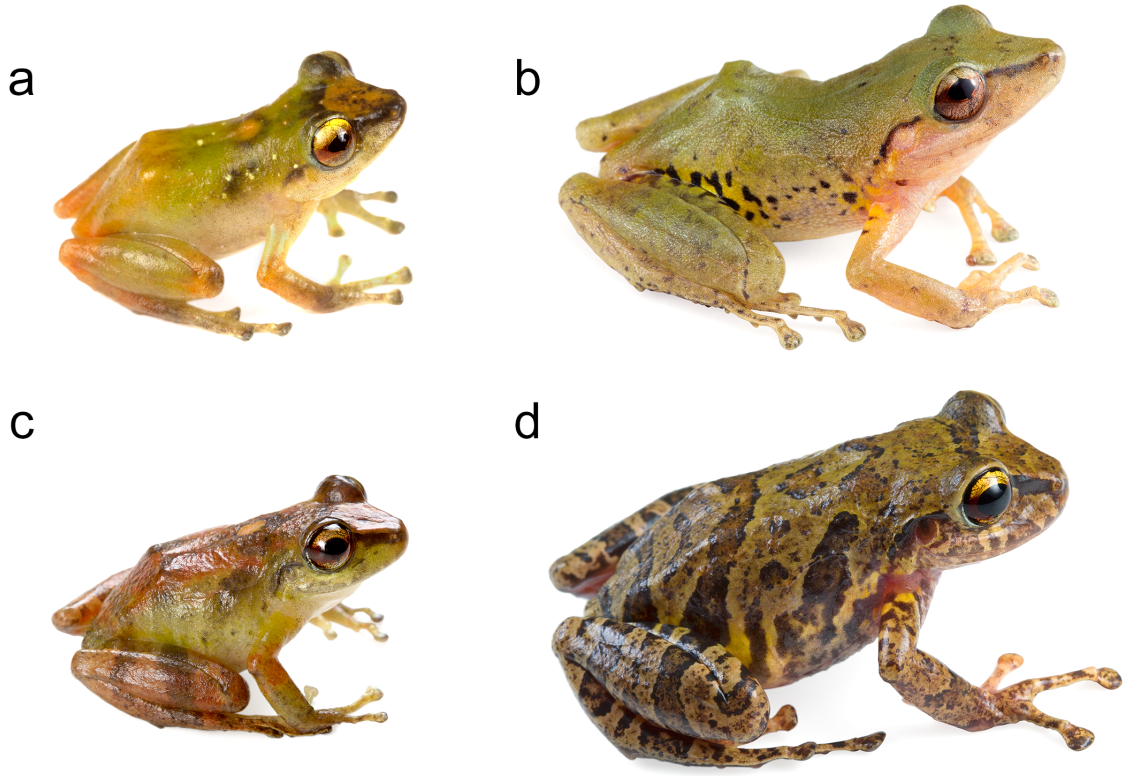

Figure 13. Morphological variation within sampled species of the *Pristimantis lacrimosus* species group. (a) Adult male of *Pristimantis subsigillatus* (MZUTI 2228). (b) Adult female of *P. subsigillatus* (MZUTI 2653). (c) Adult male of *P. mindo* (MZUTI 1382). (d) Adult female of *P. mindo* (MZUTI 1766).

