The effect of landscape on functional connectivity and shell shape in the land snail *Humboldtiana durangoensis* (#45428)

First submission

Guidance from your Editor

Please submit by **2 Mar 2020** for the benefit of the authors (and your $200 publishing discount) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Author notes
Have you read the author notes on the guidance page?

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

5 Figure file(s)
6 Table file(s)
3 Other file(s)

Structure and Criteria

2

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

🗋 You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- ✓ Clear, unambiguous, professional English language used throughout.
- ✓ Intro & background to show context. Literature well referenced & relevant.
- ✓ Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- ✓ Figures are relevant, high quality, well labelled & described.
- ✓ Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- ✓ Original primary research within Scope of the journal.
- ✓ Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- ✓ Rigorous investigation performed to a high technical & ethical standard.
- ✓ Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- ℹ Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- ✓ All underlying data have been provided; they are robust, statistically sound, & controlled.
- ✓ Speculation is welcome, but should be identified as such.
- ✓ Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	***Example***
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

The effect of landscape on functional connectivity and shell shape in the land snail *Humboldtiana durangoensis*

Benjamín López [1] , **Omar Mejia** Corresp., [1] , **Gerardo Zúñiga** [1]

[1] Laboratorio de Variación Biológica y Evolución, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas Instituto Politécnico Nacional, Mexico City, Mexico

Corresponding Author: Omar Mejia
Email address: homarmejia@hotmail.com

The populations of *Humboldtiana durangoensis* have experienced a drastic reduction in the effective population size; in addition, the species is threatened by anthropogenic activities. For the aforementioned, landscape genetics will serve as a tool to define the potential evolutionarily significant units (ESU) for this species. To complete our objective, we evaluated the effect of cover vegetation and climate on the functional connectivity of the species from the LGM to the present as well as the effect of climate on shell shape. Partial Mantel tests, distance-based redundance analysis and a Bayesian framework were used to evaluate connectivity. On the other hand, geometric morphometrics, phylogenetic principal component analysis and redundancy analysis were used for the analysis of shell shape. Our results suggest that the suitable areas have been decreasing since the LGM; also, vegetation cover rather than climate has influenced the genetic connectivity among land snail populations, although temperature had a high influence on shell shape in this species. In conclusion, vegetation cover was the main factor that determined the functional connectivity for the land snail; however, local selective pressures led to different phenotypes in shell shape that allowed us to postulate that each one of the previously defined genetic groups must be considered as a different ESU.

The effect of landscape on functional connectivity and shell shape in the land snail *Humboldtiana durangoensis*

Benjamin López[1], Omar Mejía[1*] and Gerardo Zúñiga[1]

[1] Laboratorio de Variación Biológica y Evolución, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México

Corresponding author: homarmejia@hotmail.com; hmejiag@ipn.mx

Abstract

The populations of *Humboldtiana durangoensis* have experienced a drastic reduction in the effective population size; in addition, the species is threatened by anthropogenic activities. For the aforementioned, landscape genetics will serve as a tool to define the potential evolutionarily significant units (ESU) for this species. To complete our objective, we evaluated the effect of cover vegetation and climate on the functional connectivity of the species from the LGM to the present as well as the effect of climate on shell shape. Partial Mantel tests, distance-based redundance analysis and a Bayesian framework were used to evaluate connectivity. On the other hand, geometric morphometrics, phylogenetic principal component analysis and redundancy analysis were used for the analysis of shell shape. Our results suggest that the suitable areas have been decreasing since the LGM; also, vegetation cover rather than climate has influenced the genetic connectivity among land snail populations, although temperature had a high influence on shell shape in this species. In conclusion, vegetation cover was the main factor that determined the functional connectivity for the land snail; however, local selective pressures led to different phenotypes in shell shape that allowed us to postulate that each one of the previously defined genetic groups must be considered as a different ESU.

Introduction

Species dispersal can be affected not only by essential processes (*e.g.,* the movement, mating, and reproductive fitness of the individuals) but also by ecological and topographical factors (*e.g.,* abiotic variables, land cover, line features and landforms) associated with the landscape (Manel et al. 2003; McRae et al. 2008). Especially in land snails, dispersal is a process that is highly dependent on a set of variables associated with the landscape, such as climate and vegetation cover, which represent a high physiological cost for the snail (Dörge et al. 1999; Schweiger et al. 2004; Hylander et al. 2005; Aubry et al. 2006). Thus, in a heterogeneous landscape, the differentiation between populations may be increased not only by the historical events and microevolutionary factors but also by the ecological and topographical factors that determine the habitat or structural connectivity (McRae 2006; McRae et al. 2008; Bell et al. 2010).

Due to their low vagility, patchy distribution and preference for particular microhabitats (Dörge et al. 1999; Hylander et al. 2005; Aubry et al. 2006), land snails are excellent models for exploring the effects of landscape on the movement of individuals among suitable patches, or in other words, on the functional connectivity (Tischendorf and Fahrig 2000). The effect of the Pleistocene climate changes on the phylogeographical structure and demographic history of land snails has been widely documented (Ross 1999; Haase et al. 2003; Davison and Chiba 2006; Holland and Cowie 2007; Dépraz et al. 2008; Guiller and Madec 2010); as well as changes in vegetation cover that have caused a decline in abundance and species density (Hylander et al. 2004). However, neither the effect of vegetation cover nor the effect of the climate on functional connectivity have been explored yet.

The snails of the genus *Humboldtiana* represent a group of nearly 60 species that have an insular distribution in the mountainous regions from South Texas and New Mexico to Central Mexico (Thompson 2006, Mejía and Zuñiga 2007). Many species have very small ranges, with the exception of three species that are widely distributed (Mejía et al. 2018). *H. durangoensis* is distributed in the Madrense Centro ecoregion of the Sierra Madre Occidental in Durango state, mainly in cold temperate forests in an altitudinal gradient ranging from 1600 to 2800 m asl. This vegetation community has historically been exploited in Durango state and has also experienced droughts and fires that have led to fragmentation and habitat loss (Aragón-Piña et al. 2010). For these reasons, forest loss has turned into a global conservation issue due to its effect on biodiversity (Fahrig 2003).

Conservation efforts in several countries have traditionally been focused on "surrogate" species, which can create the umbrella effect for other sympatric species and, at the same time, serve to attract attention and funding (Caro and O'Doherty 2001). Illustrative examples of this situation in Mexico are the efforts to recover the tiny vaquita porpoise (*Phocoena sinus*) and the Mexican wolf (*Canis lupus baileyi*). Nevertheless, very few efforts have been conducted to preserve "non-charismatic species" such as land snails. In fact, none of the nearly 1500 species of native land snails that occur in Mexico (Thompson and Hubert 2011) is included in the Mexican law for endangered species or in the IUCN Red List, a situation that highly contrasts with European land snails (Cuttelod et al. 2011); at the same time, few studies of the phylogeographic structure or population genetics have been performed with Mexican land snails (López et al. 2017; López et al. 2019).

