PONTIFICIA UNIVERSIDAD CATÓLICA DEL ECUADOR

FACULTAD DE CIENCIAS EXACTAS Y NATURALES

ESCUELA DE CIENCIAS BIOLÓGICAS

Importancia de la distancia y el ambiente en la estructura genética de anuros neotropicales

Disertación previa a la obtención del título de Licenciada en Ciencias Biológicas

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Quito, 2014

A mi familia

Importance of Distance and Environment in the Genetic Structure of Neotropical Frogs

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Abstract

The study of landscape features that influence genetic structure of wild populations can help us understand the first steps of biological divergence. Populations diverge when there is restriction to gene flow, which can happen due to environmental and geographic factors. However, the relative importance of these processes in generating genetic diferentiation at the neotropical landscape is poorly understood. This study aims to quantify the effect of isolation by environment (IBE) and isolation by distance (IBD) in the spatial genetic structure of nine neotropical frog species by using Structural Equation Modelling. Our analysis shows that IBD is the most important factor in all the species (0.61 IBD vs. 0.33 IBE on average) although IBE also has a significant effect. This suggests that spatial genetic structure is mainly correlated to geographic rather than ecological factors in the neotropical landscape.

Introduction

Understanding the processes that shape the genetic structure of populations is a central quest in evolutionay biology. The geographic distribution of genetic diversity influences the potential for local adaptation and the viability of populations, two key features for the survival of species on a changing environment. Although this has been an active field of research in recent years [1], most studies have focused on temperate regions. Studying the factors that influence population genetic structure in megadiverse regions is important because the higher complexity of communities in those regions may generate qualitative differences on the processes shaping genetic structure relative to temperate regions.

There are two important processes that influence gene flow among separate populations: (1) isolation by distance (IBD), where landscape features and geographic distances between populations restrict gene flow, and (2) isolation by environment or isolation by ecology (IBE) [2] which restricts gene flow between populations that inhabit different environments. IBE can happen by divergent selection of populations inhabiting different environments due to ecological factors or by reduced establishment success of inmigrants caused by local adaptation [3].

Several environmental factors have been tested as drivers of genetic divergence (e.g., elevation, climate factors, latitude/longitude, among others [1]) which has led to the recognition of isolation by environment as the main process affecting genetic structure on animals. Despite a moderate number of studies showing genetic isolation by distance in nature, population genetic divergence is thought to be mainly environmentally structured [1].

There are several limitations on the current understanding of the factors that cause population genetic structure. Most of the studies are not designed to account for both factors (IBD and IBE) at the same time, while certainly organisms are subject to both ecological and geographic influences simultaneously [1].

Amphibians are great model organisms for this type of studies because of their high endemism and low dispersal ability [4] allowing the study of generalized spatial patterns at lower spatial scales. There have been a large number of population genetic studies on amphibians because of the increasing availability of genetic information. Nonetheless the mayority of studies are still carried at temperate zones, while tropical regions nest the highest amphibian biodiversity and most of the threatened species worldwide [5].

Herein we examine how geographic and environmental distances influence the distribution of genetic diversity among nine species of amphibians in the tropical Andes, high Amazon, and Chocó regions, three of the Earth's biodiversity hotspots.

Our results, indicate that isolation by distance is the major process shaping spatial genetic structure at the neotropic, while both IBD and IBE have significant effects. This investigation contributes to the understanding of the microevolutionary processes that shape genetic structure in the poorly studied tropical landscape.

Materials and Methods

1. Study region and taxa

This study covers three hotspot areas: the Chocó, Andes and Amazon (Table 1). The Chocó region encompasses Pacific tropical rain forests, and ranges between 0 and 300 m.a.s.l. The Andean region starts at 1300 m.a.s.l., and is formed by cloud and montane forests at the Andean slopes, and páramo at the high Andes. The Amazonian region is restricted to elevations under 600 meters east of the Andes, and includes a highly diverse tropical rain forest [6].

We obtained mitochondrial and nuclear sequence data from nine frog species distributed in Ecuador and Peru (Table 1; Fig. 1): *Hypsiboas cinerascens*, *Hypsiboas pellucens*, *Dendropsophus parviceps*, *Osornophryne guacamayo* (12S and 16S), *Rhinella margaritifera*, and *Pristimantis curtipes*. All sequences were newly generated with the exception of GenBank sequences for *O. guacamayo* [7] (Table S1).

2. Locality data

Locality data for each individual were obtained from the Museo de Zoología QCAZ database. Most coordinates were measured with a GPS in the field using the WGS84 datum system. A few records lacking coordinates were georeferenced using high-resolution digital maps. Coordinates were verified using ArcGIS 10.0 (ESRI) by comparing them to the verbal description of the locality in the museum database.

To decrease spatial autocorrelation, we used a 5 km buffer around each locality. When points had overlapping buffer zones we randomly chose one to represent the locality and eliminated the others.

