



Divergence of tropical pitvipers promoted by independent colonization events of dry montane Andean habitats

David Salazar-Valenzuela^{1,2} | Ulrich Kuch³ | Omar Torres-Carvajal⁴ |
Jorge H. Valencia⁵ | H. Lisle Gibbs¹

¹Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, Ohio, USA

²Centro de Investigación de la Biodiversidad y Cambio Climático (BioCamb) e Ingeniería en Biodiversidad y Recursos Genéticos, Facultad de Ciencias de Medio Ambiente, Universidad Tecnológica Indoamérica, Quito, Ecuador

³Institute of Occupational Medicine, Social Medicine and Environmental Medicine, Goethe University, Frankfurt am Main, Germany

⁴Museo de Zoología, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

⁵Fundación Herpetológica Gustavo Orcés, Quito, Ecuador

Correspondence

David Salazar-Valenzuela, Facultad de Ciencias de Medio Ambiente, Universidad Tecnológica Indoamérica, Machala y Sabanilla EC170301, Quito, Ecuador.
Email: davidvalenzarv@gmail.com

Funding information

Fulbright Program; Secretaría de Educación Superior, Ciencia, Tecnología e Innovación; Tinker Foundation; The Ohio State University Graduate School; Deutsche Forschungsgemeinschaft, Grant/Award Number: KU2328/5-1

Handling Editor: David Chapple.

Abstract

Aim: A poorly explored feature of the origin and maintenance of Neotropical biodiversity is how the evolutionary dynamics of colonization and differentiation in relation to lowland and highland habitats has impacted lineage formation. Most speciation models for this region have focused on vicariant events, whereas the need to assess the influence of demographic processes has been recognized only recently. We evaluate if the origin of Andean montane lineages of terciopelo pitvipers is explained by either of two historical processes that represent distinct phylogeographic mechanisms: differentiation by isolation within the highlands or different dispersal events from the lowlands.

Location: Western Ecuador.

Taxon: Terciopelo pitvipers (*Bothrops asper* species complex).

Methods: We use genomic data and genetic clustering analyses, evaluation of historical migration between genetic clusters and demographic model selection to investigate recent diversification events in South America using a vertebrate group rarely explored in phylogeographic studies: tropical Andean snakes. Specifically, the origin of two Ecuadorian montane lineages of terciopelo pitvipers was evaluated given ambiguous phylogenetic relationships with the presumably ancestral Pacific lowland lineage.

Results: Discrepancies of evolutionary relationships previously obtained with tree-like methods are resolved through the use of modelling approaches. We found strong support for the independent origin of montane lineages based on topologies inferred by maximum-likelihood trees and modelling approaches that take into account possible gene flow. This suggests dispersal rather than in-situ differentiation as the most likely mechanism by which the montane lineages originated.

Main conclusions: Recent large-scale studies have found support for identifying dispersal events as important drivers of diversification in the Neotropical region. We contribute to these ideas by identifying a fine-scale case in a rarely studied group of animals -Andean snakes- in which river valleys acted as an entrance for the upward colonization of montane dry habitats and subsequent ecological diversification.



KEYWORDS

dispersal, evolutionary lineages, historical demography, Neotropics, northern Andes, RAD sequencing, terciopelo pitvipers

Andean valleys are adorned with banana and palm trees; at a higher elevation one can find a beneficial tree whose bark is the fastest and healthiest fever reducer. In this temperate elevation where quinquinas are found, and in higher elevations where there are *Escallonia*, there one finds oaks, pines, *Berberis*, *Alnus*, *Rubus* and a host of genera that we think belong only in the northern lands. Thus, the inhabitants of equinoctial regions know all the species that nature placed around them: the earth offers to their eyes a spectacle as varied as the azure vault of the heavens which hides none of its constellations from view.

Alexander von Humboldt & Aimé Bonpland, *Essai sur la géographie des plantes*, 1805

1 | INTRODUCTION

The Neotropics harbour the largest portion of the biodiversity present on the planet (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Rull, 2011; Smith et al., 2014). For decades, molecular studies have centred on exploring different hypotheses to explain the origin of this diversity (Bagley & Johnson, 2014; Leite & Rogers, 2013). Most studies have used gene genealogies to infer the timing and geographical location of divergence across taxa, and have primarily linked patterns of evolutionary relationships to specific vicariant events generated by Pliocene/Miocene landscape changes (e.g. Andean orogeny, emergence of the Panama Isthmus, drainage shifts, sea transgressions) or Pleistocene climatic oscillations (i.e. glacial-interglacial cycles) (Rull, 2011; Turchetto-Zolet, Pinheiro, Salgueiro, & Palma-Silva, 2013). However, recent meta-analyses have shown that such generalizations are not warranted, and the origin and maintenance of extant neotropical biodiversity is a complex phenomenon that depends on synergistic environmental drivers that have operated at different spatial and temporal scales (Antonelli et al., 2018; Rull, 2008; Turchetto-Zolet et al., 2013). Consequently, a thorough understanding of this topic requires a larger number of systems to be explored using data and analytical approaches to infer evolutionary history (Rull, 2013).

One of Alexander von Humboldt's (1769–1859) greatest contributions to global science was to unify concepts about nature based on careful observations and measurements made in different parts of the world (Wulf, 2015). His 22-month-long expedition along the northern Andes in the early 19th century certainly constituted a pivotal time for the establishment of his revolutionary ideas about the natural world. Although great advances have been made in how biodiversity is generated in the Neotropics, an understanding of the

origin and maintenance of Andean diversity is still incomplete (Hazzi, Moreno, Ortiz-Movliav, & Palacio, 2018). In South America, the uplift of the Andes mountain range undoubtedly played an important role in the diversification of several taxonomic groups (Antonelli et al., 2010; Esquerré, Brennan, Catullo, Torres-Pérez, & Keogh, 2019; Hoorn et al., 2010; Lagomarsino, Condamine, Antonelli, Mulch, & Davis, 2016). However, one aspect that remains poorly understood are the recent evolutionary dynamics of colonization and differentiation in lowland and highland habitats once the Andes reached their current altitudes (~2.6 Ma) (Brumfield & Edwards, 2007). For example, recent studies in different lineages of Neotropical birds have suggested the importance of dispersal events into and out of montane habitats as a cause of increased diversification rates during the Pleistocene (Brumfield & Edwards, 2007; Hazzi et al., 2018; Smith et al., 2014; Weir, 2006). These findings represent a shift from the paradigm that most speciation events in the region are primarily related to isolation due to landscape change (i.e. vicariance), to a consideration of a broader range of mechanisms. They also demonstrate the need to assess the influence of demographic processes in the structuring of present-day phylogeographic patterns in diverse taxa (Harvey & Brumfield, 2015).

Moving beyond a sole focus on vicariance-based hypotheses to a broader range of scenarios that incorporate other historical demographic mechanisms is now possible due to the increasing availability of genome-scale datasets and model-based approaches in phylogeography (Garrick et al., 2015; Hickerson et al., 2010). The inclusion of a larger number of variable sites from more regions of the genome has proven to be especially useful for this discipline due to the increased accuracy and precision of parameter estimation (Leaché et al., 2015; McCormack, Hird, Zellmer, Carstens, & Brumfield, 2013; Pyron, 2015). Such improvements have also been matched by the implementation of coalescent-based modelling techniques that increase the statistical rigor of hypothesis testing by comparing alternative historical scenarios (Beaumont et al., 2010; Knowles & Maddison, 2002). Recent computationally efficient approaches for analysing genomic datasets allow the evaluation of more complex and realistic historical processes (Excoffier, Dupanloup, Huerta-Sanchez, Sousa, & Foll, 2013; Gutenkunst, Hernandez, Williamson, & Bustamante, 2009; Pickrell & Pritchard, 2012; Rittmeyer & Austin, 2015). Specifically, modelling divergence in this framework allows the incorporation of multiple demographic processes (e.g. population size changes, migration) into inferences about true divergence patterns which is not possible with tree-based methods (Solís-Lemus, Yang, & Ané, 2016; Sousa & Hey, 2013).

Neotropical snakes of the family Viperidae (i.e. pitvipers) have become model organisms for exploring historical patterns and

ecological processes that mould diversity in this region. These snakes possess several characteristics that make them ideal for such studies: (a) Pitvipers have radiated extensively in the New World and currently occupy a wide range of environments and vegetation types (Campbell & Lamar, 2004); (b) as ectotherms, their moderate vagility and strong response to local environmental factors make them well suited for studies assessing the impact of environment and geography on lineage formation (Pyrone & Burbrink, 2009); (c) due to their impact on public health, a robust hypothesis of phylogenetic relationships exists for most groups of pitvipers (Alencar et al., 2016; Quijada-Mascareñas & Wüster, 2010; Wüster, Peppin, Pook, & Walker, 2008), and (d) distinct lineages are distributed widely across North and South America, or broadly co-distributed in specific regions, making them good choices for addressing diverse phylogeographic questions (Castoe et al., 2009; Wüster et al., 2005).

In this study, we use genomic data and demographic modelling to investigate recent diversification events in South America using a vertebrate group rarely explored in historical demographic studies: tropical Andean snakes. Specifically, we evaluate the origin of montane lineages of terciopelo pitvipers (*Bothrops asper* Garman, 1884 species complex) in the northern Andes. This group of venomous snakes mainly occurs in lowland rainforests from central Mexico all the way through Central America and into Venezuela and Peru in north-western South America (Campbell & Lamar, 2004) (Figure 1a). They are also among the most common pitvipers in Latin America and the leading cause of snakebite accidents in humans across their distribution (Otero-Patiño, 2009; Warrell, 2004). Recent analyses based on mitochondrial DNA and genomic-scale single-nucleotide polymorphism (SNP) datasets have shown that this species complex comprises 7–10 lineages, with the deepest divergence between (a) a Nuclear Central American (region comprising Chiapas in Mexico, Guatemala, Belize, El Salvador, Honduras and northern Nicaragua) (Cano, Schuster, & Morrone, 2018) clade and (b) an Isthmian Central American and South American clade estimated to have occurred around 3.2 Ma (Salazar-Valenzuela, 2016; Saldarriaga-Córdoba, Parkinson, Daza, Wüster, & Sasa, 2017) (Figure 1b).