On the other hand, while there is a lack of agreement on how to define an evolutionarily significant unit (ESU) (but see the review in Fraser and Bernatchez 2001), we agree with those proposals that suggest that ESUs must include genetic, ecological and morphological differentiation (Crandall et al. 2000) that reflect the adaptive distinctiveness. Previous papers have evaluated the population genetics and phylogeographic structure of *H. durangoensis* in the Madrense Centro region using microsatellite DNA markers and mitochondrial and nuclear DNA (López et al. 2017; López et al. 2019). The microsatellite analysis recovered seven genetic groups and signals of a strong genetic bottleneck in the populations, while the mitochondrial and nuclear DNA sequences found three main genetic groups that also showed signals of drastic reduction in the effective population size.

To evaluate the effects of vegetation cover and local climatic variables on the genetic differentiation of the snail *H. durangoensis*, we analyzed the functional connectivity in three temporal frames: the last glacial maximum (21,000 years bp), the middle Holocene (6000 years bp) and the present. In addition, we evaluated the effect of the climate on shell size and shape using phylogenetic comparative methods. Despite the lack of agreement regarding the effects of the climate on shell traits, a strong relationship between the phenotype, genetic variation and climate would be expected (Dowle et al. 2015), because land snails as other groups with low vagility and dispersal abilities, tend to develop local morphological adaptations due to restrictive gene flow (Fitzpatrick 2012; Pfenninger and Posada 2002). Both approaches together will allow us to postulate ~~the ESU~~ for this land snail in the Sierra Madre Occidental in Western Mexico.

Methodology

Resistance surfaces

The geographic centroids of each one of the seven genetic groups of *H. durangoensis* previously defined by microsatellite loci by López et al. (2017) were used to determine the effect of the landscape on functional connectivity (Fig. 1). Whereas the landscape can include a large number of variables, in the present work, we followed two approximations to evaluate the functional connectivity between snail populations. The first was to use an approximation of the Grinnellian niche defined from a set of bioclimatic variables (Bell et al. 2010; Ortego et al. 2012; Poelchau and Hamrick 2012); the second was to analyze the effect of vegetation cover, because it is known that it affects the dispersion of terrestrial snails (Labaune and Magnin 2002; Armbruster et al. 2007; Ström et al. 2009; Edworthy et al. 2012; Kappes et al. 2009), especially in mountain populations where periods of glaciation and deglaciation promoted the contraction and expansion of vegetation cover (Armbruster et al. 2007). In both cases, the different models were generated for three different time frames, including the current period and two time periods representing the extreme conditions experienced during the late quaternary: the middle Holocene (6000 years bp), which was warmer and wetter than the present, and the last glacier maximum (LGM), which was characterized by dry and colder climates (21,000 years bp).

To reduce the error in the parameterization, validation, and comparison of the models (Barve et al. 2011), the available geographic space for the taxon (M) was defined as the Ecoregion Madrense Centro (González-Elizondo et al. 2013). Grinnellian niche models were constructed with the 19 climatic variables available in WorldClim (Hijmans et al. 2005) and the 18 climatic and topographic variables available in ENVIREM (Title and Bemmels 2018). The models were made at a resolution of 30 arc-seconds, but in the case of the last glacial maximum (LGM), the variables were used at their native resolution of 2.5 minutes, and a bilinear interpolation was performed to decrease the resolution to 30 arc-seconds with the disaggregate function of the raster library ver. 2.6-7 in R (Hijmans 2017). The atmospheric circulation model used was the MPI-ESM-P, since it has shown better performance with respect to other models of circulation (Tang et al. 2017).~~.~~ The bioclimatic variables were clipped to the geographic space with the crop and mask functions of the raster library ver. 2.6-7 in R (Hijmans 2017).

Species niche model

The environmental suitability areas were defined by a maximum entropy algorithm (MAXENT v. 3.2.19, Phillips et al. 2006) from 28 records of *H. durangoensis* available in museums and our own collections. We selected this algorithm because it produces reliable results even with a small quantity of data (Elith et al. 2006; Heikkinen et al. 2006; Hernandez et al. 2006). In a preliminary analysis, the 19 WorldClim and 18 ENVIREM variables were included with the default parameters and log output to minimize the correlation and maximize their contributions to the model. The relative importance of each variable was determined from its percentage of contribution and for the loss of predictive power when each variable was excluded using a jackknife test. In addition, to select those variables with correlation coefficients lower than 0.6, environmental information was extracted from each geographic point, and a Pearson correlation test was performed with the function corr.test in the psych library of R (Revelle 2018). Thus, the geographic distribution model was obtained with the selected variables and assumed 10,000 pseudoabsence points separated by one kilometer from the presence records (Barbet-Massin et al. 2012). The statistical evaluation of the model was carried out in 10 repetitions and the data were partitioned into 75% for training and 25% for evaluation with a logistical output. The predictive power of the model was evaluated using a partial ROC test with 100 bootstrap replicates (Barve

2008). Finally, the suitability area available for the species in each temporal frame was estimated with the DEM surface tools in ArcGIS 10.

Vegetation models

The random forest (RF) classification algorithm was used to obtain the modeled vegetation cover (Breiman 2001). This method categorizes a set of data based on the classification and regression of the trees from a bootstrap analysis (Breiman 2001). The INE-INEGI (1997) vegetation cover map was used as an input file. Because this classification contains many vegetation types for the Madrense Centro ecoregion, prior to the analysis, the vegetation types were reclassified into five categories based on the ombrothermal horizons of the Sierra Madre Occidental (Macías-Rodríguez et al. 2017): (1) temperate forests, (2) cold temperate forests, (3) grasslands, (4) tropical forests and (5) drylands. The model was trained to take into account the variables sets of BIOCLIM and ENVIREM and to select only those that explained more than 50% of the variation based on the mean decrease accuracy criterion; these models were made with the randomForest 4.6-14 library in R (Breiman 2001).

Isolation by resistance

To evaluate the resistance of the landscape between the genetic groups, the resistance isolation model (IBR) was implemented in CIRCUITSCAPE 3.4.2 (McRae 2006). This method produces a resistance/conductance matrix between the pairs of sites that are obtained by assigning an arbitrary resistance/conductance value per pixel corresponding to the relative resistance of the landscape to the genetic flow. The result was a resistance value that depended on the distance between the localities, the number of possible pathways and the heterogeneity of the landscape (McRae 2006). The following resistance values were assigned to the forest structure: 60 (cold temperate forests), 110 (temperate forests), 200 (grasslands), 300 (tropical forests) and 360 (drylands).