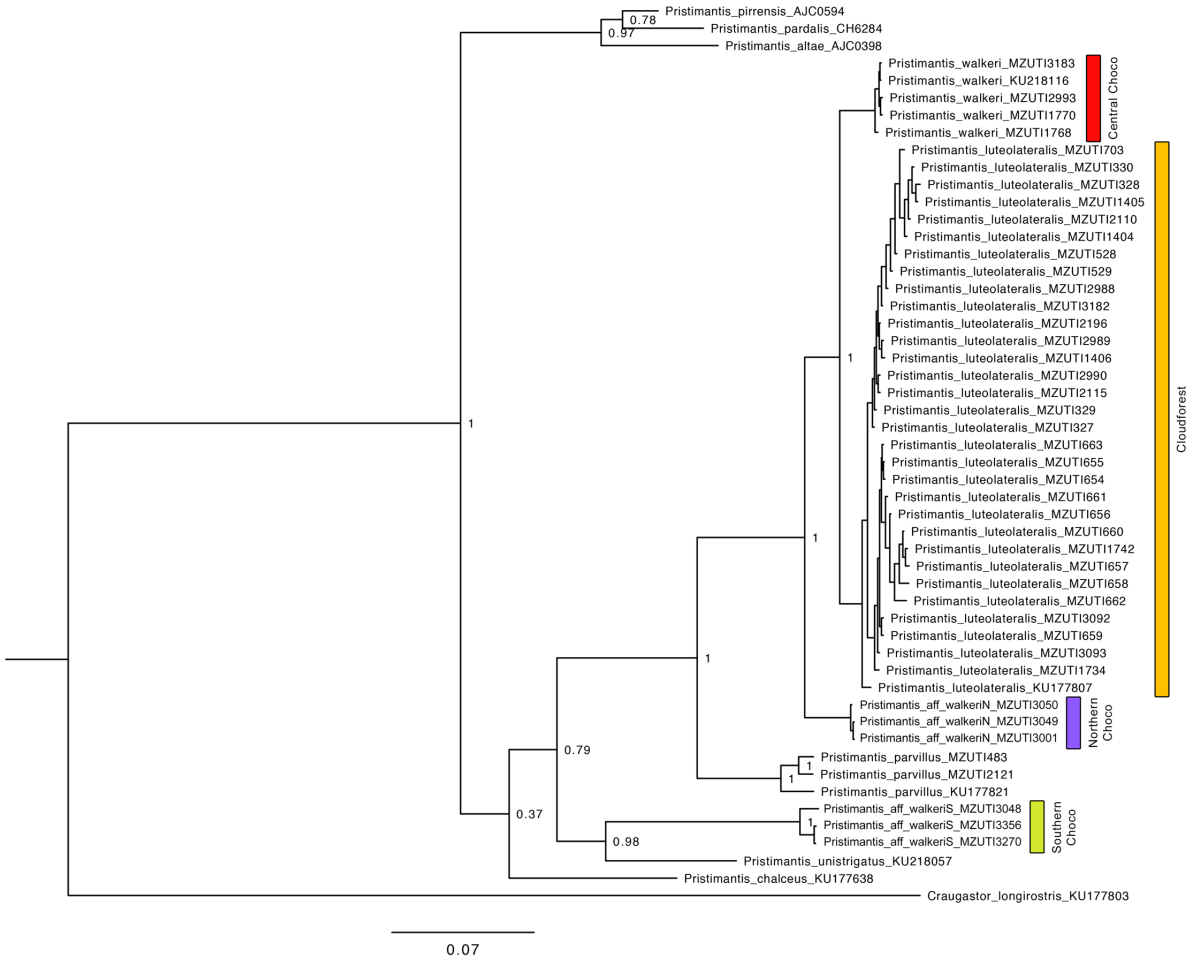

Figure 14. Maximum likelihood phylogram depicting relationships of the yellow-groined Trans-Andean *Pristimantis* of Ecuador. The phylogram was derived from analysis of 1905 bp of mitochondrial DNA (gene fragments 12S and 16S). Posterior probabilities and voucher numbers are shown.