3. Genetic Data

We sequenced mitochondrial and nuclear DNA genes of the study species (Table 1). Sequences were obtained by PCR amplification using standard protocols. PCR products were cleaned by ExoSAP digest and sequenced in Macrogen (Macrogen Inc., Seúl, Corea). Sequences were edited and aligned using Geneious Pro 5.4.6 (Biomatters, available at www.geneious.com/) and Mesquite 2.75 [8].

We constructed fused matrices including all the genes available for each species. Nuclear and mitochondrial genes were not analized separately because previous work [9] found consistency on analysis carried using both kinds of genes separated and concatenated.

To assess genetic diferentiation we constructed a genetic distance matrix between localities for each species, using a maximum composite likelihood model of nucleotide evolution with gamma distributed rate variation among sites [9] with MEGA 5.1 [10].

4. Environmental Data

Environmental data were collected from 12 raster digital maps. We obtained eight 30-second resolution bioclimatic variables from the WorldClim dataset (www.worldclim.org): Annual Mean Temperature, Mean Diurnal Range, Temperature Seasonality, Maximum Temperature of Warmest Month, Minimum Temperature of Coldest Month, Annual Precipitation, Precipitation of Warmest Quarter and Precipitation of Coldest Quarter. These layers describe annual trends in ecologically meaningfull variables like extremes of temperature and precipitation or seasonality. We selected the variables that had the lowest correlation among them based on criteria specified by Menéndez-Guerrero and Graham [11].

We also obtained four 30-m resolution layers. Two vegetation index layers: Leaf Area Index (LAI) and Normalized Difference Vegetation Index (NDVI), and a Gross Primary Production (GPP) layer derived from NASA's Moderate Resolution Imaging Spectroradiometer (MODIS) accessed at Reverb [12]. An ASTER Satellite, ASTGTM v.2 elevation layer was obtained from METI and NASA [13].

Data was extracted from these layers for each point locality using ArcGIS 10.0 and differences between localities were calculated to create dissimilarity matrices for each species.

5. Geographic Distances

To calculate geographic distances between point localities we used two approaches. First, the traditionally used Euclidean and topographic distances. Euclidean measures account for the direct distance between two point localities or populations without considering topography [14].

Topographic distances follow the same principal but in a more realistic way by accounting for the additional distance produced by topography. Euclidean and topographic distances were calculated using a script developed in R [15], for topographic distance calculations we used the ASTER digital elevation model detailed before. Euclidean and topographic distances are the simplest standard to explain genetic structure among populations [16].

In addition, we used a recent approach based on the diferential capacity of organisms to move across an environmentally heterogeneous landscape. These resistance-based distances [17,18] are based on environmental niche models and operate under the assumption that gene flow is reduced between populations with different environments either because of local adaptation or isolation by dispersal limitation.

For resistance-based distance calculation it is necessary to assign resistance values to a map of grid-cells, which reflects the landscape resistance to genetic flow. We made this by transforming the niche model idoneity values into resistances following a metholodogy developed by Pröhl et al. [19] and Wang et al. [9].

We used the same 12 environmental layers described at the environmental data section to create a niche model for each species with MaxEnt [20]. Default parameter values were used to run the model. To assess model accuracy we ran the model twice; first using 25% of the point localities as a random test percentage, and then using all the point localities as predictors. Models were considered accurate when the AUC (Area Under the Curve) was higher than 0.90 [21] in the first model, in which case the second model was used to calculate the resistance layer.

In the niche model every grid had a value from 0 to 1 representing habitat suitability (higher values for higher suitability). We created a new layer as the inverse of the niche model by substacting 1 – niche model, using the Spatial Analyst tool from ArcGIS. The resulting GIS layer was used as our resistance layer.

Resistance-based distances were calculated using two approaches. First, Least Cost Path, a commonly used method that looks for the path that represents the least cost to move between two points in a landscape where every grid has a cost value [22]. Least Cost Path was calculated using the gdistance package in R [23].

We also calculated resistance-based distances using an application of circuit theory to biological systems [24]. This method uses as a conceptual base the analogue properties of gene flow in a subpopulation network and conductancy in linear electric circuits [18]. Circuit theory models the landscape as a conductive surface, where different features represent different resistances to gene flow. We used CircuitScape to calculate circuit-distances [24].

6. Structural Equation Modelling

Structural Equation Modelling (SEM) is a second generation multivariate method that evaluates complex relations between multiple variables simultaneously [25]. It uses a series of regressions and model fit analysis to calculate correlations between variables whose relations are hypothesized a priori [9].

To be able to determine how landscape shapes gene flow variation, we considered that isolation by distance generates a correlation between geographic and genetic distance among pairs of populations. While to understand the importance of the environment to this phenomenon, we asume that isolation by environment generates a correlation between genetic divergence and environmental dissimilarity [9].

We used the methodology developed by Wang, et al. [9] and constructed two structural equation models (Fig. 2) to test the importance of isolation by distance and isolation by environment. We defined two latent variables: Environmental Dissimilarity and Geographic Distance. Both variables were evaluated against the observed variable Genetic Dissimilarity.