For the surface derived from the niche modeling, resistance values were assigned considering five symmetrical categories defined by the range between the minimum training presence (MTP) and the highest suitability value obtained by the Maxent model. The values were assigned with the ifelse and raster R libraries (Hijmans et al. 2005), and three different approaches were used to evaluate the relationship between the paired F_{ST} values among the seven genetic groups (López et al. 2017) and resistance values. Three matrices were considered in this analysis: the genetic paired distances, the log10-transformed Euclidean geographical distances, and the paired resistance distances obtained from CIRCUITSCAPE for the two evaluated resistance surfaces (climate and vegetation). In the first approximation, the Mantel partial test was used to evaluate the effects of the two variables while controlling for the effect of a third. The significance of the partial correlation of the Mantel test was obtained by 1000 random permutations using the partial.mantel.test function of the NFC library (Bjørnstad 2013). In the second approach, a distance-based redundancy analysis (dbRDA) was used in the vegan 2.5 library (Oksanen et al. 2013) considering the genetic distances, geographic distances, and the effect of vegetation cover, as well as the effect of the climatic distances on the mean of the resistance values (Noguerales et al. 2016). The characterization of the environmental space was performed with the randomPoints function in R that generated 1000 random geographic points and with the extract function to obtain the climatic point value per site. Then, the main function in R was used to perform the PCA, and later, the dist function in R was used to obtain the eigenvalues of the environmental distances for the first three components considering only the loadings of the geographic points corresponding to the genetic groups; finally, the significance of the dbRDA was evaluated with the anova.cca function in R. Lastly, in the third approach, given that in the two previous analyses the climate component was not significant (see Tables 1 and 2), we only evaluate the effects of geography (G), vegetation cover (E) and both (G + E) on functional connectivity through a Bayesian framework implemented in the Sunder 0.0.4 library (Botta et al. 2015). The algorithm implemented in SUNDER assumed that the covariance of the allelic frequencies among the populations would decrease as a function of the geographical and environmental distances (Botta et al. 2015). Thus, to estimate the effect of the set of G, E, and G+E variables, 10 independent chains with 10^7 iterations and sampling every 1000 steps were used with uniform priors with large upper bounds (Botta et al. 2015).

Shell morphometrics

A total of 129 shells of *H. durangoensis* adults from the seven genetic groups used by López et al. (2017) were analysed: Las Peñas (20), El Salto (8), Progreso (3), Topia (7), Potrero (46), Los Herreras (25) and Guanaceví (20). The shape of the shell was obtained using two approaches: a classical approach that assumed four linear shell measurements (height, SH; width, SW; aperture height, AH; maximum aperture width, AW) obtained with a digital micrometer with an accuracy of 0.01 mm; in addition, globosity (G=SH/WD), spiral height (SP=SH-AH) and shell volume (V) were calculated (Fig. S1). These variables were transformed to log10 to remove the allometric effect associated with growth following the method described by Mosimann (1970). Finally, the eigenvalues of the mean and the centroid values for each one of the genetic groups were recovered from a principal component analysis (PCA) for posterior analysis (Harigan et al. 1979). On the other hand, the shell shape was evaluated from 11 landmarks according to Mumladze et al. (2013) (Fig. S1). Following the method proposed by Kistner and Dybdahi (2012), a total of five photos were taken per individual to eliminate the error associated with the orientation. The X/Y coordinates were digitized in TPSDIG ver 2.12 (Rohlf 2008). The average shape per genetic group was obtained from a generalized Procrustes analysis (GPA) with the gpagen function implemented in geomorph 3.0.7 (Adams et al. 2019). To eliminate the phylogenetic effect on the variation in shell shape, a phylogenetic principal component analysis (pPCA) was performed considering the shell shape of both, classical and geometric morphometrics approaches and a tree based on distances generated from F_{ST} values with the function phyl.pca in phytools (Revell 2012). Lastly, to determine whether there was a relationship between the shell shape and environmental conditions, a redundancy analysis (RDA) was performed considering the three matrices generated (means, centroids and geometric morphometrics) with the rda function following the method proposed by Borcard et al. (2018) in the vegan library ver 2.5 (Oksanen et al. 2013).

Results

Six variables made the greatest contribution to the model of the potential distribution: isothermality (Bio3), the minimum temperature of the coldest month (Bio6), the precipitation of the wettest month (Bio 13), the precipitation of the driest month (Bio 14), the precipitation of the coldest month (Bio 19), and the climatic humidity index. For the potential vegetation model, 11 variables were selected: isothermality (Bio 3), temperature seasonality (Bio 4), the annual temperature range (Bio 7), the annual precipitation (Bio 12), the driest month precipitation (Bio 13), the seasonality of precipitation (Bio 15), the coldest quartile precipitation (Bio 19), the average monthly evapotranspiration potential of driest quarter (PETDriestQuarter), the monthly variability in evapotranspiration potential (PETseasonality), the average monthly evapotranspiration potential of the warmest quarter (PETWarmestQuarter), and the average evapotranspiration potential of the wettest quarter (PETWettestQuarter).

Environmental suitability and vegetation models

The results obtained for the modeling of the distribution area of *H. durangoensis* in the Madrense Centro region showed that the models constructed for the three temporal frames were satisfactory (P = 0). In general, our findings suggested that the areas of environmental suitability had decreased considerably in the last 21,000 years (38197 km^2 or 28.5% of the total area in the LGM, 32945 km^2 or 24.5% in the mid Holocene and 23620 km^2 or 17.6% in the current). Our findings show that at present, the areas with high probability of occurrence are restricted to the northern portion of the distribution area (Fig. 1). The model of vegetation cover generated from the current vegetation map with random forest showed that the estimated success rate was 76.77% for the LGM, 77.48% for the Middle Holocene and 75.57% for the current period (Table S1). In addition, in the last 21,000 years, a variation in the coverage area of each plant community was estimated, and the temperate forests increased the most, while the grasslands decreased the most (Table S1, Fig. S2).

Resistance and functional connectivity

The maps generated by CIRCUITSCAPE considering the structure of the vegetation cover suggested that the connectivity routes between the *H. durangoensis* genetic groups in the Central Madrense region have changed little in the last 21,000 years, although in the actual period, the areas of high resistance are larger compared to those in the LGM (Table 1, Fig. S2). The resistance surface from the environmental suitability models for the Mantel test and Mantel partial tests were not significant (Table 2, Fig. S3). On the other hand, when considering the effects of vegetation cover, the Mantel test between the values of F_{ST} and vegetation cover was once again not significant in any of the three time frames; however, the Mantel partial tests yielded significant correlations when controlling for the effects of geography and vegetation cover in the three time periods (Table 2). In the case of the redundancy analysis, the marginal tests for the three time frames showed a significant association between the genetic differentiation and geographic distance, explaining 24.26% of the variance, but were not significant when the resistance distances generated from the vegetation cover or from the climatic variables were considered (Table 3). In contrast, in the conditional tests as in the Mantel partial test, a relationship was again observed with the structure of the vegetation cover but not with that of the climate (Table 3). With respect to the results generated by SUNDER, when the climatic component was no longer considered, it was observed that during the LGM, it was the geographic component that best explained the variation, while for the Middle Holocene and the actual period, both the geographic component and the vegetation cover were important (Table 4).