Only three of these lineages are distributed in Nuclear Central America, where *B. asper* populations have been observed from sea level to 1,200–1,300 m, while most of the other genetic groups are present in South America where populations can be found as high as 2,640 m in the Andes (Campbell & Lamar, 2004). This mountain range had attained its current elevations by the time these snakes colonized the northern Andes and lineages arose in isolated dry inter-Andean valleys in the highlands of Colombia and Ecuador (Gutberlet & Harvey, 2004; Salazar-Valenzuela, 2016; Saldarriaga-Córdoba et

al., 2017; Werman, 2005). As such, an evaluation of the recent evolutionary dynamics involved in the lowland and highland differentiations of this ecologically dissimilar group should add to the limited knowledge of how recent evolutionary diversification in vertebrates has occurred in this highly diverse region.

We examine the distinctness and evolutionary history of three lineages of *B. asper*: one lowland Pacific lineage (PEC) and two highland lineages (HEC1 and HEC2), all present in southern Ecuador (Figure 1a). The degree to which these lineages are distinct has remained unknown and their particular evolutionary histories unresolved based on phylogenetic tree-based methods alone, due to high levels of shared polymorphism (Salazar-Valenzuela, 2016). Here, we use a population-level modelling approach and a genomic SNP dataset to generate results from genetic clustering analyses, evaluation of historical migration between genetic clusters, and demographic model selection. We use these results to evaluate if the origin of the montane lineages is explained by either of two historical processes that represent fundamental phylogeographic mechanisms: (a) differentiation by isolation within the highlands or (b) different dispersal events from the lowlands.

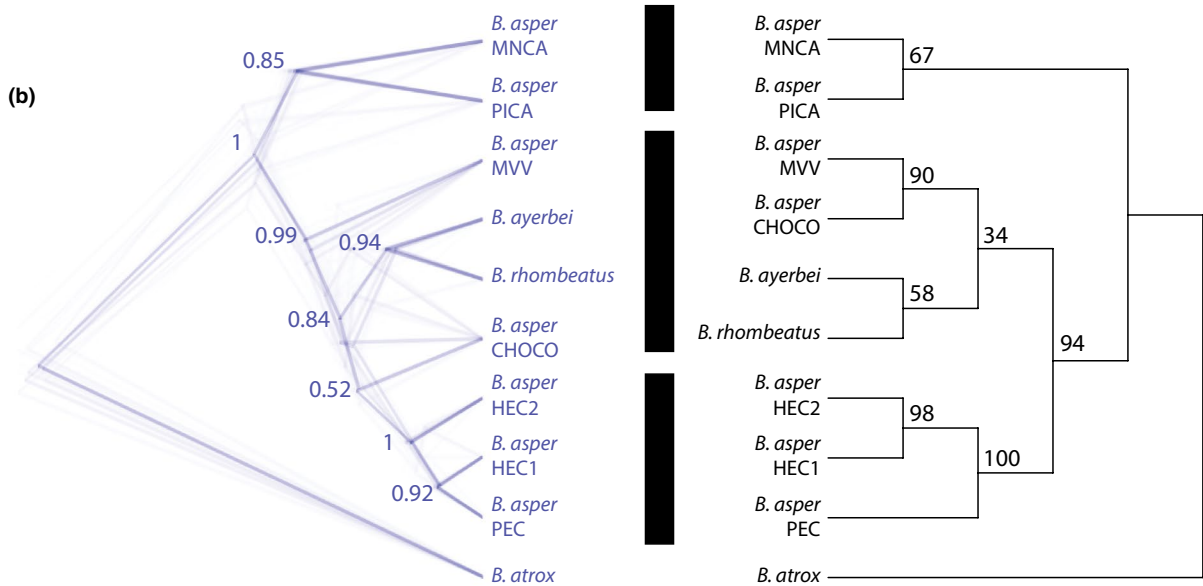
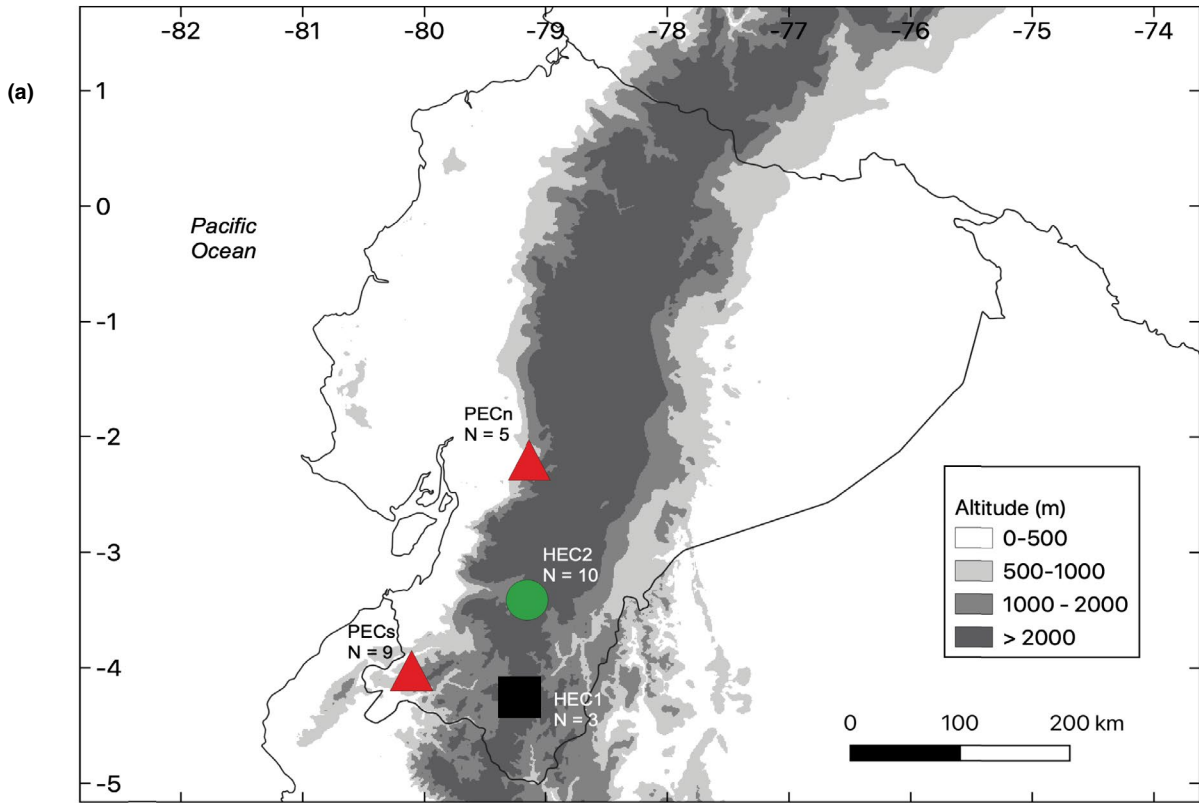
2 | MATERIALS AND METHODS

2.1 | Study system

The historical demography of lowland and montane lineages of terciopelo pitvipers (*B. asper*) in Ecuador was assessed. We targeted Ecuadorian populations because of the recently identified presence in the country of four different lineages of this venomous snake (Salazar-Valenzuela, 2016) and the high level of habitat heterogeneity in western Ecuador (Anderson & Martínez-Meyer, 2004; Lynch & Duellman, 1997; Ridgely & Greenfield, 2001), where *B. asper* is present from sea level up to 2,000 m (Campbell & Lamar, 2004; Cisneros-Heredia & Touzet, 2004; Valencia, Garzón-Tello, & Barragán-Paladines, 2016; pers. obs.). Consensus species trees obtained with SNAPP (Bryant, Bouckaert, Felsenstein, Rosenberg, & RoyChoudhury, 2012) and SVDQUARTETS (Chifman & Kubatko, 2014) by Salazar-Valenzuela (2016) show similar topologies; however, incongruence of relationships between lineages in the Pacific lowlands of Ecuador and those in the highlands suggests different mechanisms of origin of the HEC lineages and thus illustrates the need for additional analyses (Figure 1b).

Four lineages of *B. asper* have been identified in Ecuador (Salazar-Valenzuela, 2016). We obtained blood or tissue samples for three of

FIGURE 1 (a) Topographic map of Ecuador showing sampled populations of *Bothrops asper* complex pitvipers from the Pacific lowlands and the montane lineages described in the text: PEC (red triangles), HEC 1 (black square) and HEC 2 (green circle). Two populations (north and south) were used to represent the PEC lineage. (b) Consensus species trees obtained with SNAPP (left) and SVDQUARTETS (right) by Salazar-Valenzuela (2016) for pitvipers of the *B. asper* species complex across their distribution. Bars indicate the divergence of inferred lineages from Central America (above), those present mainly in Colombia (middle), and those mainly from Ecuador (below). Posterior probabilities and bootstrap support values are shown on nodes. Abbreviations: MNCA, Mexico and Nuclear Central America; PICA, Pacific Isthmian Central America; CHOCO, Darien Panama and Choco; MVV, Magdalena Valley Colombia and Venezuela; HEC1, Highlands Ecuador 1; HEC2, Highlands Ecuador 2; PEC, Pacific Ecuador. Picture of an adult *B. asper* from an Andean lineage of Ecuador by Jaime Culebras



these lineages that are geographically distributed in close proximity in the central and southern portions of the country. From these lineages, we sampled 27 *B. asper* individuals from four populations in western Ecuador ($N = 3\text{--}10$ per population): Cumandá, Chimborazo province (Pacific lowland lineage north, PECn); Alamor, Loja province (Pacific lowland lineage south, PECs); Vilcabamba, Loja province (highland Ecuador lineage 1, HEC1); and Río León, Azuay province (highland Ecuador lineage 2, HEC2) (Figure 1a, see Appendix S1). These localities are situated in the Pacific lowlands and western slope of the Andes (300–1,000 m), as well as in the highlands (1,500–2,000 m) of southern Ecuador. Despite our best efforts, we were only able to secure three samples from animals representing HEC1; terciopelo pitvipers in the locality where this lineage is present are rare (pers. obs.), and this area has been highly impacted by deforestation recently (Tapia-Armijos, Homeier, Espinosa, Leuschner, & de la Cruz, 2015). Individuals collected were considered to belong to a single population if they were found within a 5 km radius of a given locality. Based on Alencar et al., 2016; Fenwick Gutberlet Evans and Parkinson (2009), Parkinson, Campbell, and Chippindale (2002), Saldarriaga-Córdoba et al. (2017), and Wüster, Salomao, Quijada-Mascareñas, Thorpe, and BBBSP. (2002), we used three *B. atrox* samples from a single population in Amazonian Brazil as an appropriate outgroup.