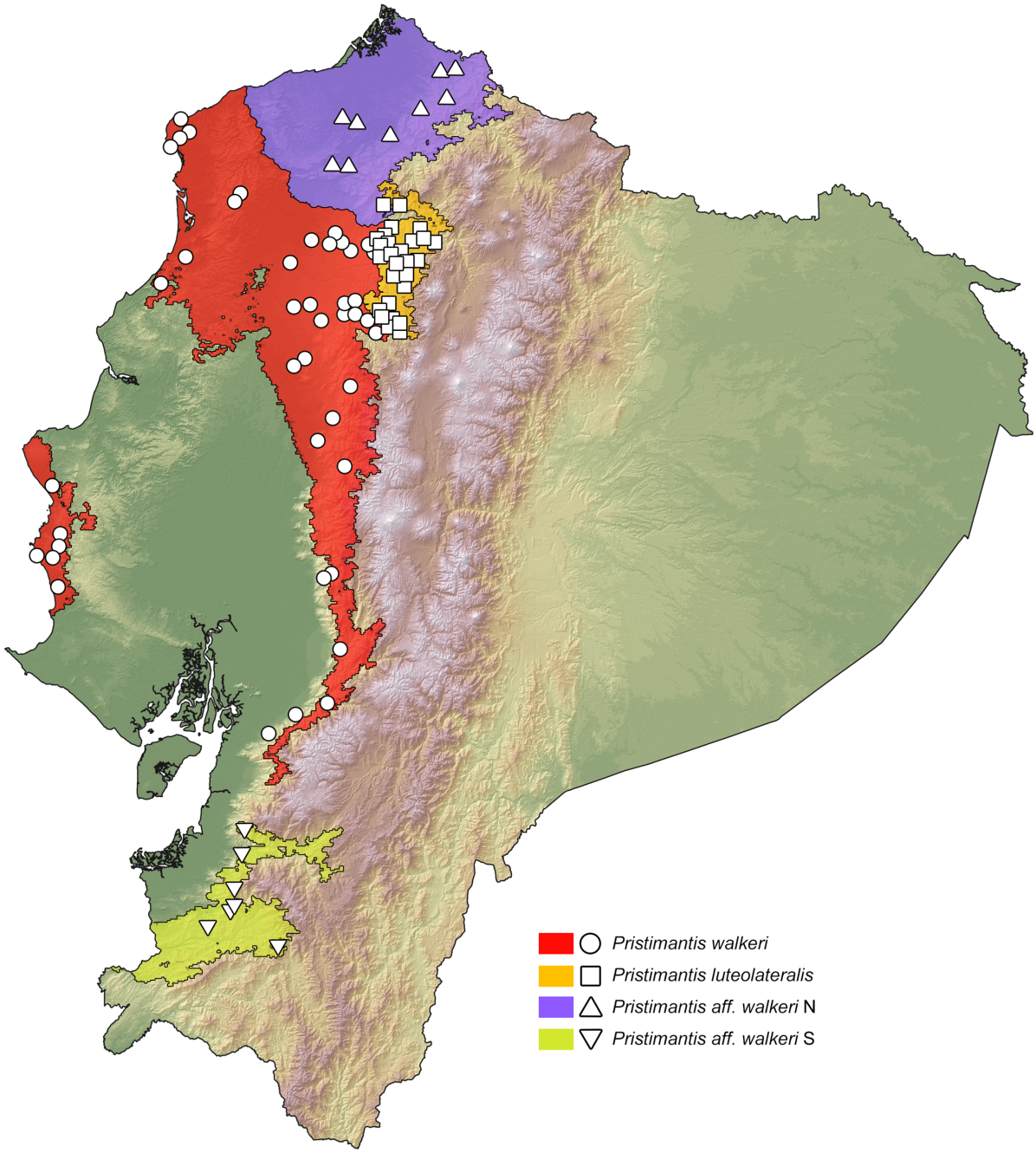

Figure 15. Distribution of *P. aff. walkeri* S, *P. luteolateralis*, *P. aff. walkeri* N and *P. walkeri* in Ecuador. White dots represent known localities. Each colored area represents the potential distribution of one of the clades recovered in the phylogeny of Figure 14.