Variation in shell size and shape

The values estimated from the morphometrics classical approach allow us to establish that the populations located in the north of the distribution area (Topia, Potrero, Los Herreras and Guanaceví) had larger sizes and higher spires in comparison with the populations in the center (Progreso) and south (Las Peñas and El Salto) of the distribution area (Table 5). The percentage of variance explained by the first three phylogenetic components was 99.47% for the means of the linear variables, 99.98% for the centroid size and 91.84% for the average shape obtained from the analysis of the geometric morphometrics. Finally, the redundancy analysis obtained from the analysis of the first three phylogenetic components was statistically significant ($P<0.05$). The bioclimatic variables associated with each dataset were different, although in all cases, they were exclusively temperature variables, with the temperature annual range (Bio 7) being the only common variable (Fig. 2). Although it was difficult to establish a pattern, the data retrieved from geometric morphometric analysis allowed us to suggest that larger shells with higher spirals are related to the max temperature of the warmest month (Bio 5), while smaller shells with the lower spirals were related to the temperature annual range (Bio 7) and mean temperature of the wettest quarter (Bio 8) (Fig. 2).

Discussion

Effects of the landscape on functional connectivity

The functional connectivity in terrestrial snails was determined by the availability of microhabitats suitable for dispersal. Our findings showed that the variables related to the humidity and relative aridity of the terrain, as well as the precipitation of the driest and the wettest month, had a greater contribution to the potential distribution model generated by Maxent. These variables were related to the apparent rupture of the estivation period in May and to the period of activity and dispersion between July and September, as has been suggested for other members of the group (Baur 1986; Aubry et al. 2006). However, the climate component defined through the environmental suitability analysis with the MAXENT maximum entropy algorithm and by the method proposed by Noguerales et al. (2016) did not contribute significantly to explaining the functional connectivity of *H. durangoensis* populations.

A possible explanation for this phenomenon might be related to the spatial resolution provided by the bioclimatic layers. It has been demonstrated that the geographic patterns of the areas of environmental suitability in the terrestrial mollusks were particularly dependent on the resolution of the grid, since this increases or diminishes the heterogeneity of the geographic space (Kadmon and Heller 1998). However, the models generated for land snails at a resolution of 30 arc-seconds (1 km^2), as used in this study, have been shown to be efficient in explaining the historical demographic reductions that are the consequence of contractions in the areas of environmental suitability (Horsák et al. 2010; Pfenninger et al. 2014; Mumladze 2014; Patrao et al. 2015). In this sense, the areas of environmental suitability for *H. durangoensis* have decreased from 28.5% in the LGM to 17.6% at the present, a result congruent with the population reductions recovered for this species with microsatellite markers and DNA sequences (López et al. 2017; López et al. 2019). Therefore, although the climate component apparently did not make a significant contribution to functional connectivity, its influence on the taxon cannot be denied because *H. durangoensis* likely experienced environmental tracking as a consequence of climate change, as has been demonstrated in alpine populations of *Arianta arbustorum* (Baur and Baur 2013).

On the other hand, the random forest algorithm has been shown to perform well in predicting the current vegetation types in heterogeneous geographic areas, as it was very robust in relation to the number of classes in which plant communities were clustered, as has been verified by paleopalinological records for models generated for LGM (Waske and Braun 2009; Rodriguez-Galiano et al. 2012; Vanselow and Samimi 2014; Hais et al. 2015). Thus, the results of efficiency in the assignment to plant categories with the random forest algorithm (Table S1) fall within the values obtained in other works (Waske and Braun 2009; Hais et al. 2015), suggesting that predictions of vegetation cover in this study are correct. Although our paleovegetation maps apparently did not show significant changes in vegetation cover (Fig. S2), the resistance results from Circuitscape suggested that these changes have occurred and that resistance values have increased from the LGM to the present (Table 1). One of the main limitations of analyses based on resistance surfaces is that the values assigned to each of the categories are arbitrary; however, it has been shown that the assigned resistance values have no effect on the habitat categories in a fragmented landscape (Schweiger et al. 2004; Wang et al. 2009). Consequently, as has been reported for other mountain snails (Schell and Hausdorf 2012; Hugall et al. 2002; Sherpa et al. 2018), the altitudinal displacement of plant communities in mountainous regions during Quaternary climate changes could explain the dynamics of functional connectivity in *H. durangoensis* as has been postulated for other species distributed in the SMOc (Metcalfe et al. 2000; Anducho-Reyes et al. 2008; Bryson et al. 2011; López-González et al. 2014),

Based on these findings, we hypothesized that the functional connectivity of *H. durangoensis* on different temporal scales has been promoted by the presence of both temperate and cold temperate forests and that two patterns can be distinguished as has been suggested in *Helix aspersa* and *Cepaea nemoralis* (Arnaud et al. 2003; Schweiger et al. 2004; Barahona-Segovia et al. 2019), for which two patterns can be distinguished. The first is a model of isolation by distance on a larger geographic scale (Pfenninger and Posada 2002; Arnaud et al. 2003; Schweiger et al. 2004), and the second is possible dynamic metapopulation promoted both by environmental and landscape heterogeneity on a fine geographic scale, as has been documented for other land snails (Arnaud et al. 2001; Baur and Baur 2013).

Variation of the shell in H. durangoensis

The relationship between shell size and shape in land snails with climatic variables of temperature and precipitation has been widely studied and is well known (see review in Goodfriend 1986). However, while the effect of the genetic component on shell shape variation has been studied (Goodacre 2001; Dowle et al. 2015; Sherpa et al. 2018), few studies have attempted to control this effect (Webster et al. 2012; Kotsakiozi et al. 2013), and none so far have evaluated this effect at the intraspecific level. Our findings showed, after controlling for the genetic effects, that the shell size and shape were determined by climatic variables of temperature and precipitation (Fig. 2). However, whereas these variables were not significant to explain the genetic relationships among the groups, they suggested that both the phenotype and genotype were the results of independent processes (Haase and Misof 2009); that is, the microhabitat conditions had a great effect on the shell despite the existence of gene flow (Chiba and Davison 2007; Fiorentino et al. 2013; Stankowski 2013; Proćków et al. 2017). Thus, whereas has been suggested that the use of comparative phylogenetic methods at intrapopulation levels may generate poor informative results (Niewiarowski et al. 2004), the power of resolution of these methods may depend on the taxon and the assessed trait (Martins and Housworth 2002), as has been found in this study.

In addition, our results suggested that populations with larger shells and apertures are distributed to the north, while populations with smaller shells and apertures were distributed to the south. The altitudinal interval of the sampled localities in the northern region (1,702-2,400 m asl) was lower than the altitudinal interval in which the populations in the southern region were collected (2,587-2,759 m asl), which was consistent with the results previously found in intrapopulation studies of the species of the genera *Arianta*, *Vestia* and *Trochulus* (Burla and Stahel 1983; Baur and Raboud 1988; Sulikowska-Drozd 2001; Proćków et al. 2017), where the populations from colder climates had smaller shells. This could be related to a greater probability of survival of organisms with small shells in unfavorable climatic conditions (Baur et al. 2014) and the greater resistance to crystallization temperatures (Ansart et al. 2014). At the same time, at higher altitudes, the duration of individual growth time is shorter (Anderson et al. 2007; Proćków et al. 2017). However, there were also differences in the sizes of the aperture and the heights of the spires between the north and south regions. These shells attributes could reflected microclimatic conditions, where small apertures tended to occur in the drier and higher altitude regions, meanwhile large apertures and higher spires ocuured at lower altitudes as has been reported in other species (Anderson et al. 2007; Haase and Misof 2009; Dowle et al. 2015).