2.2 | Genomic library preparation, sequencing and bioinformatic methods

We extracted genomic DNA from each sample using a Qiagen DNA blood and tissue kit (Qiagen, Valencia, CA). The concentration of DNA isolates was examined on a Qubit 2.0 fluorometer using a dsDNA BR assay kit (Life Technologies, Carlsbad, CA). We followed Sovic, Carstens, and Gibbs (2016) for the construction of double-digest RADseq libraries (DaCosta & Sorenson, 2014; Peterson, Weber, Kay, Fisher, & Hoekstra, 2012). Briefly, the method consists of (a) digestion of approximately 250 ng of DNA from each individual, using 15 units of EcoRI and SbfI restriction enzymes (New England Biolabs, Ipswich, MA), (b) ligation of Illumina unique barcoded adapters to each DNA sample, (c) size selection of fragments ranging between 300 and 450 bp by extraction from agarose gels, (d) qPCR quantification (KAPA Biosystems kit, Wilmington, MA) of gel extraction products in order to prevent high levels of missing data; a minimum threshold of 150,000 molecules was chosen and samples not attaining this number were discarded from the library and prepared again, (e) PCR amplification of the libraries using a Phusion polymerase kit (New England Biolabs), and (f) purification of the products with AmpPure beads (Beckman Coulter Inc., Pasadena, CA) and a second qPCR quantification in order to pool equimolar concentrations of each individual into a single library. Sequencing was performed in 50-bp-runs using 10%–20% of a lane of an Illumina HiSeq 2000 at the Genomics Shared Resource of the Ohio State University Comprehensive Cancer Center.

We used AFRTRAD 4.1 (Sovic, Fries, & Gibbs, 2015) to assemble and genotype the RADseq data, as well as to produce input

files for downstream analyses. We used default settings, except for the parameters described below. Only loci scored in at least 95% of the individuals were retained; this level was chosen to reduce the effects of allele drop out (Arnold, Corbett-Detig, Hartl, & Bomblies, 2013; Gautier et al., 2013). A maximum of four indels were allowed between reads to consider them alternative alleles from the same locus, and a minimum of five reads was required at a given locus to call a genotype. Finally, we used AFRTRAD to plot the location of each SNP along the reads to identify the position at which spurious SNPs form at the end of reads due to locus assembly methods; based on this, only SNPs occurring in the first 34 positions were retained after removal of barcodes and restriction sites (Sovic et al., 2015).

2.3 | Genetic structure analyses

We examined the structure of the populations using the Bayesian clustering algorithm implemented in the program STRUCTURE 2.3 (Pritchard, Stephens, & Donnelly, 2000), which clusters samples into populations by minimizing Hardy–Weinberg disequilibrium. We used an admixture model and iteratively conducted five independent runs of K values ranging from 1 to 8. A burn-in of 50,000 generations was used and each analysis sampled every 100 iterations for 500,000 generations. STRUCTURE HARVESTER 0.6.94 (Earl & vonHoldt, 2012) was used to implement the ΔK statistic of Evanno, Regnaut, and Goudet (2005) to identify an appropriate number of clusters. Results were summarized with CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) using the FullSearch algorithm and visualized with the program DISTRUCT 1.1 (Rosenberg, 2004). Additionally, we used the k -means clustering method available in 'adegenet' 1.4-2 (Jombart, 2008; Jombart & Ahmed, 2011). This program identifies the most appropriate clustering solutions based on Bayesian information criterion (BIC) scores from axes derived from a principal components analysis (PCA), and therefore does not rely on the Hardy–Weinberg assumptions that Structure considers. We evaluated K values ranging from 1 to 15 and performed a discriminate function analysis of PCAs (DAPC) based on the optimal clustering solution suggested by 'adegenet'. These functions are available in the 'ADE4' package and were conducted in R 3.1.3 (R Core Team, 2015). For analyses run on both programs we excluded the *B. atrox* outgroup samples.

2.4 | Population splits and mixtures

We used TREEMIX 1.12 (Pickrell & Pritchard, 2012) to infer the hypothesized evolutionary history of the sampled populations. The method implemented in this software models both population splits and admixture or migration events by assessing the relationship among sample populations with their ancestral population using genome-wide allele frequency data and a Gaussian approximation of genetic drift. The input file for TREEMIX consisted of the first SNP from each locus and was obtained by converting



the diploid genotype calls for each individual into population-level allele counts using *AFTRRAD*. We constructed maximum-likelihood trees allowing 0–10 migration events and performed 100 bootstrap replicates while sampling blocks of 10 contiguous SNPs in order to control for stochastic sampling error. We identified the information contribution of each migration model by calculating the *f* index, which represents the fraction of the variance in the sample covariance matrix (\hat{W}) accounted for by the model covariance matrix (*W*) (Barbato et al., 2017). Finally, we also used *TREEMIX* to perform the three-population and four-population tests to calculate the *f*₃ and *f*₄ statistics for all population combinations (Pickrell & Pritchard, 2012; Reich, Thangaraj, Patterson, Price, & Singh, 2009).

2.5 | Demographic modelling

Although *TREEMIX* is a useful method to infer recent population histories that consist of splits and instances of gene flow, it only models migration as discrete events and does not consider continuous gene flow (Pickrell & Pritchard, 2012; Sousa & Hey, 2013). Therefore, we used the coalescent-based modelling package *FASTSIMCOAL* 2.5.2 (Excoffier et al., 2013) to statistically compare the relative fit of more complex historical demographic models using our genomic data. Due to computational demands of the program and based on our clustering analyses (no genetic difference between PECn and PECs; see Results 3.1), we only used the southern population of PEC to represent this lineage under the assumption that both populations are genetically equivalent. For the demographic model selection tests, we specifically evaluated isolation and isolation with migration models that clustered highland lineages (Figure 2a,c) against models that

included a topology that clustered the Pacific lineage and the HEC1 lineage (Figure 2b,d). These represent fundamentally different mechanisms of phylogeographic differentiation (Avice, 2000). We used representative populations of these lineages with the goal of establishing if the origin of these montane groups could be attributed to diversification within the highlands of southern Ecuador (i.e. support for models A or C) or to independent dispersal events from Pacific lowland populations to montane habitats (i.e. support for models B or D).

The models differed in the topology: ((PEC, HEC1) HEC2) in Figure 2a versus ((HEC1, HEC2) PEC) in Figure 2b, and whether they allowed current migration between the different lineages (Figure 2c vs. d). We selected a subset of our original data when building the unfolded site frequency spectrum (SFS) and retained only loci scored in 100% of the samples in order to define the ancestral allele from *B. atrox* at all loci. Likelihoods for each model were calculated based on the SFS; we performed 100 independent runs of *FASTSIMCOAL* (200,000 simulations per run), and the run with the highest likelihood for each model was chosen to perform model selection with AIC. Maximum likelihood estimates for parameters were obtained from the optimal model/run and used to generate confidence intervals by a parametric bootstrapping approach in which 50 independent SFS were simulated for the optimal model and parameter estimates. These simulated datasets were treated as observed data, and parameter estimation was performed for each as above.

To convert estimates of time from generations to years, we used data from Sasa, Wasko, and Lamar (2009) and the method suggested by Graziotin, Monzel, Echeverrigaray, and Bonatto (2006) for *B. jararaca*. Generation time was estimated as 8 years and was obtained as the average of the youngest reported age at maturity (3 years) and the shortest reported life span (15 years) minus 1 year to compensate for survival probability until old ages.

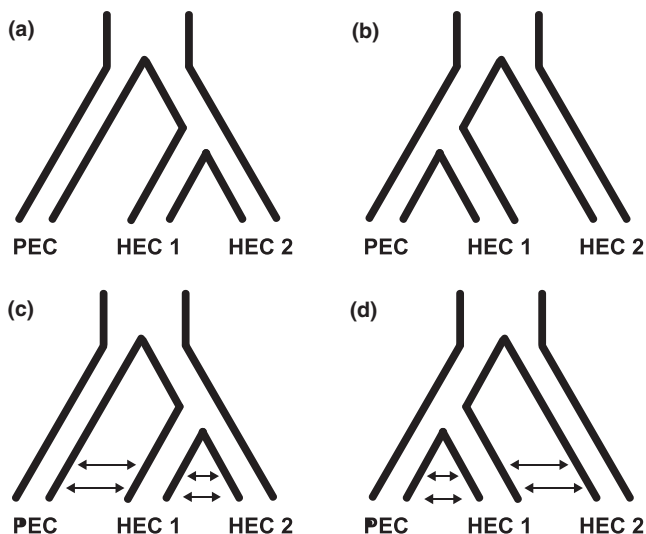


FIGURE 2 Isolation models (a,b) and isolation with migration models (c,d) tested in *FASTSIMCOAL* for sampled populations of *Bothrops asper* complex pitvipers in Ecuador. Abbreviations: HEC1, Highlands Ecuador 1; HEC2, Highlands Ecuador 2; PEC, Pacific Ecuador

2.6 | Data archiving

Data sets and historical demographic models used in analyses are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.pf8011h>.

3 | RESULTS

3.1 | Genotyping of RADseq data

We recovered a mean of 717,094 sequence reads for individuals included in our RADseq dataset (range: 85,395–1,964,552) after quality filtering. The mean read depth per locus was 86.2 reads while the median read depth was 54 reads. A total of 15,393 non-paralogous loci were identified: 12,442 were monomorphic and the remaining 2,951 contained at least one polymorphic site. Of the 2,951 polymorphic loci, 1,241 were scored in at least 95% of the samples and were used for subsequent analyses.