We constructed two models to assess the differences of the measurement model in Geographic Distance (Fig. 1). In Model A we employed resistance-based distances, as defined by the least cost path and circuit distances between localities. In Model B we employed Euclidean and topographic distances. All the data were included in the model as dissimilarity matrices.

Environmental Dissimilarity was defined by dissimilarity matrices between point localities, one matrix for each environmental variable. Each latent variable was connected by a path to the genetic distances variable. These two paths represented Isolation by Distance and Isolation by Environment in each model. With Structural Equation Modelling we obtained a maximum likelihood estimate value for these paths, which represents the relative importance of each process to explain genetic variation.

Results

For nine frog species distributed across Ecuador, we analyzed a total of 696 occurence points and 409 DNA sequences with an average of 2478 bp, sequence length ranged from 1440 to 4456 bp. Data information by species is detailed in Table 1.

For all nine species both processes (IBE and IBD) explained a significant proportion of the genetic variation. Isolation by distance was more important than isolation by environment in all the species. The structural equation model results as maximum likelihood estimates are shown in Table 2. Overall, isolation by distance explained 0.61 of the genetic variation while isolation by environment explained 0.33. We found the same pattern in both models but the geographic distance measured by Euclidean and topographic distances (Model B) explained a lower proportion of the variation than the resistance-based distances (Model A) (Table 2; Fig. 3). *Hypsiboas pellucens* showed the highest difference in IBD between Model A (79.3%) and B (70.5%).

Environmental data layers showed a wide range of variation across Ecuador, especially in the Andean region, whereas the Amazonian region was more homogeneous. The spatial distribution of the populations is shown in Fig. 1.

CircuitScape current maps revealed similarity in gene flow patterns among the Amazonian species *Dendropsophus parviceps*, *D. triangulum*, *Hypsiboas cinerascens* and *Rhinella margaritifera*. As seen in Fig. 4 these four species show a line connecting populations at northeastern Ecuador which corresponds to the Napo river, an Amazon river affluent. Only two Amazonian species (*Dendropsophus bifurcus* and *D. sarayacuensis*) did not show this pattern, probably as a result of lack of enough samples on this area.

Discussion

1. IBD vs. IBE

Disentagling the effects of IBD and IBE on spatial genetic structure can help understand which processes are relevant to generate genetic divergence. Our results show that both factors were significant in all the species. However, we found an unexpected general pattern: in all of the nine neotropical frog species studied, isolation by distance was more important than isolation by environment (Fig. 3).

A recent review on the effects of IBD and IBE on population structure [1] showed that in most studies IBE shows a stronger effect than IBD. The contrasting pattern in our study (all species showing stronger IBD) is likely a consequence of methodological differences. Most published studies do not evaluate the relative effects of IBD and IBE [26–30] and only consider a single environmental variable (e.g., elevation, vegetation type; [27,28,31–33]). Our study is unique in comparing simultaneously the effect of IBD and IBE while considering multiple environmental variables. There is only one previous study that adresses this question with the same methodological framework. Wang et al. [9] in a survey with 17 species of Caribbean anoles found similar results with IBD playing a more important role than IBE in 14 of the 17 species studied. As in our results, both processes (IBE and IBD) were significant in explaining interpopulation genetic variation.

2. The Napo River as a gene flow highway

Large Amazonian rivers have been proposed as barriers to gene flow that isolate populations on land and cause them to diverge [34,35]. This is just one of several hypotheses that intend to explain the extraordinary biodiversity of the Amazonian region. We found a completely opposite and unexpected pattern at Napo river (Fig. 4). CircuitScape current maps suggest that this river not only represents no barrier, but it serves as a gene flow pathway between populations geographically distant but connected through it.

The Napo river is a main tributary of the upper Amazon and is the most important river of eastern Ecuador with an annual discharge of 2210 m^3s^{-1} at Nuevo Rocafuerte. It drains almost 3% of the total Amazonian catchment in Ecuador [36].

Funk, et al. [37] tested the riverine barrier hypothesis at the Napo river with a widely distributed Amazonian frog, *Engystomops petersi*. Using a phylogenetic approach, they found no support for Napo river as a gene flow barrier. This is consistent with our results.

Large pieces of plant material drifting on the Napo river could be acting as floating islands, transporting individuals through the river to other populations, and though converting the Napo river in a gene flow highway. No previous study has found similar results, and further study is needed to corroborate the hypothesis of the Napo river as a gene flow highway.

3. Resistance-based vs. Euclidean distances

Isolation by Distance showed very similar results (Table 2) when measured by resistance-based distances (Model A) and Euclidean distances (Model B). This is an unexpected

pattern, considering that Euclidean and topographic distances are very raw measures that do not account for any environmental factor affecting individual movement.

On the other hand, *Hypsiboas pellucens*, our only Choco species, displays an interesting difference between models, IBD based on environmental-resistance (79.3% of the total genetic divergence explained) was almost 9% higher than based on Euclidean distances (70.5%), this is the highest difference among all species.