How many ESUs?

In the literature, only two published works that addressed the definition of the ESUs of land snails have been published (Holland and Hadfield 2002; Ursenbacher et al. 2010); however, they did not remove the phylogenetic effects, which impacted their results. In the first study, a fragment of the mtCOI DNA was used and only the phylogenetic trees, genetic distances and AMOVA analysis in the 12 populations of the tree snail *Achatinella mustelina* were recognized as six ESUs that were reproductively isolated and distributed throughout a longitudinal transect of 24 km (Holland and Hadfield 2002). In the second study, which used microsatellite loci and performed a genetic structure analysis, two main clusters were found in *Trochulus aureatus*, although the authors decided to define each one of the nine sampled populations as different ESUs, even though they were separated by less than 200 meters (Ursenbacher et al. 2010). In opposition, our results suggest that each of the seven genetic groups previously identified by the analysis performed by López et al. (2017) must be considered an ESU, not only because of their genetic distinctiveness but also due to the phenotypical differences. The removal of the phylogenetic effect shows that temperature and precipitation variables were strong determinants of the shell size and shape of the species, which explained the morphological differentiation (Fig. 2).

Conclusions

The main conclusion of this work is that vegetation cover has a high impact on the functional connectivity of the land snail, as does climate, which is a strong determinant of shell shape in this species. Previous studies have found that young restored forests can achieve even higher snail diversities than old unperturbed forests (Hylander et al. 2004; Ström et al. 2009), although this could depend on survival in microrefugia or dispersal from other patches. Forestry is one of the main economic activities in the state of Durango, Mexico, that exerts strong pressure on the populations of the land snail *H.*

durangoensis due to habitat loss and degradation. Nevertheless, the development of comprehensive management plans for the state (Conafor 2006) could guarantee the long-term survival of *H. durangoensis*, although further studies need to be performed to evaluate the potential effects of global climate warming on the species.

Acknowledgments

This work was partially funded by the Consejo Nacional de Ciencia y Tecnología project number 165990. We also thank Angus Davison for their comments.

References

Adams D, Collyer M, Kaliontzopoulou A (2019) Geometric Morphometric Analyses of 2D/3D Landmark Data.

Anderson TK, Weaver KF, Guralnick RP (2007) Variation in adult shell morphology and life-history traits in the land snail *Oreohelix cooperi* in relation to biotic and abiotic factors. J Mollus Stud 73:129-137. https://doi.org/10.1093/mollus/eym006

Anducho-Reyes MA, Cognato AI, Hayes JL, Zúñiga G (2008) Phylogeography of the bark beetle *Dendroctonus mexicanus* Hopkins (Coleoptera: Curculionidae: Scolytinae). Mol Phylogenet Evol 49:930-940. https://doi.org/10.1016/j.ympev.2008.09.005

Ansart A, Guiller A, Moine O, Martin MC, Madec L (2014) Is cold hardiness size-constrained? A comparative approach in land snails. Evol Ecol 28:471-493. https://doi.org/10.1007/s10682-013-9680-9

Aragón-Piña EE, Garza-Herrera A, González-Elizondo MS, Luna-Vega I (2010) Composición y estructura de las comunidades vegetales del rancho El Durangueño, en la Sierra Madre Occidental, Durango, México. Rev Mex Biodivers 81:771-787. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1870-34532010000300018&lng=es&tlng=es

Armbruster GF, Hofer M, Baur B (2007) Effect of cliff connectivity on the genetic population structure of a rock-dwelling land snail species with frequent self-fertilization. Biochem Syst Ecol 35:325-333. https://doi.org/10.1016/j.bse.2006.12.005

Arnaud JF, Madec L, Guiller A, Bellido A (2001) Spatial analysis of allozyme and microsatellite DNA polymorphisms in the land snail *Helix aspersa* (Gastropoda: Helicidae). Mol Ecol 10:1563-1576. https://doi.org/10.1046/j.1365-294X.2001.01292.x

Arnaud JF (2003) Metapopulation genetic structure and migration pathways in the land snail *Helix aspersa*: influence of landscape heterogeneity. Landsc Ecol 18:333-346. https://doi.org/10.1023/A:1024409116214

Aubry S, Labaune C, Magnin F, Roche P, Kiss L (2006) Active and passive dispersal of an invading land snail in Mediterranean France. J Anim Ecol 75:802-813. https://doi.org/10.1111/j.1365-2656.2006.01100.x

Barahona-Segovia RM, Riveros-Díaz AL, Zaror S, Catalán R, Araya JF (2019) Shelter, ecophysiology and conservation status of *Plectostylus araucanus* (Pulmonata: Bothriembryontidae) in the fragmented Maulino Forest, central Chile. Rev Mex Biodivers 90:1-11. https://doi.org/10.22201/ib.20078706e.2019.90.2703

Barbet-Massin M, Jiguet F, Albert CH, Thuiller W (2012) Selecting pseudo-absences for species distribution models: how, where and how many?. Methods Ecol Evol 3:327-338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Barve N (2008) Tool for Partial-ROC ver. 1.0. Biodiversity Institute, Lawrence, Kansas

Barve N, Barve V, Jiménez-Valverde A, Lira-Noriega A, Maher SP, Peterson AT, Villalobos F (2011) The crucial role of the accessible area in ecological niche modeling and species distribution modeling. Ecol Model 222:1810-1819. https://doi.org/10.1016/j.ecolmodel.2011.02.011

Baur B (1986) Patterns of dispersion, density and dispersal in alpine populations of the land snail *Arianta arbustorum* (L.)(Helicidae). Ecography 9:117-125. https://doi.org/10.1111/j.1600-0587.1986.tb01200.x

Baur B, Raboud C (1988) Life history of the land snail *Arianta arbustorum* along an altitudinal gradient. J Anim Ecol 57:71-87. https://www.jstor.org/stable/4764

Baur B, Baur A (2013) Snails keep the pace: shift in upper elevation limit on mountain slopes as a response to climate warming. Can J Zool 91:596-599. https://doi.org/10.1139/cjz-2013-0036

Baur B, Meier T, Baur A, Schmera D (2014) Terrestrial gastropod diversity in an alpine region: disentangling effects of elevation, area, geometric constraints, habitat type and land-use intensity. Ecography 37:390-401. https://doi.org/10.1111/j.1600-0587.2013.00312.x