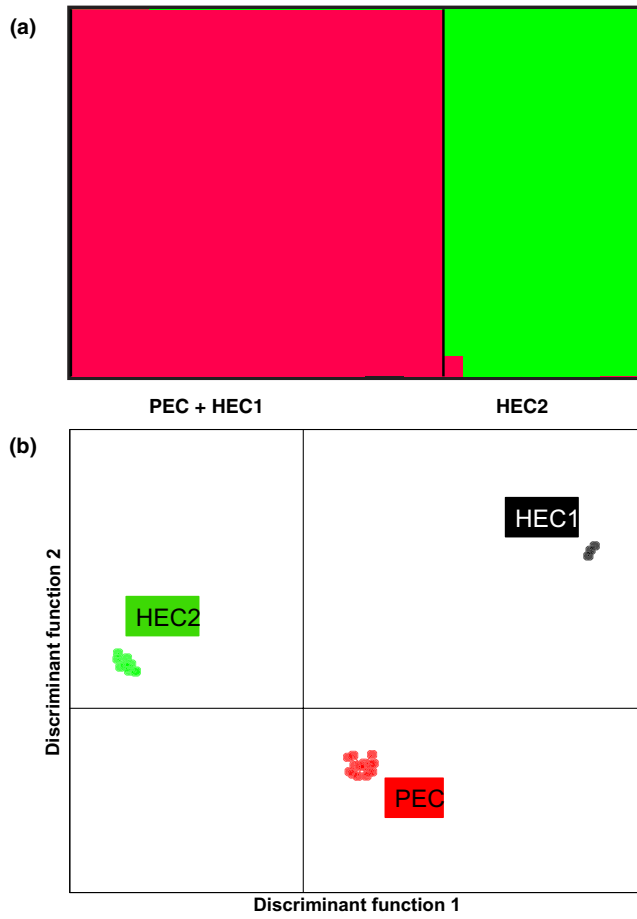


FIGURE 3 (a) STRUCTURE plot ($K = 2$) and (b) results from the DAPC clustering analysis in 'adegenet' ($K = 3$) generated from 1,241 polymorphic loci for sampled populations of *Bothrops asper* pitvipers in Ecuador. Abbreviations: DAPC, discriminate function analysis of principal components analysis; HEC1, Highlands Ecuador 1; HEC2, Highlands Ecuador 2; PEC, Pacific Ecuador

3.2 | Genetic clustering

Genetic clustering in STRUCTURE suggested an optimal K of two with one group consisting of both PECn and PECs populations and HEC1 lineage, and another group represented by HEC2. These groups form distinct clusters with almost no evidence of admixture between them (Figure 3a). In contrast, BIC values for the DAPC approach in 'adegenet' suggested three independent groups; however, we note that both PEC populations and HEC1 samples clustered closely in the multivariate space relative to HEC2 (Figure 3b; see Appendix S2).

3.3 | Population splits and mixtures

Similar to the clustering solutions suggested above, ML population trees from TREEMIX inferred a topology that groups the PEC (PECn and PECs populations) and HEC1 lineages together (Figure 4a). The f_3 and f_4 statistics suggested non-significant levels of admixture

between the sampled populations (i.e. positive values for f_3 statistics and non-significant non-zero values for f_4 statistics). Accordingly, of all the migration models tested ($m = 0-10$), most of the variance (98.1%) in the dataset was explained by $m = 0$ and when allowed one migration event it only increased to 99.9% (see Appendix S3). The unique migration event that was identified occurred from the out-group represented by *B. atrox* samples from Brazil to the PEC lineage suggesting historical migration between populations of the *B. atrox* complex located in the Amazon Basin and Pacific populations of *B. asper* (Figure 4b).

3.4 | Historical demography

Our modelling approach suggested strong support for a ((PEC, HEC1) HEC2) relationship (model 2B) as this model received 98.1% of the relative weight based on AIC (Table 1). These results indicate that there is a clear differentiation of one of the highland lineages (HEC2), and that the other highland group (HEC1) is sister to the PEC lineage. There is no evidence that significant levels of ongoing migration exist between any of these lineages.

Maximum likelihood parameter estimates and confidence intervals under the best-supported model (Table 2) indicate that effective population sizes for the highland lineage populations (HEC1: 11,915; HEC2: 19,138) are smaller than the PEC population (85,918) consistent with a founding event. Divergence times for the Pacific and HEC1 lineages occurred approximately 60,976 years before present (CI 33,376–121,800 ybp), while estimated times for the divergence from the HEC2 lineage occurred approximately 201,656 ybp (CI 116,008–298,000 ybp).

4 | DISCUSSION

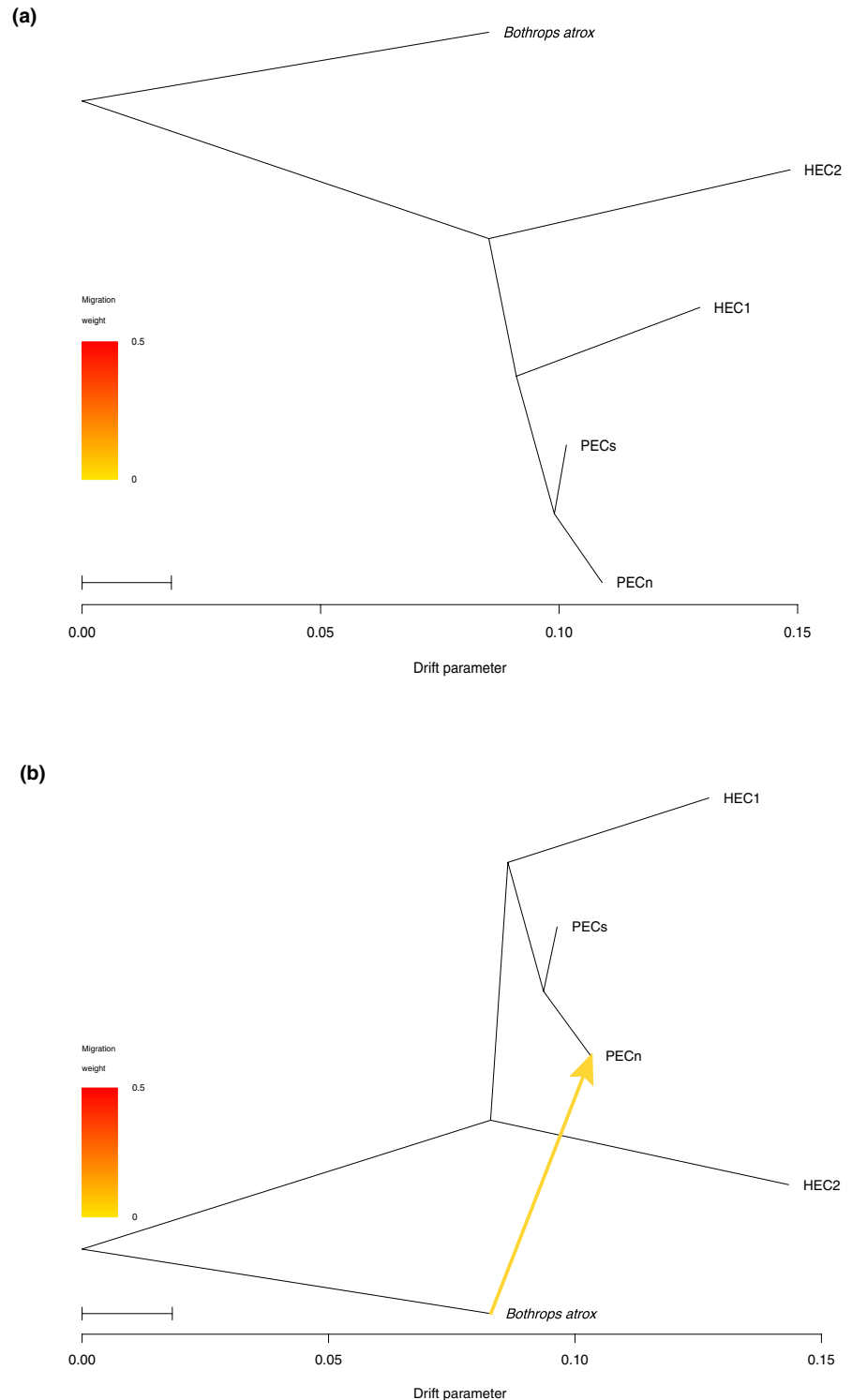
The main results of our study are that (a) the discrepancy of evolutionary relationships obtained with tree-like methods is resolved through the use of historical modelling approaches and (b) there is strong support for the presence and independent origin of montane lineages based on clustering solutions and topologies inferred by ML trees and modelling approaches. This evidence supports the importance of dispersal events across altitudinal habitat gradients and subsequent ecological differentiation for explaining diversification patterns in terciopelo pitvipers inhabiting the Tropical Andes. We discuss additional evolutionary, taxonomic and biomedical implications of our findings below.

4.1 | Evolutionary relationships among Ecuadorian lineages of *B. asper*

In addition to potentially being affected by incomplete lineage sorting, coalescent-based inferences in systems characterized by rapid speciation events could also be affected by gene flow because methods for lineage identification based on coalescent methods assume that it has ceased upon species formation (Burbrink & Guiher, 2015).



FIGURE 4 (a) ML population tree inferred with TREEMIX for sampled populations of *Bothrops asper* pitvipers in Ecuador and a population of *B. atrox* from Brazil used as outgroup; no migration events allowed and (b) ML population tree and one migration event allowed. The graphs depict splits among different populations and the weight associated with migration events. Abbreviations: HEC1, Highlands Ecuador 1; HEC2, Highlands Ecuador 2; PEC, Pacific Ecuador



Thus, gene flow could reduce the accuracy of species tree inferences, resulting in underestimates of lineage divergence times and/or inference of an erroneous topology (Gruenstaedl, Reid, Wheeler, & Carstens, 2015). Newly acquired population genomic datasets can be used to disentangle the conflicting role of gene flow during the divergence process (Sousa & Hey, 2013), and we have employed them here to explore patterns of diversification between lowland

and highland lineages of terciopelo pitvipers present in the Andes of southern Ecuador.

Contrasting topologies between these lineages were inferred in a previous study of this widespread species complex (Salazar-Valenzuela, 2016). A closer relationship between highland (HEC1 and HEC2) lineages was recovered with analyses of mtDNA data and some approaches used to analyse RADSeq data (estimation of

TABLE 1 Akaike information criterion (AIC) model selection results for FASTSIMCOAL analyses performed using Ecuadorian lineages of *Bothrops asper* pitvipers

Model	No. parameters	Ln likelihood	AIC	Akaike weight
Isolation highland lineages (Figure 2a)	10	-3,862.055	17,805.4205	0.00000
Isolation pacific + HEC1 versus HEC2 (Figure 2b)	10	-3,854.965	17,772.7699	0.98101
Isolation + migration highland lineages (Figure 2c)	14	-3,856.737	17,788.9302	0.00030
Isolation + migration pacific + HEC1 versus HEC2 (Figure 2d)	14	-3,854.948	17,780.6916	0.01869

Note: Models are those shown in Figure 2.

Abbreviations: HEC1, Highlands Ecuador 1; HEC2, Highlands Ecuador 2.

TABLE 2 Maximum likelihood estimates for demographic parameters estimated in the FASTSIMCOAL analysis for sampled populations of *Bothrops asper* complex pitvipers in Ecuador

Parameter	Point estimate	Confidence interval
Effective population sizes		
HEC1 (current)	11,915	6,220–25,785
HEC2 (current)	19,138	11,454–28,236
PEC (current)	85,918	53,381–121,541
<i>B. atrox</i> (current)	194,993	143,273–225,409
PEC + HEC1 (ancestral)	102,705	30,850–120,622
<i>B. asper</i> (ancestral)	83,367	66,314–112,991
<i>B. asper</i> + <i>B. atrox</i> (ancestral)	223,371	182,374–253,718
Divergence times		
PEC – HEC1	60,976 ybp	33,376–121,800 ybp
PEC, HEC1 – HEC2	201,656 ybp	116,008–298,000 ybp
<i>B. asper</i> – <i>B. atrox</i>	929,968 ybp	672,808–1,160,736 ybp

Note: Results correspond to the best-supported model: ((PEC, HEC1) HEC2).