Broquet et al. [38] compared spatial patterns of genetic isolation between fragmented and continuous habitat, and showed that in disturbed environments resitance-based distances best represent the pattern of IBD compared to Euclidean distances, while in continuous, undisturbed landscapes Euclidean measures where almost as good indicators as resistance-distances.

The Choco is the most degraded Ecuadorian region, 75% of the forest has been destroyed by human activities [6]. Habitat fragmentation could be the cause of the dissimilarity between models' IBD for *Hypsiboas pellucens,* our only chocoan species. In an heterogeneous landscape where populations are surrounded by large areas of non-suitable habitat, resistance-based distances resemble more accurately the real life process of individual dispersal because movement only takes place through small portions of suitable areas.

Our results suggest that in more continuous landscapes as the Amazonian and Andean regions, raw measures of distance are good estimators of genetic isolation by distance, contrary to the leading hypothesis [18,24,39].

In all the species IBE didn't vary significantly between models, on average it was 0.334 in Model A and 0.333 in Model B.

4. Ecological and life-history factors

We studied one high Andean species; *Pristimantis curtipes*, which inhabits montane forest and paramo habitats [6]. The island-type separations of these habitats should represent an extra difficulty of connection between populations because the complex topography of the Andes generates steep environmental gradients. This would cause a greater difficulty to move between populations than in Amazonian species, as a result we could expect a greater relative importance of geographic distance. *Pristimantis curtipes* is consistent with this expectation as it is the species with the lowest value for isolation by environment; IBD (0.5) explained five times more genetic variation that IBE (0.1). Nonetheless, this pattern was not visible on the other species, we found no correlation between elevation and IBD/IBE ratio, probably because our data is mainly from lowland species and though do not cover a complete altitudinal gradient.

A number of different gene flow scenarios can happen in nature, which certainly depend on the type of organism, life-history, landscape features, and demographic history [40].

Pristimantis curtipes reproduces by direct development [6] which means there is no need for juvenile dispersal to different habitats after metamorphosis. This could increase the importance of distance in gene flow restriction, because of less dispersal in regards of metamorphosing anurans. However, the effects of life-history type –direct-development

vs. metamorphosing amphibians– on gene flow is difficult to assess due to the small number of studies carried on direct developing species [5].

We identify isolation by distance as the prime process in shaping genetic structure at the neotropical landscape, isolation by environment is also significative but plays a less important role. Our results coupled with Wang et al.'s [9] suggest this could be a generalized pattern for ectotherm vertebrates in the tropics. Our findings differ from most studies, carried mainly in temperate regions, [1] showing the need to study evolutionary processes at the highly complex tropical regions.

Acknowledgements

We greatly thank Sueny Paloma Lima, Francy Mora, Daniela Pareja-Mejía, and Daniel Rivadeneira-M. for clarifying the evolutionary relationships and providing data of *Rhinella margaritifera*, *Hypsiboas pellucens*, *Hypsiboas cinerascens* and *Dendropsophus parviceps*, respectively. We are very thankfull to Andrés Merino-Viteri who created the R script used for direct distance calculations, greatly helped with R, GIS layers and valuable comments, and Pablo Menéndez-Guerrero for his help with GIS layers, advice and helpful comments on the methods. We thank Gabriela Castillo, Liliana Jaramillo, and all the technicians at the Molecular Biology Lab of Museo de Zoología QCAZ for DNA extraction and PCR amplifications on genetic samples. We also thank Gabriela Barragán, Simón E. Lobos, Andrés Mármol, Nadia B. Paéz and Omar Torres-Carvajal for their help and comments.

Finally, we thank the Ecuadorian Secretaría Nacional de Ciencia, Tecnología e Innovación SENESCYT and the General Administrative Direction of the Pontificia Universidad Católica del Ecuador, for funding this research.

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Figure 1. **Geographic distribution of point localities by species.**