Bell RC, Parra JL, Tonione M, Hoskin CJ, Mackenzie JB, Williams SE, Moritz C (2010) Patterns of persistence and isolation indicate resilience to climate change in montane rainforest lizards. Mol Ecol 19:2531-2544. https://doi.org/10.1111/j.1365-294X.2010.04676.x

Bjørnstad ON (2013) Package ‘ncf’: Spatial nonparametric covariance functions. R package version 1.1-3. http://CRAN.R-project.org/package=ncf

Borcard D, Gillet F, Legendre P (2018) Numerical ecology with R. Springer, New York

Botta F, Eriksen C, Fontaine MC, Guillot G (2015) Enhanced computational methods for quantifying the effect of geographic and environmental isolation on genetic differentiation. Methods Ecol Evol 6:1270-1277. https://doi.org/10.1111/2041-210X.12424

Breiman L (2001) Random forests. Mach Learn 45:5-32. https://doi.org/10.1023/A:1010933404324

Bryson Jr RW, Murphy RW, Graham MR, Lathrop A, Lazcano D (2011) Ephemeral Pleistocene woodlands connect the dots for highland rattlesnakes of the *Crotalus intermedius* group. J Biogeogr 38:2299-2310. https://doi.org/10.1111/j.1365-2699.2011.02565.x

Buria H, Stahel W (1983) Altitudinal variation in *Arianta arbustorum* (Mollusca, Pulmonata) in the Swiss alps. Genetics 62:95-108. https://doi.org/10.1007/BF00116631

Chiba S, Davison A (2007) Shell shape and habitat use in the North-west Pacific land snail *Mandarina polita* from Hahajima, Ogasawara: current adaptation or ghost of species past?. Biol J Linn Soc 91:149-159. https://doi.org/10.1111/j.1095-8312.2007.00790.x

CONAFOR (2006) Programa estrátegico forestal Durango. http://www.conafor.gob.mx:8080/documentos/docs/12/177Programa%20Estrat%c3%a9gico%20Forestal%20de%20Durango.pdf

Crandall KA, Bininda-Emonds OR, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. Trends Ecol Evol 15:290-295. https://doi.org/10.1016/S0169-5347(00)01876-0

Cuttelod A, Seddon M, Neubert E (2011) European red list of non-marine molluscs. Publication office of the European Union. Luxembourg, Publications Office of the European Union

Davison A, Chiba S (2006) Labile ecotypes accompany rapid cladogenesis in an adaptive radiation of *Mandarina* (Bradybaenidae) land snails. Biol J Linn Soc 88:269-282. https://doi.org/10.1111/j.1095-8312.2006.00624.x

Dépraz A, Cordellier M, Hausser J, Pfenninger M (2008) Postglacial recolonization at a snail's pace (*Trochulus villosus*): confronting competing refugia hypotheses using model selection. Mol Ecol 17:2449-2462. https://doi.org/10.1111/j.1365-294X.2008.03760.x

Dörge N, Walther C, Beinlich B, Plachter H (1999) The significance of passive transport for dispersal in terrestrial snails (Gastropoda, Pulmonata). Z Ökol Nat schutz 8:1- 10.

Dowle EJ, Morgan-Richards M, Brescia F, Trewick SA (2015) Correlation between shell phenotype and local environment suggests a role for natural selection in the evolution of *Placostylus* snails. Mol Ecol 24:4205-4221. https://doi.org/10.1111/mec.13302

Edworthy AB, Steensma KMM, Zandberg HM, Lilley PL (2012) Dispersal, home-range size, and habitat use of an endangered land snail, the Oregon forestsnail (*Allogona townsendiana*). Can J Zool 90:875-884. https://doi.org/10.1139/z2012-056

Elith JH, Graham CP, Anderson R, Dudík M, Ferrier S, Guisan A, Li J (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography 29:129-151. https://doi.org/10.1111/j.2006.0906-7590.04596.x

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annu Rev Ecol Evol Syst 34:487-515. https://doi.org/10.1146/annurev.ecolsys.34.011802.132419

Fiorentino V, Manganelli G, Giusti F (2008) Multiple scale patterns of shell and anatomy variability in land snails: the case of the Sicilian *Marmorana* (Gastropoda: Pulmonata, Helicidae). Biol J Linn Soc 93:359-370. https://doi.org/10.1111/j.1095-8312.2007.00940.x

Fiorentino V, Manganelli G, Giusti F, Tiedemann, R, Ketmaier V (2013) A question of time: the land snail *Murella muralis* (Gastropoda: Pulmonata) reveals constraints on past ecological speciation. Mol Ecol 22:170-186. https://doi.org/10.1111/mec.12107

Fitzpatrick BM (2012) Estimating ancestry and heterozygosity of hybrids using molecular markers. BMC Evol Biol 12:131. https://doi.org/10.1186/1471-2148-12-131

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741-2752. https://doi.org/10.1046/j.0962-1083.2001.01411.x

Goodacre SL, Wade CM (2001) Patterns of genetic variation in Pacific island land snails: the distribution of cytochrome b lineages among Society Island Partula. Biol J Linn Soc 73:131-138. https://doi.org/10.1111/j.1095-8312.2001.tb01351.x

González-Elizondo MS, González-Elizondo M, Ruacho-González L, López-Enríquez IL, Renata-Rentería FI, Tena-Flores JA (2013) Ecosystems and diversity of the Sierra Madre Occidental. In: Merging science and management in a rapidly changing world: Biodiversity and management of the Madrean Archipelago III and 7th Conference on Research and Resource Management in the Southwestern Desert, 2012 May 1-5, Tucson, AZ (Gottfried GJ, Ffolliott P F, Gebow BS, Eskew LG, Collins LC, eds) Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 204–211

Goodfriend G A (1986) Variation in land-snail shell form and size and its causes: a review. Syst Biol 35:204-223. https://doi.org/10.1093/sysbio/35.2.204

Guiller A, Madec L (2010) Historical biogeography of the land snail *Cornu aspersum*: a new scenario inferred from haplotype distribution in the Western Mediterranean basin. BMC Evol Biol 10:1-18. https://doi.org/10.1186/1471-2148-10-18

Haase M, Misof B, Wirth T, Baminger H, Baur B (2003) Mitochondrial differentiation in a polymorphic land snail: evidence for Pleistocene survival within the boundaries of permafrost. J Evol Biol 16:415-428. https://doi.org/10.1046/j.1420-9101.2003.00542.x

Haase M, Misof B (2009) Dynamic gastropods: stable shell polymorphism despite gene flow in the land snail *Arianta arbustorum*. J Zool Syst Evol Res 47:105-114. https://doi.org/10.1111/j.1439-0469.2008.00488.x

Hais M, Komprdová K, Ermakov N, Chytrý M (2015) Modelling the last glacial maximum environments for a refugium of Pleistocene biota in the Russian Altai Mountains, Siberia. Palaeogeogr Palaeoclimatol Palaeoecol 438:135-145. https://doi.org/10.1016/j.palaeo.2015.07.037