Figure 16. Ecuadorian Trans-Andean *Pristimantis* characterized by their yellow to orange pigmentation in the hidden surfaces of the hind limbs. (a) Adult male of *P. aff. walkeri* S (MZUTI 3270). (b) Adult male holotype of *P. aff. walkeri* S (MZUTI 3480). (c) Adult female paratype of *P. af.f walkeri* S (MZUTI 3356). (d) Adult male of *P. aff. walkeri* N

(MZUTI 3913). (e) Adult male of *P. af.f walkeri* N (MZUTI 3914). (f) Adult male of *P. aff. walkeri* N (MZUTI 3915). (g) Adult male of *P. luteolateralis* (MZUTI 3092). (h) Adult male of *P. luteolateralis* (MZUTI 3904). (i) Adult female of *P. luteolateralis* (Not vouchered). (j) Adult male of *P. parvillus* (Not vouchered). (k) Adult male of *P. walkeri* (MZUTI 1768). (l) Adult female of *P. walkeri* (MZUTI 1769). (m) Adult female of *P. scolodiscus* (Not vouchered). (n) Adult male of *P. esmeraldas* (MZUTI 3545). (o) Adult female of *P. esmeraldas* (MZUTI 3375).

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- Lists any opposed reviewers

Your cover letter should **not** include requests to reduce or waive publication fees. Should your manuscript be accepted, you will have the opportunity to include your requests at that time. See *PLOS ONE* Editorial Policy for more information regarding publication fees.

Manuscript Organization

PLOS ONE considers manuscripts of any length. There are no explicit restrictions for the number of words, figures, or the length of the supporting information, although we encourage a concise and accessible writing style. We will **not** consider monographs.

All manuscripts should be double-spaced and include line numbers and page numbers.

Manuscripts should begin with the ordered sections:

- Title
- Authors
- Affiliations
- Abstract
- Introduction

and end with the sections of:

- Acknowledgments
- References
- Supporting Information Captions

Figures should be cited in ascending numeric order upon first appearance. Each figure caption should then be inserted immediately after the first paragraph in which it is cited in the article file.

Figures should not be included in the main manuscript file. Each figure must be prepared and submitted as an individual file. Find more information about preparing figures here.

Tables should be cited in ascending numeric order upon first appearance. Each table should then be inserted immmediately after the first paragraph in which it is cited in the article file.

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

There are no explicit requirements for section organization between these beginning and ending sections. Articles may be organized in different ways and with different section titles, according to the authors' preference. In most cases, internal sections include:

- Materials and Methods
- Results
- Discussion
- Conclusions (optional)

PLOS ONE has no specific requirements for the order of these sections, and in some cases it may be appropriate to combine sections. Guidelines for individual sections can be found below. Limit section and sub-sections to three headings levels.

Abbreviations should be kept to a minimum and defined upon first use in the text. Non-standard abbreviations should not be used unless they appear at least three times in the text.

Standardized nomenclature should be used as appropriate, including appropriate usage of species names and SI units.

PLOS articles do not support text footnotes. If your accepted submission contains footnotes, you will be asked to move that material into either the main text or the reference list, depending on the content.

Manuscript File Requirements

Authors may submit their manuscript files in Word (as .doc or .docx), LaTeX (as .pdf), or RTF format. Word files must not be protected.

LaTeX Submissions. If you would like to submit your manuscript using LaTeX, you must author your article using the *PLOS ONE* LaTeX template and BibTeX style sheet. Articles prepared in LaTeX may be submitted in PDF format for use during the review process. After acceptance, however, .tex files will be required. Please consult our LaTeX guidelines for a

list of what will be required.

Microsoft Word Submissions with Equations. If your manuscript is or will be in Microsoft Word and contains equations, you must follow the instructions below to make sure that your equations are editable when the file enters production.

1. Format display equations only in MathType (http://www.dessci.com/en/products/mathtype/).
2. Inline equations should be completely input via MathType. Do not include an equation that is part text, part MathType.
3. Do not use graphic objects.