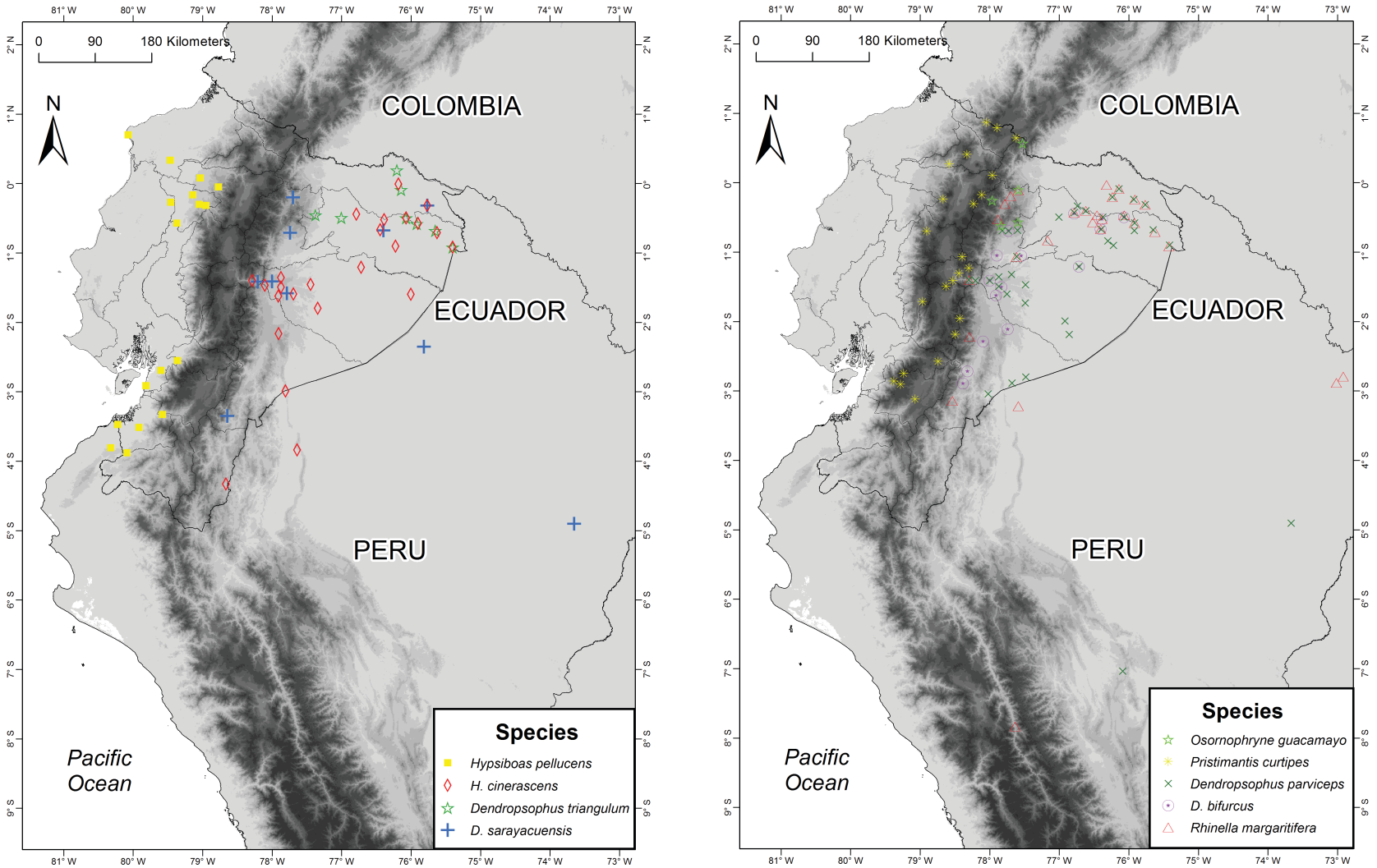

Figure 2. **Graphic representation of Structural Equation models A and B.** Observed variables are represented by rectangles and latent variables by ovals. Simple one-way arrows indicate the observed variables used to infer latent variables, double-headed straight arrows indicate regression pathways and double-headed curved arrows represent covariance pathways. Second level covariance pathways are not shown. *Modified from Wang, et al. (2012).*