Abbreviations: HEC1, Highlands Ecuador 1; HEC2, Highlands Ecuador 2; PEC, Pacific Ecuador.

a maximum likelihood tree based on the concatenated dataset and species tree analyses with SVDQUARTETS, Chifman & Kubatko, 2014). In contrast, species tree analyses of the same data with SNAPP (Bryant et al., 2012) inferred a topology that clustered the Pacific Ecuadorian lineage (PEC) with one of the highland (HEC1) lineages. All the methods we used here to analyse our population genomic dataset strongly support the latter hypothesis ((PEC, HEC1) HEC2). A closer relationship between the two highland lineages can therefore be accounted for by the fact that incompletely sorted ancestral polymorphisms were not taken into account with single-locus analyses or the evaluation of a concatenated multilocus dataset.

Burbrink and Guiher (2015) recently used multilocus data and alternative population assignments of admixed individuals to demonstrate the impact of gene flow on the identification and delimitation of lineages of North American *Agkistrodon* pitvipers. The approach implemented in our study goes one step further by using TREEMIX

and model-based analyses in FASTSIMCOAL for our population genomic data in order to disentangle the role of gene flow during the origin of montane lineages of terciopelo pitvipers in Ecuador.

4.2 | Evolutionary dynamics of lowland and highland lineages of *B. asper* in Ecuador

Vicariance events have been emphasized as the primary mechanism explaining the speciation of Andean colonists from lowland ancestors (Guarnizo, Amézquita, & Bermingham, 2009; Winger et al., 2015). Support for this allopatric model has been found in several studies that have established that sister lineages in different groups of Andean vertebrates occupy areas of similar altitude (Brumfield & Edwards, 2007; Patton & Smith, 1992; Roberts et al., 2006). Alternatively, ecological gradients, availability of new habitats and absence of competition are mechanisms of rapid lineage diversification proposed to occur on recently formed mountain ranges such as the northern Andes (Brumfield & Edwards, 2007; Caro, Caycedo-Rosales, Bowie, Slabbekoorn, & Cadena, 2013; Chapman, 1926; Endler, 1977; Graham, Ron, Santos, Schneider, & Moritz, 2004; Hughes & Eastwood, 2006; Kattan, Tello, Giraldo, & Cadena, 2016). Parapatric speciation along a mountain vertical axis should follow the differential adaptations associated with these ecological gradients (Guarnizo et al., 2009). Our results for Ecuadorian lineages of *B. asper* indirectly support this alternate model of diversification since we rejected the hypothesis of highland lineages being each other's closest relatives. Divergent ecological regimes influencing genetic differentiation patterns have been proposed to be more significant in taxa with more limited ecological niches (e.g. anurans, insects) (Brumfield & Edwards, 2007). Although few studies have been conducted with snakes, these organisms could also be regarded as heavily influenced by ecotones given their moderate vagility and strong response to local environmental factors (Pyron & Burbrink, 2009).

Our findings indicate that no genetic structure exists between the two PEC populations of *B. asper* analysed here. This is in agreement with a more extensive analysis of genetic variation for different Pacific Ecuador populations south of the Chocó region, which were always clustered together (Salazar-Valenzuela, 2016). Our results also indicate that *B. asper* populations from the Pacific lowlands of



southern Ecuador independently colonized highland habitats and then underwent differentiation in isolation. Although the limited information on movement patterns for *B. asper* suggests that these snakes are highly sedentary in their daily activity, there are also reports of individuals capable of long-distance movements (e.g. one specimen moved more than 1,200 m in two consecutive nights) and snakes usually found near water bodies (i.e. swamps and river banks) (Sasa et al., 2009). Therefore, colonization of Andean habitats along river valleys in southwestern Ecuador seems plausible and in support of our results.

The HEC1 population analysed here is located in the Inter-Andean valley of Vilcabamba, Loja province. Isolation of this lineage agrees with previous reports of morphological differences for these populations relative to other *B. asper* populations in Ecuador (Campbell & Lamar, 2004), and with present-day distribution patterns that show no published records of collection localities between the Vilcabamba valley and populations close to the Pacific coast along the Catamayo river basin (Bustamante & Arteaga, 2013; Cisneros-Heredia & Touzet, 2004; Rodríguez-Guerra, 2011; Valencia et al., 2016). The distinctiveness of the HEC2 lineage is more intriguing as there are at least some intervening populations present in the foothills of the Andes (DSV, pers. obs.). Nevertheless, the Inter-Andean valley where HEC2 populations are located (Jubones river valley, Azuay and Loja provinces) seems to be effectively isolated from surrounding areas, as it is part of a phylogeographic barrier that has been recognized as important for other montane organisms, like Andean plants and birds (Weigend, 2002), and several other species of reptiles (e.g. *Holcosus orcesi*, *Phyllodactylus leoni*, *Stenocercus rhodomelas* and *Tantilla insulamontana*) are endemic to this valley (Torres-Carvajal, Pazmiño-Otamendi, & Salazar-Valenzuela, 2019). This barrier, known as the Huancabamba Depression, could also explain our results of historical migration between snakes of the *B. atrox* complex and Pacific populations of *B. asper*, as it has been suggested that this region of low altitude in the Andes could have facilitated the exchange of organisms located on its eastern and western slopes (Duellman, 1979; Trénel, Hansen, Normand, & Borchsenius, 2008). Overall, our results support the recognition of the HEC2 lineage as phylogenetically significant. Moreover, based on the estimated time of isolation associated with the HEC2 population and observed differentiation in morphological traits (Salazar-Valenzuela, 2016), its recognition as a distinct species is possibly warranted. A detailed morphological description of these snakes and their taxonomic status will be provided in a forthcoming publication (E. N. Smith, The University of Texas at Arlington, personal communication).

4.3 | Recent events of pitviper colonization and diversification in South America

The recent and extensive diversification of *B. asper* lineages in South America during the Pleistocene contrasts with the findings of previous phylogeographic studies of the regional herpetofauna which emphasized older diversification events during the Pliocene and/or Miocene (Turchetto-Zolet et al., 2013). Although divergence

in lowland *B. asper* lineages in this subcontinent could still be related to large-scale landscape changes (e.g. marine transgressions, Quaternary climate changes) that fragmented previously continuous distributions (Rull, 2011), our results highlight the importance of montane habitats for diversification in the group.

Besides the *B. asper* complex, only a few additional groups of pitvipers were involved in the great American biotic interchange of taxa between North and South America (Bacon et al., 2015; Werman, 2005). Most of them dispersed from Central America and colonized lowland habitats on the eastern and western sides of the Andes. For example, the Neotropical rattlesnake (*Crotalus durissus* complex) dispersed from Central America during the middle Pleistocene and subsequently diversified in open and dry habitats in South America east of the Andes (Wüster et al., 2005). In contrast, the ancestors of the *B. asper* species complex originated in north-western South America approximately 3.3 Ma and then independently colonized Central America and dispersed south through the narrow strip of lowlands west of the Andes (Salazar-Valenzuela, 2016; Saldarriaga-Córdoba et al., 2017). The present-day distribution of the group is mainly restricted to lowland tropical rainforests in these areas (Campbell & Lamar, 2004), but as with other vertebrates (Miller, Bermingham, Klicka, Escalante, & Winker, 2010) its southern limit occurs in north-western Peru where highly arid conditions during the last 3 Ma may have restricted their dispersal further south (Hartley & Chong, 2002).

Based on the overall assessment of tree methods (Salazar-Valenzuela, 2016) and demographic modelling presented here, our results suggest that inter-Andean valleys have been a previously unrecognized yet important driver of montane lineage divergence in terciopelo pitvipers. Interestingly, the environmental conditions in these valleys of the highlands of Ecuador and Colombia are similar. Both are populated by seasonally dry forests, which in the last 15 years have been recognized as an important and threatened biome rich in endemic species (Miles et al., 2006; Pennington et al., 2010; Pennington, Prado, & Pendry, 2000; Werneck, Costa, Colli, Prado, & Sites, 2011). There is evidence that these types of forests have been present in the area since 10–15 Ma (Särkinen, Pennington, Lavin, Simon, & Hughes, 2012), and therefore may have provided ecological opportunities for terciopelo pitviper populations to colonize and diverge in highland habitats. Our findings, coupled with recent descriptions of endemic snakes and lizards from Ecuador and Peru (see Koch, Venegas, Santa Cruz, & Böhme, 2018 for a recent review; Torres-Carvajal, Carvajal-Campos, Barnes, Nicholls, & Pozo-Andrade, 2013), suggest that dry montane habitats may play a previously unappreciated role as drivers of diversification in Andean reptiles. This finding broadens our understanding of the mechanisms involved in diversification events identified in other pitvipers from the Neotropics. Castoe et al. (2009) established coincident Miocene and Pliocene divergences for highland lineages of pitvipers from Middle America. However, diversification in those lineages seems to be associated with vicariant events rather than with the more recent availability

of dry montane habitats as is the case with terciopelo pitvipers colonizing the Andes of South America.

4.4 | Neotropical diversification implications and future directions

Dispersal is a biotic process recently confirmed to be an important driver of the rich and heterogeneous patterns of biodiversity present in the Neotropics. Antonelli et al. (2018), for example, found extensive rates of biotic interchange across different taxonomic groups and between major Neotropical regions – Amazonia being the primary source of diversity. Similarly, Hazzi et al. (2018) found that patterns of spatial and temporal evolution in birds from the Tropical Andes were explained by a combination of vicariance driven by mountain uplift and downslope dispersal events using river valley habitats. We complement these findings by identifying a fine-scale case occurring in an ecologically distinct group of animals (i.e. pitviper snakes), in which river valleys acted as an entrance for the upward colonization of montane dry habitats and subsequent ecological diversification. A similar case of lineage diversification through dispersal across an ecological gradient of major habitat types in the Amazon river has recently been identified in a congener, *B. atrox* (Gibbs et al., 2018).

Historical demographic approaches like those used in this study could be applied to analyse divergence between other lineages identified in the *B. asper* complex. Diversification patterns of terciopelo pitvipers present in the inter-Andean valleys of southern Colombia seem to be similar to those analysed here and could provide an independent assessment of the importance of montane habitats for the divergence processes that have acted in this group.