If you have already composed your article in Microsoft Word and used its built-in equation editing tool, your equations will become unusable during the typesetting process. To resolve this problem, re-key your equations using MathType.

If you do not follow these instructions, PLOS will not be able to accept your file.

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2. Guidelines for Standard Sections

Title

Manuscripts must be submitted with both a full title and a short title, which will appear at the top of the PDF upon publication if accepted. Only the full title should be included in the manuscript file; the short title will be entered during the online submission process.

The full title must be 250 characters or fewer. It should be specific, descriptive, concise, and comprehensible to readers outside the subject field. Avoid abbreviations if possible. Where appropriate, authors should include the species or model system used (for biological papers) or type of study design (for clinical papers).

Examples:

- Impact of Cigarette Smoke Exposure on Innate Immunity: A *Caenorhabditis elegans* Model
- Solar Drinking Water Disinfection (SODIS) to Reduce Childhood Diarrhoea in Rural Bolivia: A Cluster-Randomized, Controlled Trial

The short title must be 50 characters or fewer and should state the topic of the paper.

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Authors and Affiliations

All author names should be listed in the following order:

- First names (or initials, if used),
- Middle names (or initials, if used), and

- Last names (surname, family name)

Each author should list an associated department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. If the article has been submitted on behalf of a consortium, all author names and affiliations should be listed at the end of the article.

This information cannot be changed after initial submission, so please ensure that it is correct.

To qualify for authorship, one should contribute to **all** of the following:

1. Conception and design of the work, acquisition of data, or analysis and interpretation of data
2. Drafting the article or revising it critically for important intellectual content
3. Final approval of the version to be published
4. Agreement to be accountable for all aspects of the work

All persons designated as authors should qualify for authorship, and all those who qualify should be listed. Each author must have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments.

When a large group or center has conducted the work, the author list should include the individuals whose contributions meet the criteria defined above, as well as the group name.

All authors must approve the final manuscript before submission. PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission of the manuscript.

One author should be designated as the corresponding author, and his or her email address or other contact information should be included on the manuscript cover page. This information will be published with the article if accepted.

See the PLOS Editorial and Publishing Policies for more information.

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Abstract

The abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should **not** include:

- Citations
- Abbreviations, if possible

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Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

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Materials and Methods

This section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

We encourage authors to submit detailed protocols for newer or less well-established methods as Supporting Information. Further information about formatting Supporting Information files, can be found here.

Methods sections of papers on research using **human or animal subjects and/or tissue or field sampling** must include required ethics statements. See the Reporting Guidelines for human research, clinical trials, animal research, and observational and field studies for more information.

Methods sections of papers with **data that should be deposited in a publicly available database** should specify where the data have been deposited and provide the relevant accession numbers and version numbers, if appropriate. Accession numbers should be provided in parentheses after the entity on first use. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication. A list of recommended repositories for different types of data can be found here.

Methods sections of papers using **cell lines** must state the origin of the cell lines used. See the Reporting Guidelines for cell line research for more information.

Methods sections of papers adding **new taxon names** to the literature must follow the Reporting Guidelines below for a new zoological taxon, botanical taxon, or fungal taxon.

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Results, Discussion, and Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled "Results and Discussion") or a mixed Discussion/Conclusions section (commonly labeled "Discussion"). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn. Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* Publication Criteria for more information.

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Acknowledgments

People who contributed to the work but do not fit the *PLOS ONE* authorship criteria should be listed in the acknowledgments, along with their contributions. You must ensure that anyone named in the acknowledgments agrees to being so named.

Funding sources should **not** be included in the acknowledgments, or anywhere in the manuscript file. You will provide this information during the manuscript submission process.

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References

General guidelines

- PLOS uses the reference style as outlined in the ICMJE sample references, also referred to as the “Vancouver” style.
- References must be listed at the end of the manuscript and numbered in the order that they appear in the text.
- In the text, citations should be indicated by the reference number in brackets.
- Authors may cite any and all available works in the reference list.
- Authors may not cite unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., “unpublished work,” “data not shown”).
- If an article is submitted to a journal and also publicly available as a pre-print, the pre-print may be cited.
- If related work has been submitted to PLOS ONE or elsewhere, authors should include a copy with the submitted article as confidential supplementary information, for review purposes only.