Hartigan JA, Wong MA (1979) Algorithm AS 136: A k-means clustering algorithm. J R Stat Soc. Series C (Applied Statistics) 28:100-108. https://www.jstor.org/stable/2346830

Hernandez PA, Graham CH, Master LL, Albert DL (2006) The effect of sample size and species characteristics on performance of different species distribution modeling methods. Ecography 29:773-785. https://doi.org/10.1111/j.0906-7590.2006.04700.x

Heikkinen RK, Luoto M, Araújo MB, Virkkala R, Thuiller W, Sykes MT (2006) Methods and uncertainties in bioclimatic envelope modelling under climate change. Prog Phys Geog 30:751-777. https://doi.org/10.1177/0309133306071957

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965-1978. https://doi.org/10.1002/joc.1276

Hijmans RJ (2017) Raster: geographic analysis and modeling with raster data. R Package versión 2.6-7. https://CRAN.R-project.org/package=raster

Holland BS, Hadfield MG (2002) Islands within an island: phylogeography and conservation genetics of the endangered Hawaiian tree snail Achatinella mustelina. Mol Ecol 11:365-375. https://doi.org/10.1046/j.1365-294X.2002.01464.x

Holland BS, Cowie RH (2007) A geographic mosaic of passive dispersal: population structure in the endemic Hawaiian amber snail *Succinea caduca* (Mighels, 1845). Mol Ecol 16:2422-2435. https://doi.org/10.1111/j.1365-294X.2007.03246.x

Hylander K, Nilsson C, Gunnar Jonsson B, Göthner T (2005) Differences in habitat quality explain nestedness in a land snail meta-community. Oikos 108:351-361. https://doi.org/10.1111/j.0030-1299.2005.13400.x

Hylander K, Nilsson C, Göthner T (2004) Effects of buffer-strip retention and clearcutting on land snails in boreal riparian forests. Conserv Biol 18:1052-1062. https://doi.org/10.1111/j.1523-1739.2004.00199.x

Horsák M, Chytrý M, Pokryszko BM, Danihelka J, Ermakov N, Hájek M, Lustyk P (2010) Habitats of relict terrestrial snails in southern Siberia: lessons for the reconstruction of palaeoenvironments of full-glacial Europe. J Biogeogr 37:1450-1462. https://doi.org/10.1111/j.1365-2699.2010.02280.x

Hugall A, Moritz C, Moussalli A, Stanisic J (2002) Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proc Natl Acad Sci 99:6112-6117. https://doi.org/10.1073/pnas.092538699

INE-INEGI~~,~~ (1997) Uso del suelo y vegetación, escala 1:250000, serie I (continuo nacional), escala: 1:250000. Instituto Nacional de Ecología – Dirección Ordenamiento Ecológico Nacional e Instituto de Estadística, Geografía e Información. Digitalización de las cartas de uso de suelo y vegetación elaboradas por INEGI entre los años 1980-1991 con base en fotografías aéreas de 1968-1986. México, D.F. http://www.conabio.gob.mx/informacion/metadata/gis/usv250kcs1agw.xml?_httpcache=yes&_xsl=/db/metadata/xsl/fgdc_html.xsl&_indent=no. Accessed 9 January 2019

Kadmon R, Heller J (1998) Modelling faunal responses to climatic gradients with GIS: land snails as a case study. J Biogeogr 25:527-539. https://doi.org/10.1046/j.1365-2699.1998.2530527.x

Kappes H, Jordaens K, Hendrickx F, Maelfait JP, Lens L, Backeljau T (2009) Response of snails and slugs to fragmentation of lowland forests in NW Germany. Landsc Ecol 24:685-697. https://doi.org/10.1007/s10980-009-9342-z

Kistner EJ, Dybdahl MF (2013) Adaptive responses and invasion: the role of plasticity and evolution in snail shell morphology. Ecol Evol 3:424-436. https://doi.org/10.1002/ece3.471

Kotsakiozi P, Rigal F, Valakos ED, Parmakelis A (2013) Disentangling the effects of intraspecies variability, phylogeny, space, and climate on the evolution of shell morphology in endemic Greek land snails of the genus *Codringtonia*. Biol J Linn Soc 110:796-813. https://doi.org/10.1111/bij.12169

Labaune C, Magnin F (2002) Pastoral management vs land abandonment in Mediterranean uplands: impact on land snail communities. Glob Ecol Biogeogr 11:237-245. https://doi.org/10.1046/j.1466-822X.2002.00280.x

López B, Gómez R, Mejía O (2017) Strong genetic structure and signs of population bottlenecks in the land snail *Humboldtiana durangoensis* in the Sierra Madre Occidental of Western Mexico. J Zool Syst Evol Res 55:288-297. https://doi.org/10.1111/jzs.12177

López B, Zúñiga G, Mejía O (2019) Phylogeographic structure in the apparent absence of barriers: a case study of the Mexican land snail *Humboldtiana durangoensis* (Pulmonata: Humboldtianidae). J Molluscan Stud 85:244-252. https://doi.org/10.1093/mollus/eyz007

López-González C, Correa-Ramírez MM, García-Mendoza DF (2014) Phylogeography of Peromyscus schmidlyi: an endemic of the Sierra Madre Occidental, Mexico. J Mammal 95: 254-268. https://doi.org/10.1644/13-MAMM-A-166

Macías-Rodríguez MÁ, Giménez de Azcárate-Cornide J, Gopar-Merino LF (2017) Sistematización bioclimática de la Sierra Madre Occidental (México) y su relación con los pisos de vegetación. Polibotanica 1:125-163. http://dx.doi.org/10.18387/polibotanica.43.6.

Mejía O, Zuniga G (2007) Phylogeny of the three brown banded land snail genus *Humboldtiana* (Pulmonata: Humboldtianidae). Mol phylogenetics Evol 45:587-595. https://doi.org/10.1016/j.ympev.2007.07.013

Mejía O, López B, Reyes-Gomez JP (2018) Three new species of the genus *Humboldtiana* (Gastropoda: Pulmonata: Humboldtianidae) from Mexico. Nautilus 132:124-130.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:198-197. https://doi.org/10.1016/S0169-5347(03)00008-9

Martins EP, Housworth EA (2002) Phylogeny shape and the phylogenetic comparative method. Syst Biol 51:873-880. https://doi.org/10.1080/10635150290102573

McRae BH (2006) Isolation by resistance. Evolution 60:1551-1561. https://doi.org/10.1111/j.0014-3820.2006.tb00500.x

McRae BH, Dickson BG, Keitt TH, Shah VB (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. Ecology 89:2712-2724. https://doi.org/10.1890/07-1861.1

Metcalfe SE, O'Hara SL, Caballero M, Davies SJ (2000) Records of Late Pleistocene–Holocene climatic change in Mexico—a review. Quaternary Sci Rev 19:699-721. https://doi.org/10.1016/S0277-3791(99)00022-0

Mosimann JE (1970) Size allometry: size and shape variables with characterizations of the lognormal and generalized gamma distributions. J Am Stat Assoc 65:930-945.