Finally, since terciopelo snakes are the leading cause of snakebite accidents across their distribution (Otero-Patiño, 2009; Warrell, 2004), our study provides a much-needed historical framework for this system that has practical implications. Snakebite envenomation is recognized by the World Health Organization as a priority neglected tropical disease, and terciopelo pitvipers are model organisms in toxinology (Chippaux, 2017; Gutiérrez, 2009). In this sense, our findings of multiple recently evolved and previously unrecognized lineages could help (a) inform future comparative toxinological studies, (b) establish a better understanding of the appropriate selection and mixture of venoms for immunization in order to produce more effective antivenoms, and (c) better understand the role of geographic variation in venom in complex clinical manifestations of snakebite accidents produced by these organisms in humans and livestock. It is indeed this holistic treatment of a phenomenon that Alexander von Humboldt understood as key to the advancement of the natural sciences.

ACKNOWLEDGEMENTS

We thank W. Wüster, E.N. Smith, M. Terán and D. Amazonas for generously providing key samples. We are grateful to M. Sovic, J. Díaz and P. Santacruz-Ortega for advice and help in the laboratory. For advice and comments on the manuscript, we thank

M. Sovic, A. Fries, R. Denton, M. Holding, S. Smiley-Walters, J. Freudenstein, B. Carstens, T. Hetherington, P. Fuerst, G. Silva, and two anonymous reviewers. We also thank E. Arbeláez, A. Loaiza-Lange, D. Núñez, P. Santacruz-Ortega, S. Harris, A. Freire-Lascano, F. Ayala and R. Molina for assistance in the field. We thank J.A. Campbell and W.W. Lamar for valuable discussion and for bringing the Río León population of pitvipers to our attention. Specimens were collected under collection permit 008-09 IC-FAU-DNB/MA and were deposited at Museo de Zoología (QCAZ), Pontificia Universidad Católica del Ecuador. Funding was provided by a Fulbright-Secretaría de Educación Superior, Ciencia y Tecnología del Ecuador (Senescyt) grant, a Tinker Foundation grant and an Alumni Grant from The Ohio State University Graduate School to DSV; a German Research Foundation grant to UK (Deutsche Forschungsgemeinschaft DFG KU2328/5-1); and a Senescyt grant under the 'Arca de Noé' Initiative (PIs: S.R. Ron and O. Torres-Carvajal) to OTC.

ORCID

David Salazar-Valenzuela  <https://orcid.org/0000-0002-3874-7690>

Ulrich Kuch  <https://orcid.org/0000-0002-5091-7752>

Omar Torres-Carvajal  <https://orcid.org/0000-0003-0041-9250>

Jorge H. Valencia  <https://orcid.org/0000-0002-6492-7388>

H. Lisle Gibbs  <https://orcid.org/0000-0001-7461-3393>

REFERENCES

- Alencar, L. R. V., Quental, T. B., Graziotin, F. G., Alfaro, M. L., Martins, M., Venzon, M., & Zaher, H. (2016). Diversification in vipers: Phylogenetic relationships, time of divergence and shifts in speciation rates. *Molecular Phylogenetics and Evolution*, 105, 50–62. <https://doi.org/10.1016/j.ympev.2016.07.029>
- Anderson, R. P., & Martínez-Meyer, E. (2004). Modeling species' geographic distributions for preliminary conservation assessments: An implementation with the spiny pocket mice (*Heteromys*) of Ecuador. *Biological Conservation*, 116, 167–179. [https://doi.org/10.1016/S0006-3207\(03\)00187-3](https://doi.org/10.1016/S0006-3207(03)00187-3)
- Antonelli, A., Quijada-Mascareñas, A., Crawford, A. J., Bates, J. M., Velasco, P. M., & Wüster, W. (2010). Molecular studies and paleogeography of Amazonian tetrapods and their relation to geological and climatic models. In C. Hoorn, & F. P. Wesselingh (Eds.), *Amazonia: Landscape and species evolution. A look into the past* (pp. 386–404). Chichester, UK: Wiley-Blackwell. <https://doi.org/10.1002/978144306408.ch24>
- Antonelli, A., Zizka, A., Carvalho, F. A., Scharn, R., Bacon, C. D., Silvestro, D., & Condamine, F. L. (2018). Amazonia is the primary source of Neotropical biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, 115, 6034–6039. <https://doi.org/10.1073/pnas.1713819115>
- Arnold, B., Corbett-Detig, R. B., Hartl, D., & Bomblies, K. (2013). RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling. *Molecular Ecology*, 22, 3179–3190. <https://doi.org/10.1111/mec.12276>
- Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Cambridge, MA: Harvard University Press.



- Bacon, C. D., Silvestro, D., Jaramillo, C., Smith, B. T., Chakrabarty, P., & Antonelli, A. (2015). Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 6110–6115. <https://doi.org/10.1073/pnas.1423853112>
- Bagley, J. C., & Johnson, B. J. (2014). Phylogeography and biogeography of the lower Central American Neotropics: Diversification between two continents and between two seas. *Biological Reviews*, *89*, 767–790. <https://doi.org/10.1111/brv.12076>
- Barbato, M., Hailer, F., Orozco-terWengel, P., Kijas, J., Mereu, P., Cabras, P., ... Bruford, M. W. (2017). Genomic signatures of adaptive introgression from European mouflon into domestic sheep. *Scientific Reports*, *7*, 7623. <https://doi.org/10.1038/s41598-017-07382-7>
- Beaumont, M. A., Nielsen, R., Robert, C., Hey, J., Gaggiotti, O., Knowles, L., ... Excoffier, L. (2010). In defence of model-based inference in phylogeography. *Molecular Ecology*, *19*, 436–446. <https://doi.org/10.1111/j.1365-294X.2009.04515.x>
- Brumfield, R. T., & Edwards, S. V. (2007). Evolution into and out of the Andes: A Bayesian analysis of historical diversification in *Thamnophilus antshrikes*. *Evolution*, *61*, 346–367. <https://doi.org/10.1111/j.1558-5646.2007.00039.x>
- Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N. A., & RoyChoudhury, A. (2012). Inferring species trees directly from bi-allelic genetic markers: Bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution*, *29*, 1917–1932. <https://doi.org/10.1093/molbev/mss086>
- Burbrink, F. T., & Guiher, T. J. (2015). Considering gene flow when using coalescent methods to delimit lineages of North American pitvipers of the genus *Agkistrodon*. *Zoological Journal of the Linnean Society*, *173*, 505–526. <https://doi.org/10.1111/zoj.12211>
- Bustamante, L., & Arteaga, A. (2013). *Bothrops asper*. In A. Arteaga, L. Bustamante, & J. M. Guayasamin (Eds.), *The amphibians and reptiles of Mindo* (pp. 193–195). Quito, Ecuador: Universidad Tecnológica Indoamérica.
- Campbell, J. A., & Lamar, W. W. (2004). *The venomous reptiles of the Western Hemisphere*. Ithaca, NY: Cornell University Press.
- Cano, E. B., Schuster, J. C., & Morrone, J. (2018). Phylogenetics of *Ogyges* Kaup and the zoogeography of Nuclear Central America (Coleoptera, Passalidae). *ZooKeys*, *737*, 81–111. <https://doi.org/10.3897/zookeys.737.20741>
- Caro, L. M., Caycedo-Rosales, P. C., Bowie, R. C., Slabbekoorn, H., & Cadena, C. D. (2013). Ecological speciation along an elevational gradient in a tropical passerine bird? *Journal of Evolutionary Biology*, *26*, 357–374. <https://doi.org/10.1111/jeb.12055>
- Castoe, T. A., Daza, J. M., Smith, E. N., Sasa, M. M., Kuch, U., Campbell, J. A., ... Parkinson, C. L. (2009). Comparative phylogeography of pitvipers suggests a consensus of ancient Middle American highland biogeography. *Journal of Biogeography*, *36*, 88–103. <https://doi.org/10.1111/j.1365-2699.2008.01991.x>
- Chapman, F. M. (1926). The distribution of bird-life in Ecuador. *Bulletin of the American Museum of Natural History*, *55*, 1–784.
- Chifman, J., & Kubatko, L. (2014). Quartet inference from SNP data under the coalescent model. *Bioinformatics*, *30*, 3317–3324. <https://doi.org/10.1093/bioinformatics/btu530>
- Chippaux, J. P. (2017). Snakebite envenomation turns again into a neglected tropical disease! *Journal of Venomous Animals and Toxins including Tropical Diseases*, *23*, 38. <https://doi.org/10.1186/s40409-017-0127-6>
- Cisneros-Heredia, D. F., & Touzet, J. M. (2004). Distribution and conservation status of *Bothrops asper* (Garman, 1884) in Ecuador. *Herpetozoa*, *17*, 135–141.
- DaCosta, J. M., & Sorenson, M. D. (2014). Amplification biases and consistent recovery of loci in a double-digest RAD-seq protocol. *PLoS ONE*, *9*, e106713. <https://doi.org/10.1371/journal.pone.0106713>
- Duellman, W. E. (1979). The herpetofauna of the Andes: Patterns of distribution, origin, differentiation, and present communities. In W. E. Duellman (Ed.), *The South American Herpetofauna: Its origin, evolution, and dispersal* (pp. 371–459). Lawrence, KS: The University of Kansas.
- Earl, D., & vonHoldt, B. (2012). Structure Harvester: A website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Endler, J. A. (1977). *Geographic variation, speciation, and clines*. Princeton, NJ: Princeton University Press.
- Esquerré, D., Brennan, I. G., Catullo, R. A., Torres-Pérez, F., & Keogh, J. S. (2019). How mountains shape biodiversity: The role of the Andes in biogeography, diversification, and reproductive biology in South America's most species-rich lizard radiation (Squamata: Liolaemidae). *Evolution*, *73*, 214–230. <https://doi.org/10.1111/evo.13657>
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software Structure: A simulation study. *Molecular Ecology*, *14*, 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Excoffier, L., Dupanloup, I., Huerta-Sanchez, E., Sousa, V. C., & Foll, M. (2013). Robust demographic inference from genomic and SNP data. *PLoS Genetics*, *9*, e1003905. <https://doi.org/10.1371/journal.pgen.1003905>
- Fenwick, A. M., Gutberlet, R. L. Jr, Evans, J. A., & Parkinson, C. L. (2009). Morphological and molecular evidence for phylogeny and classification of South American pitvipers, genera *Bothrops*, *Bothriopsis*, and *Bothrocophias* (Serpentes: Viperidae). *Zoological Journal of the Linnean Society*, *156*, 617–640. <https://doi.org/10.1111/j.1096-3642.2008.00495.x>
- Garrick, R. C., Bonatelli, I. A. S., Hyseni, C., Morales, A., Pelletier, T. A., Perez, M. F., ... Carstens, B. C. (2015). The evolution of phylogeographic data sets. *Molecular Ecology*, *24*, 1164–1171. <https://doi.org/10.1111/mec.13108>
- Gautier, M., Gharbi, K., Cezard, T., Foucaud, J., Kerdelhué, C., Pudlo, P., ... Estoup, A. (2013). The effect of RAD allele dropout on the estimation of genetic variation within and between populations. *Molecular Ecology*, *22*, 3165–3178. <https://doi.org/10.1111/mec.12089>
- Gibbs, H. L., Sovic, M., Amazonas, D., Chalkidis, H., Salazar-Valenzuela, D., & Moura-da-Silva, A. M. (2018). Recent lineage diversification in a venomous snake through dispersal across the Amazon river. *Biological Journal of the Linnean Society*, *123*, 651–665. <https://doi.org/10.1093/biolinnean/blx158>
- Graham, C. H., Ron, S. R., Santos, J. C., Schneider, C. J., & Moritz, C. (2004). Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution*, *58*, 1781–1793. <https://doi.org/10.1111/j.0014-3820.2004.tb00461.x>
- Grazziotin, F. G., Monzel, M., Echeverrigaray, S., & Bonatto, S. L. (2006). Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): Past fragmentation and island colonization in the Brazilian Atlantic Forest. *Molecular Ecology*, *15*, 3969–3982. <https://doi.org/10.1111/j.1365-294X.2006.03057.x>
- Gruenstaedl, M., Reid, N. M., Wheeler, G. L., & Carstens, B. C. (2015). Posterior predictive checks of coalescent models: P2C2M, an R package. *Molecular Ecology Resources*, *16*, 193–205. <https://doi.org/10.1111/1755-0998.12435>
- Guarnizo, C. E., Amézquita, A., & Bermingham, E. (2009). The relative roles of vicariance versus elevational gradients in the genetic differentiation of the high Andean tree frog, *Dendropsophus labialis*. *Molecular Phylogenetics and Evolution*, *50*, 84–92. <https://doi.org/10.1016/j.ympev.2008.10.005>
- Gutberlet, R. L. Jr, & Harvey, M. B. (2004). The evolution of New World venomous snakes. In J. A. Campbell, & W. W. Lamar (Eds.), *The venomous reptiles of the Western Hemisphere* (pp. 634–682). Ithaca, NY: Cornell University Press.

- Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, 5, e1000695. <https://doi.org/10.1371/journal.pgen.1000695>
- Gutiérrez, J. M. (2009). *Bothrops asper*: Beauty and peril in the Neotropics. *Toxicon*, 54, 901–903. <https://doi.org/10.1016/j.toxicon.2009.06.012>
- Hartley, A. J., & Chong, G. (2002). A late Pliocene age for the Atacama Desert: Implications for the desertification of western South America. *Geology*, 30, 43–46. [https://doi.org/10.1130/0091-7613\(2002\)030<0043:L-PAFTA>2.0.CO;2](https://doi.org/10.1130/0091-7613(2002)030<0043:L-PAFTA>2.0.CO;2)
- Harvey, M. G., & Brumfield, R. T. (2015). Genomic variation in a widespread Neotropical bird (*Xenops minutus*) reveals divergence, population expansion, and gene flow. *Molecular Phylogenetics and Evolution*, 83, 305–316. <https://doi.org/10.1016/j.ympev.2014.10.023>
- Hazzi, N. A., Moreno, J. S., Ortiz-Movliav, C., & Palacio, R. D. (2018). Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. *Proceedings of the National Academy of Sciences of the United States of America*, 115, 7985–7990. <https://doi.org/10.1073/pnas.1803908115>
- Hickerson, M. J., Carstens, B. C., Cavender-Bares, J., Crandall, K. A., Graham, C. H., Johnson, J. B., ... Yoder, A. D. (2010). Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution*, 54, 291–301. <https://doi.org/10.1016/j.ympev.2009.09.016>
- Hoorn, C., Wesselingh, F. P., ter Steege, H., Bermudez, M. A., Mora, A., Sevink, J., ... Antonelli, A. (2010). Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, 330, 927–931. <https://doi.org/10.1126/science.1194585>
- Hughes, C., & Eastwood, R. (2006). Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 10334–10339. <https://doi.org/10.1073/pnas.0601928103>
- Humboldt, A., & Bonpland, A. (1805). *Essai sur la géographie des plantes, accompagné d'un tableau physique des régions équinoxiales, fondé sur les mesures exécutées, depuis le dixième degré de latitude boréale jusqu'au dixième degré de latitude australe, pendant les années 1799, 1800, 1801, 1802, et 1803*. Paris, France: Chez Levrault, Schoell et Compagnie.
- Jakobsson, M., & Rosenberg, N. A. (2007). Clump: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23, 1801–1806. <https://doi.org/10.1093/bioinformatics/btm233>
- Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27, 3070–3071. <https://doi.org/10.1093/bioinformatics/btr521>
- Kattan, G. H., Tello, S. A., Giraldo, M., & Cadena, C. D. (2016). Neotropical bird evolution and 100 years of the enduring ideas of Frank M. Chapman. *Biological Journal of the Linnean Society*, 117, 407–413. <https://doi.org/10.1111/bij.12720>
- Knowles, L. L., & Maddison, W. P. (2002). Statistical phylogeography. *Molecular Ecology*, 11, 2623–2635. <https://doi.org/10.1046/j.1365-294X.2002.01410.x>
- Koch, C., Venegas, P. J., Santa Cruz, R., & Böhme, W. (2018). Annotated checklist and key to the species of amphibians and reptiles inhabiting the northern Peruvian dry forest along the Andean valley of the Marañón River and its tributaries. *Zootaxa*, 4385, 1–101. <https://doi.org/10.11646/zootaxa.4385.1.1>
- Lagomarsino, L. P., Condamine, F. L., Antonelli, A., Mulch, A., & Davis, C. C. (2016). The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytologist*, 210, 1430–1442. <https://doi.org/10.1111/nph.13920>
- Leaché, A. D., Chavez, A. S., Jones, L. N., Grummer, J. A., Gottscho, A. D., & Linkem, C. W. (2015). Phylogenomics of phrynosomatid lizards: Conflicting signals from sequence capture versus restriction site associated DNA sequencing. *Genome Biology and Evolution*, 7, 706–719. <https://doi.org/10.1093/gbe/evv026>
- Leite, R. N., & Rogers, D. S. (2013). Revisiting Amazonian phylogeography: Insights into diversification hypotheses and novel perspectives. *Organisms Diversity & Evolution*, 13, 639–664. <https://doi.org/10.1007/s13127-013-0140-8>
- Lynch, J. D., & Duellman, W. E. (1997). *Frogs of the genus Eleutherodactylus (Leptodactylidae) in western Ecuador: systematics, ecology, and biogeography*. Lawrence, KS: University of Kansas Museum of Natural History Miscellaneous Publication 69.
- McCormack, J. E., Hird, S. M., Zellmer, A. J., Carstens, B. C., & Brumfield, R. T. (2013). Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution*, 66, 526–538. <https://doi.org/10.1016/j.ympev.2011.12.007>
- Miles, L., Newton, A. C., DeFries, R. S., Ravilious, C., May, I., Blyth, S., ... Gordon, J. E. (2006). A global overview of the conservation status of tropical dry forests. *Journal of Biogeography*, 33, 491–505. <https://doi.org/10.1111/j.1365-2699.2005.01424.x>
- Miller, M. J., Bermingham, E., Klicka, J., Escalante, P., & Winker, K. (2010). Neotropical birds show a humped distribution of within-population genetic diversity along a latitudinal transect. *Ecology Letters*, 13, 576–586. <https://doi.org/10.1111/j.1461-0248.2010.01454.x>
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858. <https://doi.org/10.1038/35002501>
- Otero-Patiño, R. (2009). Epidemiological, clinical and therapeutic aspects of *Bothrops asper* bites. *Toxicon*, 54, 998–1011. <https://doi.org/10.1016/j.toxicon.2009.07.001>
- Parkinson, C. L., Campbell, J. A., & Chippindale, P. T. (2002). Multigene phylogenetic analysis of pitvipers, with comments on their biogeography. In G. W. Schuett, M. Höggren, M. E. Douglas, & H. W. Greene (Eds.), *Biology of the vipers* (pp. 93–110). Eagle Mountain, UT: Eagle Mountain Publishing.
- Patton, J. L., & Smith, M. F. (1992). mtDNA phylogeny of Andean mice: A test of diversification across ecological gradients. *Evolution*, 46, 174–183. <https://doi.org/10.1111/j.1558-5646.1992.tb01992.x>
- Pennington, R. T., Lavin, M., Sarkinen, T., Lewis, G. P., Klitgaard, B. B., & Hughes, C. E. (2010). Contrasting plant diversification histories within the Andean biodiversity hotspot. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 13783–13787. <https://doi.org/10.1073/pnas.1001317107>
- Pennington, R. T., Prado, D. E., & Pendry, C. A. (2000). Neotropical seasonally dry forests and Quaternary vegetation changes. *Journal of Biogeography*, 27, 261–273. <https://doi.org/10.1046/j.1365-2699.2000.00397.x>
- Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, 7, e37135. <https://doi.org/10.1371/journal.pone.0037135>
- Pickrell, J. K., & Pritchard, J. K. (2012). Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genetics*, 8, e1002967. <https://doi.org/10.1371/journal.pgen.1002967>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Pyron, R. A. (2015). Post-molecular systematics and the future of phylogenetics. *Trends in Ecology & Evolution*, 30, 384–389. <https://doi.org/10.1016/j.tree.2015.04.016>
- Pyron, R. A., & Burbrink, F. T. (2009). Lineage diversification in a widespread species: Roles for niche divergence and conservatism in the common kingsnake, *Lampropeltis getula*. *Molecular Ecology*, 18, 3443–3457. <https://doi.org/10.1111/j.1365-294X.2009.04292.x>