- Authors should not state 'unpublished work' or 'data not shown,' but instead include those data as supplementary material or deposit the data in a publicly available database.
- Journal name abbreviations should be those found in the NCBI databases.

Reference formatting

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial. References should be formatted as follows:

Published papers
1. Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun, B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (*Ailuropoda melanoleuca*). Genet Mol Res. 2011;10: 1576-1588.

Note: Use of a DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers:

Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005

Accepted, unpublished papers
Same as above, but "In press" appears instead of the page numbers or DOI.

Websites or online articles
1. Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available: http://www.globalizationandhealth.com/content/1/1/14.

Books
1. Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.

Book chapters
1. Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.

Deposited articles (preprints, e-prints, or arXiv)
1. Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available: arXiv:1403.3301v1. Accessed 17 March 2014.

Published media (print or online newspapers and magazine articles)
1. Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html. Accessed 17 March 2014.

New media (blogs, websites, or other written works)
1. Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.

Masters' theses or doctoral dissertations
1. Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available: http://cumincad.scix.net/cgi-bin/works/Show?2e09.

Databases and repositories (Figshare, arXiv)
1. Roberts SB. QPX Genome Browser Feature Tracks; 2013. Database: figshare [Internet]. Accessed: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214.

Multimedia (videos, movies, or TV shows)
1. Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

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Figure Legends

Figures should **not** be included in the manuscript file, but figure legends should be. Guidelines for preparing figures can be found here.

Figure legends should describe the key messages of a figure. Legends should have a short title of 15 words or less. The full legend should have a description of the figure and allow readers to understand the figure without referring to the text. The legend itself should be succinct, avoid lengthy descriptions of methods, and define all non-standard symbols and abbreviations.

Figures should be cited in ascending numeric order upon first appearance. Each figure caption should be inserted immediately after the first paragraph in which they are cited in the article file. Further information about figure captions can be found in the Figure Guidelines.

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Supporting Information Captions

Because Supporting Information is accessed via a hyperlink attached to its captions, captions must be listed in the article file. Do not submit a separate caption file. It is acceptable to have them in the file itself in addition, but they must be in the article file for access to be possible in the published version.

The file category name and number is required, and a one-line title is highly recommended. A legend can also be included but is not required. Supporting Information captions should be formatted as follows.

S1 Text. Title is strongly recommended. Legend is optional.

Please see our Supporting Information guidelines for more details.

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Data Reporting Guidelines

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article. Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (eg, GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CCBY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include: Dryad and figshare. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

Deposit data in the integrated repository of choice. Once deposition is final and complete, the repository will provide the author with a dataset DOI (provisional) and private URL for reviewers to gain access to the data. Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS Submission form. Then provide the URL passcode in the Attach Files section. If you have any questions, please contact us at plosone@plos.org

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Accession Numbers

All appropriate datasets, images, and information should be deposited in public resources. Please provide the relevant accession numbers (and version numbers, if appropriate). Accession numbers should be provided in parentheses after the entity on first use. Suggested databases include, but are not limited to:

- ArrayExpress
- BioModels Database
- Database of Interacting Proteins
- DNA Data Bank of Japan [DDBJ]
- DRYAD
- EMBL Nucleotide Sequence Database
- GenBank
- Gene Expression Omnibus [GEO]

- Protein Data Bank
- UniProtKB/Swiss-Prot
- ClinicalTrials.gov

In addition, as much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Providing accession numbers allows linking to and from established databases and integrates your article with a broader collection of scientific information.

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Striking Images

Authors are encouraged to upload a "striking image" that may be used to represent their paper online in places like the journal homepage or in search results. The striking image must be derived from a figure or supporting information file from the paper, ie. a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows. If no striking image is uploaded, a figure from the paper will be designated as the striking image.