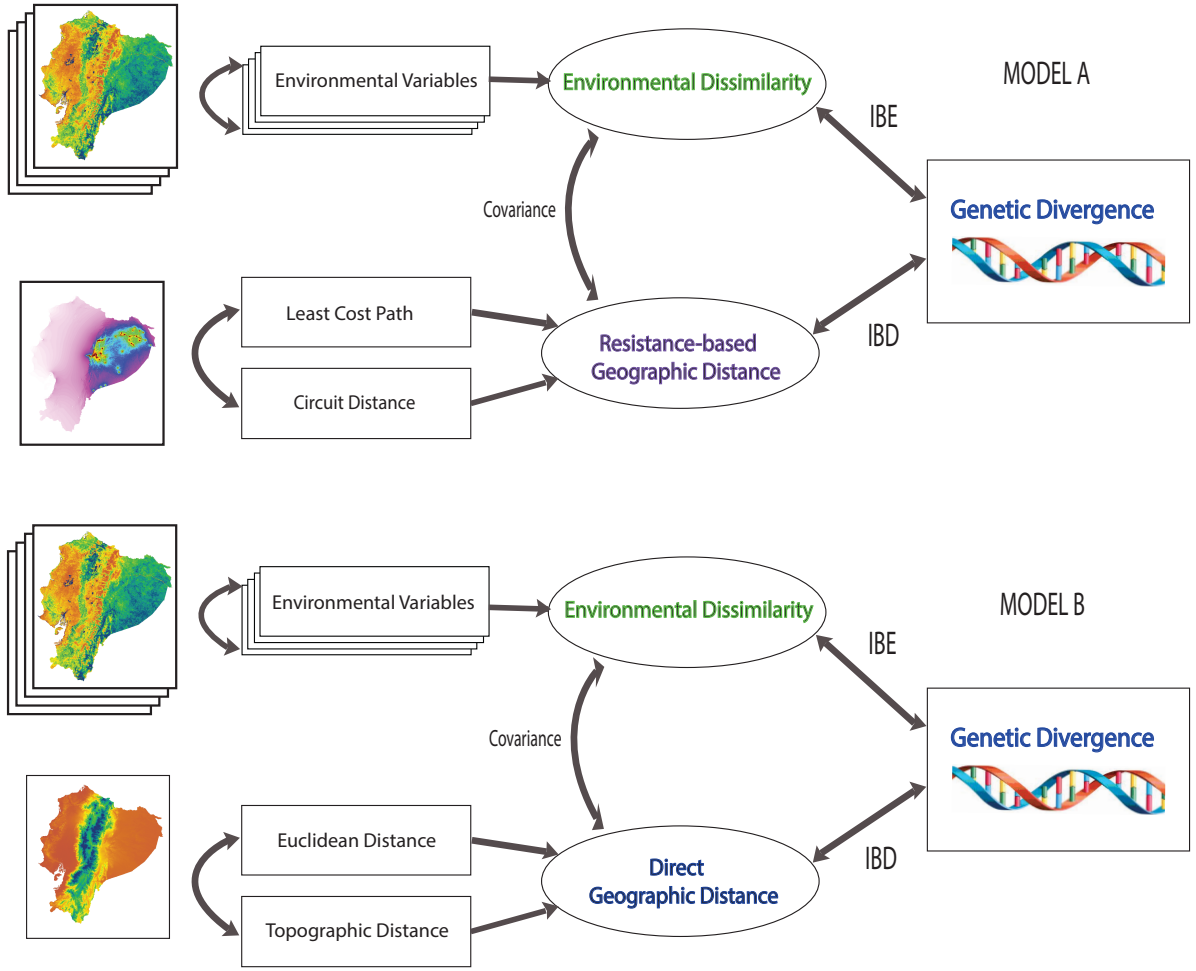

Figure 3. **Structural Equation Model results.** This figure shows the proportion of spatial genetic divergence explained by isolation by distance (purple) and isolation by environment (green), values are expressed as maximum likelihood estimates. Results for Model A are shown, where geographical distance is measured based on landscape's resistance to gene flow, Model B results are not shown because they exhibit the same pattern.