- Quijada-Mascareñas, A., & Wüster, W. (2010). Recent advances in venomous snake systematics. In S. P. Mackessy (Ed.), *Handbook of venoms and toxins of reptiles* (pp. 25–64). Boca Raton, FL: CRC Press, Taylor & Francis Group.
- R Core Team. (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Reich, D., Thangaraj, K., Patterson, N., Price, A. L., & Singh, L. (2009). Reconstructing Indian population history. *Nature*, *461*, 489–494. <https://doi.org/10.1038/nature08365>
- Ridgely, R. S., & Greenfield, P. J. (2001). *The birds of Ecuador*. Ithaca, NY: Cornell University Press.
- Rittmeyer, E. N., & Austin, C. C. (2015). Combined next-generation sequencing and morphology reveal fine-scale speciation in Crocodile Skinks (Squamata: Scincidae: *Tribolonotus*). *Molecular Ecology*, *24*, 466–483. <https://doi.org/10.1111/mec.13030>
- Roberts, J. L., Brown, J. L., May, R., Arizabal, W., Schulte, R., & Summers, K. (2006). Genetic divergence and speciation in lowland and montane Peruvian poison frogs. *Molecular Phylogenetics and Evolution*, *41*, 149–164. <https://doi.org/10.1016/j.ympev.2006.05.005>
- Rodríguez-Guerra, A. (2011). *Bothrops asper*. In: Torres-Carvajal, O., Pazmiño-Otamendi, G., & Salazar-Valenzuela, D. (2018). *Reptiles del Ecuador. Versión 2018.0*. Museo de Zoología, Pontificia Universidad Católica del Ecuador. Retrieved from <https://bioweb.bio/faunaweb/reptiliaweb/FichaEspecie/Bothrops%20asper>
- Rosenberg, N. A. (2004). Distruct: A program for the graphical display of population structure. *Molecular Ecology Notes*, *4*, 137–138. <https://doi.org/10.1046/j.1471-8286.2003.00566.x>
- Rull, V. (2008). Speciation timing and neotropical biodiversity: The Tertiary-Quaternary debate in the light of molecular phylogenetic evidence. *Molecular Ecology*, *17*, 2722–2729. <https://doi.org/10.1111/j.1365-294X.2008.03789.x>
- Rull, V. (2011). Neotropical biodiversity: Timing and potential drivers. *Trends in Ecology and Evolution*, *26*, 508–513. <https://doi.org/10.1016/j.tree.2011.05.011>
- Rull, V. (2013). Some problems in the study of the origin of neotropical biodiversity using paleoecological and molecular phylogenetic evidence. *Systematics and Biodiversity*, *11*, 415–423. <https://doi.org/10.1080/14772000.2013.865682>
- Salazar-Valenzuela, D. (2016). *Diversification in the Neotropics: Insights from demographic and phylogenetic patterns of lancehead pitvipers (Bothrops spp.)*. PhD thesis, The Ohio State University, pp. 164.
- Saldarriaga-Córdoba, M., Parkinson, C. L., Daza, J. M., Wüster, W., & Sasa, M. (2017). Phylogeography of the Central American lancehead *Bothrops asper* (Serpentes: Viperidae). *PLoS ONE*, *12*, e0187969. <https://doi.org/10.1371/journal.pone.0187969>
- Särkinen, T., Pennington, R. T., Lavin, M., Simon, M. F., & Hughes, C. D. (2012). Evolutionary islands in the Andes: Persistence and isolation explain high endemism in Andean dry tropical forests. *Journal of Biogeography*, *39*, 884–900. <https://doi.org/10.1111/j.1365-2699.2011.02644.x>
- Sasa, M., Wasko, D. K., & Lamar, W. W. (2009). Natural history of the terciopelo *Bothrops asper* (Serpentes: Viperidae) in Costa Rica. *Toxicon*, *54*, 904–922. <https://doi.org/10.1016/j.toxicon.2009.06.024>
- Smith, B. T., McCormack, J. E., Cuervo, A. M., Hickerson, M. J., Aleixo, A., Cadena, C. D., ... Brumfield, R. T. (2014). The drivers of tropical speciation. *Nature*, *515*, 406–409. <https://doi.org/10.1038/nature13687>
- Solís-Lemus, C., Yang, M., & Ané, C. (2016). Inconsistency of species tree methods under gene flow. *Systematic Biology*, *65*, 843–851. <https://doi.org/10.1093/sysbio/syw030>
- Sousa, V., & Hey, J. (2013). Understanding the origin of species with genome-scale data: Modelling gene flow. *Nature Reviews Genetics*, *14*, 404–414. <https://doi.org/10.1038/nrg3446>
- Sovic, M. G., Carstens, B. C., & Gibbs, H. L. (2016). Genetic diversity in migratory bats: Results from RADseq data for three tree bat species at an Ohio windfarm. *PeerJ*, *4*, e1647. <https://doi.org/10.5061/dryad.jh7h4>
- Sovic, M. G., Fries, A. C., & Gibbs, H. L. (2015). AftrRAD: A pipeline for accurate and efficient de novo assembly of RADseq data. *Molecular Ecology Resources*, *15*, 1163–1171. <https://doi.org/10.1111/1755-0998.12378>
- Tapia-Armijos, M. F., Homeier, J., Espinosa, C. I., Leuschner, C., & de la Cruz, M. (2015). Deforestation and forest fragmentation in south Ecuador since the 1970s – Losing a hotspot of biodiversity. *PLoS ONE*, *10*, e0133701. <https://doi.org/10.1371/journal.pone.0133701>
- Torres-Carvajal, O., Carvajal-Campos, A., Barnes, C. W., Nicholls, G., & Pozo-Andrade, M. J. (2013). A new Andean species of leaf-toed gecko (Phyllodactylidae: *Phyllodactylus*) from Ecuador. *Journal of Herpetology*, *47*, 384–390. <https://doi.org/10.1670/12-017>
- Torres-Carvajal, O., Pazmiño-Otamendi, G., & Salazar-Valenzuela, D. (2019). *Reptiles del Ecuador. Versión 2019.0*. Museo de Zoología, Pontificia Universidad Católica del Ecuador. Retrieved from <https://bioweb.bio/faunaweb/reptiliaweb>
- Trénel, P., Hansen, M. M., Normand, S., & Borchsenius, F. (2008). Landscape genetics, historical isolation and cross-Andean gene flow in the wax palm, *Ceroxylon echinulatum* (Arecaceae). *Molecular Ecology*, *17*, 3528–3540. <https://doi.org/10.1111/j.1365-294X.2008.03853.x>
- Turchetto-Zolet, A. C., Pinheiro, F., Salgueiro, F., & Palma-Silva, C. (2013). Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology*, *22*, 1193–1213. <https://doi.org/10.1111/mec.12164>
- Valencia, J. H., Garzón-Tello, K., & Barragán-Paladines, M. E. (2016). *Serpientes venenosas del Ecuador: sistemática, taxonomía, historia natural, conservación, envenenamiento y aspectos antropológicos*. Quito, Ecuador: Fundación Herpetológica Gustavo Orcés; Universidad de Texas, Arlington; Fondo Ambiental Nacional.
- Warrell, D. A. (2004). Snakebites in Central and South America: Epidemiology, clinical features, and clinical management. In J. A. Campbell, & W. W. Lamar (Eds.), *The venomous reptiles of the Western Hemisphere* (pp. 709–761). Ithaca, NY: Cornell University Press.
- Weigend, M. (2002). Observations on the biogeography of the Amotape-Huancabamba zone in northern Peru. *The Botanical Review*, *68*, 38–54. [https://doi.org/10.1663/0006-8101\(2002\)068\[0038:OOTBOT\]2.0.CO;2](https://doi.org/10.1663/0006-8101(2002)068[0038:OOTBOT]2.0.CO;2)
- Weir, J. T. (2006). Divergent timing and patterns of species accumulation in lowland and highland neotropical birds. *Evolution*, *60*, 842–855. <https://doi.org/10.1111/j.0014-3820.2006.tb01161.x>
- Werman, S. D. (2005). Hypotheses on the historical biogeography of Bothropid pitvipers and related genera of the Neotropics. In M. A. Donnelly, B. I. Crother, C. Guyer, M. H. Wake, & M. E. White (Eds.), *Ecology and evolution in the tropics: A herpetological perspective* (pp. 306–365). Chicago, IL: University of Chicago Press.
- Werneck, F. P., Costa, G. C., Colli, G. R., Prado, D. E., & Sites, J. W. Jr (2011). Revisiting the historical distribution of Seasonally Dry Tropical Forests: New insights based on palaeodistribution modelling and palynological evidence. *Global Ecology and Biogeography*, *20*, 272–288. <https://doi.org/10.1111/j.1466-8238.2010.00596.x>
- Winger, B. M., Hosner, P. A., Bravo, G. A., Cuervo, M., Aristizabal, N., Cueto, L. E., & Bates, J. M. (2015). Inferring speciation history in the Andes with reduced-representation sequence data: An example in the bay-backed antpittas (Aves: Grallariidae: *Grallaria hypoleuca* s. l.). *Molecular Ecology*, *24*, 6256–6277. <https://doi.org/10.1111/mec.13477>
- Wulf, A. (2015). *The invention of nature: Alexander von Humboldt's new world*. New York, NY: Alfred A. Knopf.
- Wüster, W., Ferguson, J. E., Quijada-Mascareñas, J. A., Pook, C. E., Da graça salomão, M., & Thorpe, R. S. (2005). Tracing an invasion: Landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). *Molecular Ecology*, *14*, 1095–1108. <https://doi.org/10.1111/j.1365-294X.2005.02471.x>
- Wüster, W., Peppin, L., Pook, C. E., & Walker, D. E. (2008). A nesting of vipers: Phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). *Molecular Phylogenetics and Evolution*, *49*, 445–459. <https://doi.org/10.1016/j.ympev.2008.08.019>



Wüster, W., Salomao, M. G., Quijada-Mascareñas, J. A., Thorpe, R. S.; BBBSP. (2002). Origins and evolution of the South American pitviper fauna: Evidence from mitochondrial DNA sequence analysis. In G. W. Schuett, M. Höggren, M. E. Douglas, & H. W. Greene (Eds.), *Biology of the vipers* (pp. 111–128). Eagle Mountain, UT: Eagle Mountain Publishing.

BIOSKETCH

David Salazar-Valenzuela is an assistant professor at Universidad Tecnológica Indoamérica, Quito, Ecuador. His current research interests include unravelling diversification patterns and processes in the Neotropics using reptiles, as well as study the evolution and function of toxins present in neotropical venomous snakes of biological and medical interest.

Authors' Contributions: DSV, UK and HLG conceived the ideas; OTC and JHV contributed samples/reagents/materials; DSV gathered data, performed the analyses and led the writing; UK, OTC, JHV and HLG contributed to the writing and revision of the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Salazar-Valenzuela D, Kuch U, Torres-Carvajal O, Valencia JH, Gibbs HL. Divergence of tropical pitvipers promoted by independent colonization events of dry montane Andean habitats. *J Biogeogr.* 2019;46:1826–1840. <https://doi.org/10.1111/jbi.13661>