Please keep in mind that PLOS's Creative Commons Attribution License applies to striking images. As such, do not submit any figures or photos that have been previously copyrighted unless you have express written permission from the copyright holder to publish under the CCAL license. Note that all published materials in PLOS ONE are freely available online, and any third party is permitted to read, download, copy, distribute, and use these materials in any way, even commercially, with proper attribution.

Care should be taken with the following image types in particular:

1. PLOS ONE is unable to publish any images generated by Google software (Google Maps, Street View, and Earth)
2. Maps in general are usually copyrighted, especially satellite maps
3. Photographs
4. Commercial or government images, slogans, or logos
5. Images from Facebook or Twitter

Authors must also take special care when submitting manuscripts that contain potentially identifying images of people. Identifying information should not be included in the manuscript unless the information is crucial and the individual has provided written consent

by completing the Consent Form for Publication in a PLOS Journal (PDF).

For license inquiries, e-mail license [at] plos.org.

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Tables

Tables should be cited in ascending numeric order upon first appearance. Each table should be inserted immediately after the first paragraph in which it is cited in the article file. All tables should have a concise title. Footnotes can be used to explain abbreviations. Citations should be indicated using the same style as outlined above. Tables occupying more than one printed page should be avoided, if possible. Larger tables can be published as Supporting Information. Please ensure that table formatting conforms to our Guidelines for table preparation.

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3. Specific Reporting Guidelines

Human Subject Research

Methods sections of papers on research using human subject or samples must include ethics statements that specify:

- The name of the approving institutional review board or equivalent committee(s). If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- Whether informed consent was written or oral. If informed consent was oral, it must be stated in the manuscript:
    - Why written consent could not be obtained
    - That the Institutional Review Board (IRB) approved use of oral consent
    - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: "Caucasian" should be changed to "white" or "of [Western] European descent" (as appropriate); "cancer victims" should be changed to "patients with cancer."

For papers that include identifying, or potentially identifying, information, authors must

download the Consent Form for Publication in a PLOS Journal (PDF), which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

> **The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.**

For more information about *PLOS ONE* policies regarding human subject research, see the Publication Criteria and Editorial Policies.

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Clinical Trials

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement website. Before the paper can enter peer review, authors must:

1. Provide the registry name and number in the methods section of the manuscript
2. Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as Supporting Information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
3. Include the CONSORT flow diagram as the manuscript's "Fig. 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The methods section must include the name of the registry, the registry number, and the URL of your trial in the registry database for each location in which the trial is registered.

For more information about *PLOS ONE* policies regarding clinical trials, see the Editorial Policies.

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Animal Research

Methods sections of manuscripts reporting results of animal research must include required ethics statements that specify:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s) (where ethical approval is not required, the manuscript should include a clear statement of this and the reason why)
- Relevant details for efforts taken to ameliorate animal suffering

For example:

> **This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Permit Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.**

The organism(s) studied should always be stated in the abstract. Where research may be confused as pertaining to clinical research, the animal model should also be stated in the title.

We ask authors to follow the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information. Please note that inclusion of a completed ARRIVE Checklist will be a formal requirement for publication at a later date.

For more information about *PLOS ONE* policies regarding animal research, see the Publication Criteria and Editorial Policies.

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Observational and Field Studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

For more information about *PLOS ONE* policies regarding observational and field studies, see the Publication Criteria and Editorial Policies.

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Cell Line Research

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate. Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers a gift, authors must follow our policies for human subject research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended - e.g. by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis - and may be required during peer review or after publication.