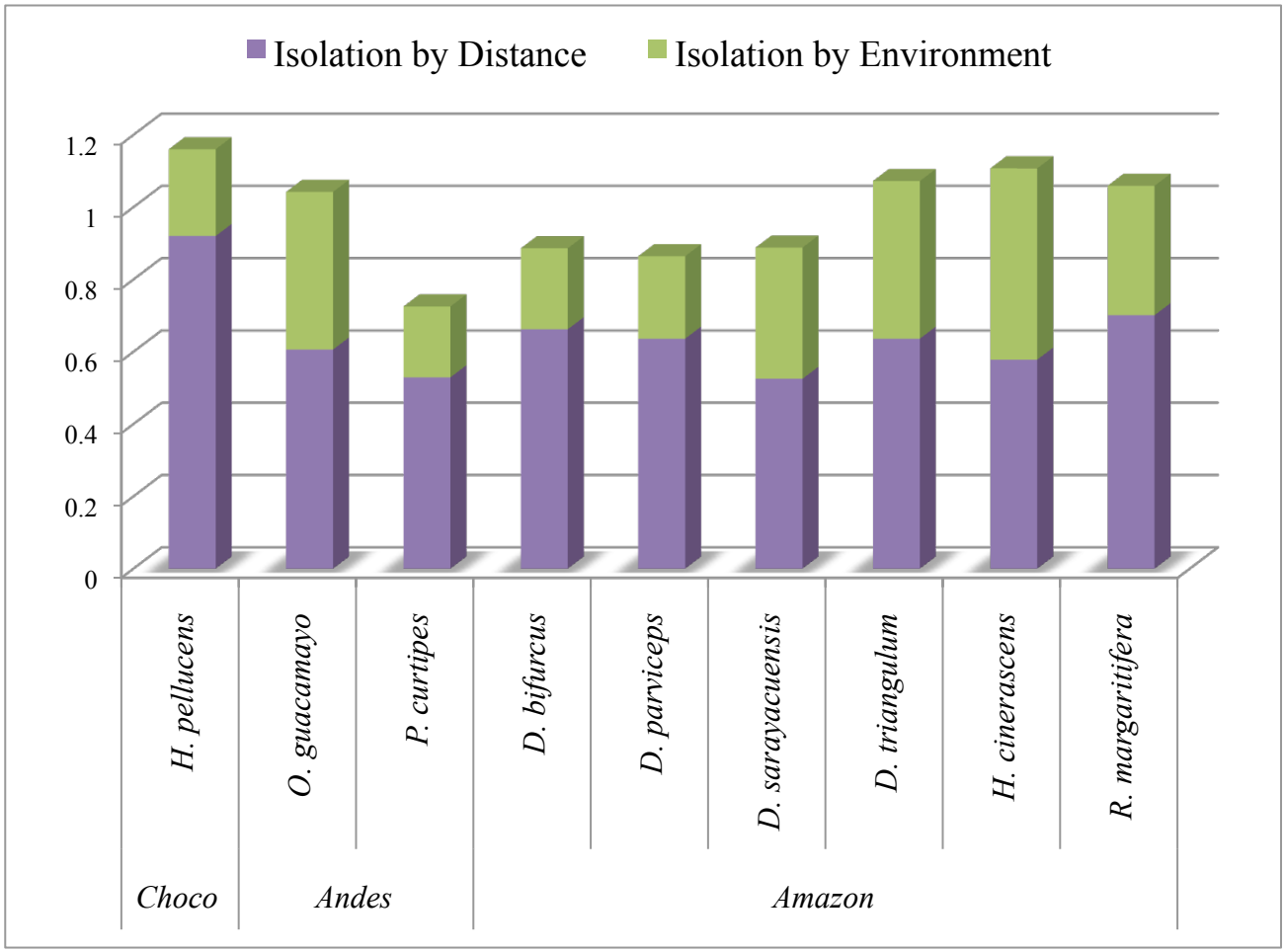

Figure 4. **Cumulative current maps from CircuitScape showing the Napo river region at northeastern Ecuador**. CircuitScape current maps reveal population connectivity based on landscape's resistance to movement. **A.** *Dendropsophus triangulum*, **B.** *Dendropsophus parviceps*, **C.** *Hypsiboas cinerascens* and **D.** *Rhinella margaritifera*.

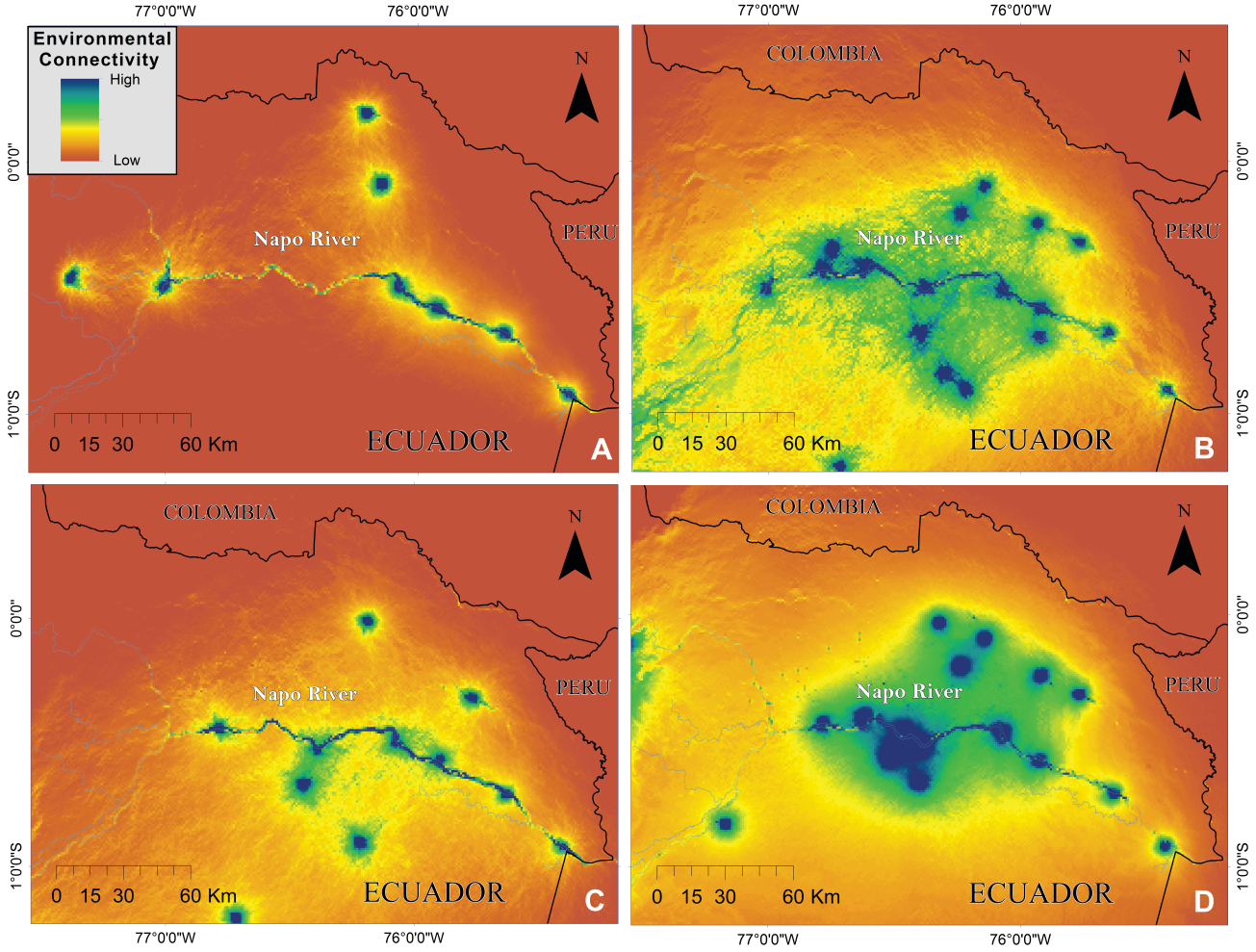

Table 1. **Data used in our study for nine frog species.** Geographical data include the number of occurence records used for niche modelling (Occ.) and the number of point localities (Loc.). Genetic data comprehend the number of sequences (Seq.), the genes used for each species, and the average and range of base pairs obtained.