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Blots and Gels

Authors of manuscripts reporting results from blots (including Western blots) and electrophoretic gels should follow these guidelines:

- In accordance with *PLOS ONE*'s policy on image manipulation, the image should not be adjusted in any way that could affect the scientific information displayed, e.g. by modifying the background or contrast
- All blots and gels that support results reported in the manuscript should be provided
- Original uncropped and unadjusted blots and gels, including molecular size markers, should be provided in either the figures or the supplementary files
- Lanes should not be overcropped around the bands; the image should show most or all of the blot or gel. Any non-specific bands should be shown and an explanation of their nature should be given
- The image should include all relevant controls, and controls should be run on the same blot or gel as the samples
- A figure panel should not include composite images of bands originating from different blots or gels. If the figure shows non-adjacent bands from the same blot or gel, this should be clearly denoted by vertical black lines and the figure legend should provide details of how the figure was made

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Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species
- The commercial supplier or source laboratory
- The catalogue or clone number and, if known, the batch number

- The antigen(s) used to raise the antibody
- For established antibodies, authors are encouraged to supply a stable public identifier from the Antibody Registry (www.antibodyregistry.org).

Authors should also report the following experimental details:

- The final antibody concentration or dilution
- A reference to the validation study if the antibody was previously validated, and if not, details of how the authors validated the antibody for the applications and species used. Authors should consider adding information on new validations to a publicly available database such as Antibodypedia or CiteAb.

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Systematic Review/Meta-Analysis

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as Supporting Information and provide the registry number in the abstract.

If your article is a Systematic Review or a Meta-Analysis you should:

- State this in your cover letter
- Select "Research Article" as your article type when submitting
- Include the PRISMA flowchart as Fig. 1 (required where applicable)
- Include the PRISMA checklist as Supporting Information

Meta-Analysis of Genetic Association Studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in “Systematic Reviews of Genetic Association Studies” by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results.

Authors will also be asked to complete a checklist outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

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Paleontology and Archaeology Research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

See the *PLOS ONE* Editorial Policies for more information regarding manuscripts describing paleontology and archaeology research.

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Software Papers

Manuscripts describing software should provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

See the *PLOS ONE* Editorial Policies for more information about submitting manuscripts.

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Database Papers

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a

direct link to the database hosting site from within the paper.

See the *PLOS ONE* Editorial Policies for more information about submitting manuscripts describing databases.

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New Zoological Taxon

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Anochetus boltoni Fisher **sp. nov.** urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB

You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called "Nomenclatural Acts":

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS [author to insert any additional repositories].

All *PLOS ONE* articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

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New Botanical Taxon

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). In association with the International Plant Names Index (IPNI), the following guidelines for publication in an online-only journal have been agreed such that any scientific botanical

name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, "the description or diagnosis required for valid publication of the name of a new taxon" can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of a *PLOS ONE* article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

> **Solanum aspersum** S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

PLOS ONE staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called "Nomenclature" using the following wording:

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a *PLOS ONE* article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All *PLOS ONE* articles are deposited in PubMed Central and LOCKSS. If your institute, or

those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

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New Fungal Taxon

When publishing papers that describe a new fungal taxon name, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific fungal name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, "the description or diagnosis required for valid publication of the name of a new taxon" can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of a *PLOS ONE* article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Hymenogaster huthii. Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called "Nomenclature" using the following wording (this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum):

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a *PLOS ONE* article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed

copies.

In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All *PLOS ONE* articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

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Qualitative Research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.

PARA GRADOS ACADÉMICOS DE LICENCIADOS (TERCER NIVEL)

PONTIFICIA UNIVERSIDAD CATÓLICA DEL ECUADOR

DECLARACIÓN Y AUTORIZACIÓN

Yo, Alejandro Federico Arteaga Navarro, C.I. 0302448956, autor del trabajo de graduación intitulado: "*Comparative phylogeography reveals cryptic diverstiy and repeated patterns of cladogenesis for amphibians and reptiles in northwestern Ecuador*", previa a la obtención del grado académico de **LICENCIADO EN CIENCIAS BIOLÓGICAS** en la Facultad de **Ciencias Exactas y Naturales**:

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