Species	**Region**	**Occ.**	**Loc.**	**Seq.**	**Genes**	**Average bp**	**Range bp**
Dendropsophus bifurcus	Amazon	177	15	28	CO1, ND1	2083.3	1846-2162
D. triangulum	Amazon	85	8	25	12S, CO1, ND1	2803.1	2258-2996
D. sarayacuensis	Amazon	94	10	13	12S, CO1, ND2	258.9	1813-2134
D. parviceps	Amazon	137	36	106	12S, CO1, ND1	2918.3	2592-3005
Rhinella margaritifera	Amazon	45	28	69	16S, 12S, CO1, Tyr	2911.9	2688-3000
Hypsiboas cinerascens	Amazon	37	24	53	12S, ND1, CO1	2710.5	1440-2996
H. pellucens	Choco	54	17	32	12S, 16S, ND1, POMC	4083.5	3165-4456
Pristimantis curtipes	Andes	41	23	67	16S, ND1, RAG1	2943.1	2547-3075
Osornophryne guacamayo	Andes	26	5	16	12S, 16S	1589.4	1551-1620
Mean		*77.3*	*18.4*	*45.4*		*2478*	

Table 2. **Results of the Structural Equation Models A and B.** SEM was used to quantify the proportion of genetic divergence explained by isolation by distance (IBD) and isolation by environment (IBE). Path coefficient results for IBD and IBE are presented as maximum likelihood estimates. We also record the sums of IBD and IBE (Total) and the covariation between these variables (Covar.). The percentage of the total explained by each process is also shown (%IBD, %IBE). Values in italics represent non-sinificant scores.

	Model A - Resistance-based distance						*Model B - Direct distance*					
Species	**IBD**	**IBE**	**Total**	**Covar.**	**% IBD**	**% IBE**	**IBD**	**IBE**	**Total**	**% IBD**	**% IBE**	**Covar.**
Dendropsophus bifurcus	0.662	0.224	0.886	0.368	74.7	25.3	0.558	*0.223*	0.781	71.4	28.6	0.463
D. triangulum	0.636	0.436	1.072	0.618	59.3	40.7	0.513	0.436	0.949	54.1	45.9	0.686
D. sarayacuensis	0.525	0.363	0.888	0.551	59.1	40.9	0.426	0.353	0.779	54.7	45.3	0.607
D. parviceps	0.636	0.228	0.864	0.217	73.6	26.4	0.648	0.228	0.876	74	26	0.006
Rhinella margaritifera	0.701	0.358	1.059	0.236	66.2	33.8	0.695	0.359	1.054	65.9	34.1	0.217
Hypsiboas cinerascens	0.578	0.529	1.107	0.012	52.2	47.8	0.543	0.528	1.071	50.7	49.3	0.242
H. pellucens	0.92	0.24	1.16	0.032	79.3	20.7	0.574	0.24	0.814	70.5	29.5	-0.017
Osornophryne guacamayo	0.606	0.436	1.042	0.235	58.2	41.8	0.674	0.438	1.112	60.6	39.4	*-0.06*
Pristimantis curtipes	0.529	0.196	0.725	0.219	73	27	0.588	0.188	0.776	75.8	24.2	*0.001*
Mean	0.644	0.334	0.978	0.276	66	33.8	0.58	0.333	0.912	64.2	35.8	0.238

Table S1. List of the GenBank accesion numbers for *Osornophryne guacamayo.*

Museum number	**12S**	**16S**
QCAZ 12240	JF907469	JX411984
QCAZ 12241	JF907470	JX411985
QCAZ 17293	JF907472	JX411988
QCAZ 17294	JF907473	JX411989
QCAZ 17295	JF907471	JX411990
QCAZ 2735	JF907466	JX411991
QCAZ 40102	JF907492	JX412001
QCAZ 40106	JF907468	JX412002
QCAZ 40138	JF907463	JX412004
QCAZ 40143	JF907464	JX412005
QCAZ 40147	JF907465	JX412006
QCAZ 43370	JF907474	JX412015
QCAZ 43554	JF907467	JX412018
QCAZ 4576	JF907491	JX412024
QCAZ 46661	--	JX412027
QCAZ 46662	JF907475	JX412028

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Quito, 27 de noviembre de 2014

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