Mumladze L, Tarkhnishvili D, Murtskhvaladze M (2013) Systematics and evolutionary history of large endemic snails from the Caucasus (*Helix buchii* and *H. goderdziana*) (Helicidae). Am Malacol Bull 31:225-235. https://doi.org/10.4003/006.031.0202

Mumladze L (2014) Sympatry without co-occurrence: exploring the pattern of distribution of two *Helix species* in Georgia using an ecological niche modelling approach. J Molluscan Stud 80:249-255. https://doi.org/10.1093/mollus/eyu045

Niewiarowski PH, Angilletta MJ, Leaché AD (2004) Phylogenetic comparative analysis of life-history variation among populations of the lizard *Sceloporus undulatus*: an example and prognosis. Evolution 58:619-633. https://doi.org/10.1111/j.0014-3820.2004.tb01684.x

Noguerales V, Cordero PJ, Ortego J (2016) Hierarchical genetic structure shaped by topography in a narrow-endemic montane grasshopper. BMC Evol Biol 16:96. https://doi.org/10.1186/s12862-016-0663-7

Oksanen J, Blanchet FG, Kindt R, Legendre P, MinchinPRO′, Hara RB, Wagner H (2013) Vegan: Community Ecology Package. R package version, 2. http://CRAN.Rproject.org/package=vegan

Ortego J, Riordan EC, Gugger PF, Sork VL (2012) Influence of environmental heterogeneity on genetic diversity and structure in an endemic southern Californian oak. Mol Ecol 21:3210-3223. https://doi.org/10.1111/j.1365-294X.2012.05591.x

Patrao C, Assis J, Rufino M, Silva G, Jordaens K, Backeljau T, Castilho R (2015) Habitat suitability modelling of four terrestrial slug species in the Iberian Peninsula (Arionidae: *Geomalacus* species). J Molluscan Stud 81:427-434. https://doi.org/10.1093/mollus/eyv018

Pfenninger M, Posada D (2002) Phylogeographic history of the land snail *Candidula unifasciata* (Helicellinae, Stylommatophora): fragmentation, corridor migration, and secondary contact. Evolution 56:1776-1788. https://doi.org/10.1111/j.0014-3820.2002.tb00191.x

Pfenninger M, Weigand A, Bálint M, Klussmann-Kolb A (2014) Misperceived invasion: the Lusitanian slug (*Arion lusitanicus* auct. non-Mabille or *Arion vulgaris* Moquin-Tandon 1855) is native to Central Europe. Evol Appl 7:702-713. https://doi.org/10.1111/eva.12177

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecol Model 190:231-259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Poelchau MF, Hamrick JL (2012) Differential effects of landscape-level environmental features on genetic structure in three codistributed tree species in Central America. Mol Ecol 21:4970-4982. https://doi.org/10.1111/j.1365-294X.2012.05755.x

Proćków M, Kuźnik-Kowalska E, Mackiewicz P (2017) The Influence of climate on shell variation in *Trochulus striolatus* (C. Pfeiffer, 1828) (Gastropoda: Hygromiidae) and its implications for subspecies taxonomy. Ann Zool 67:199-221. https://doi.org/10.3161/00034541ANZ2017.67.2.002

Revell LJ (2012) phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol 3:217-223. http://www.respond2articles.com/MEE/

Revelle W~~.~~ (2018) psych: Procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA, https://CRAN.R-project.org/package=psych Version = 1.8.12.

Rodriguez-Galiano VF, Ghimire B, Rogan J, Chica-Olmo M, Rigol-Sanchez JP (2012) An assessment of the effectiveness of a random forest classifier for land-cover classification. ISPRS J Photogramm Remote Sens 67:93-104. https://doi.org/10.1016/j.isprsjprs.2011.11.002

Rohlf FJ (2008) TPSdig, v. 2.12. NY: State University at Stony Brook

Ross TK (1999) Phylogeography and conservation genetics of the Iowa Pleistocene snail. Mol Ecol 8:1363-1373. https://doi.org/10.1046/j.1365-294x.1999.00696.x

Scheel BM, Hausdorf B (2012) Survival and differentiation of subspecies of the land snail *Charpentieria itala* in mountain refuges in the Southern Alps. Mol Ecol 21:3794-3808. https://doi.org/10.1111/j.1365-294X.2012.05649.x

Sherpa S, Ansart A, Madec L, Martin MC, Dréano S, Guiller A (2018) Refining the biogeographical scenario of the land snail *Cornu aspersum aspersum*: Natural spatial expansion and human-mediated dispersal in the Mediterranean basin. Mol Phylogenet Evol 120:218-232. https://doi.org/10.1016/j.ympev.2017.12.018

Schweiger O, Frenzel M, Durka W (2004) Spatial genetic structure in a metapopulation of the land snail *Cepaea nemoralis* (Gastropoda: Helicidae). Mol Ecol 13:3645-3655. https://doi.org/10.1111/j.1365-294X.2004.02357.x

Stankowski S (2013) Ecological speciation in an island snail: evidence for the parallel evolution of a novel ecotype and maintenance by ecologically dependent postzygotic isolation. Mol Ecol 22:2726-2741. https://doi.org/10.1111/mec.12287

Ström L, Hylander K, Dynesius M (2009) Different long-term and short-term responses of land snails to clear-cutting of boreal stream-side forests. Biol Conserv 142:1580-1587. https://doi.org/10.1016/j.biocon.2009.02.028

Sulikowska-Drozd A (2001) Shell variability in Vestia turgida (Rossmassler, 1836) (Gastropoda, Clausiliidae) along an altitudinal gradient. Folia Malacol 9:73-81. http://dx.doi.org/10.12657/folmal.009.010

Tang CQ, Dong YF, Herrando-Moraira S, Matsui T, Ohashi H, He LY, Yan HZ (2017) Potential effects of climate change on geographic distribution of the Tertiary relict tree species *Davidia involucrata* in China. Sci Rep 7:43822. https://doi.org/10.1038/srep43822

Tischendorf L, Fahrig, L. (2000). On the usage and measurement of landscape connectivity. Oikos, 90: 7-19. https://doi.org/10.1034%2Fj.1600-0706.2000.900102.x

Thompson FG (2006) Some landsnails of the genus *Humboldtiana* from Chihuahua and western Texas. Fla Mus Nat Hist 46:61-98.

Thompson FG, Hulbert RC (2011) An annotated checklist and bibliography of the land and freshwater snails of Mexico and Central America. Bull Fla Mus Nat Hist 50:1-299.

Title PO, Bemmels JB (2018) ENVIREM: an expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. Ecography 41:291-307. https://doi.org/10.1111/ecog.02880

Ursenbacher S, Alvarez C, Armbruster GF, Baur B (2010) High population differentiation in the rock-dwelling land snail (Trochulus caelatus) endemic to the Swiss Jura Mountains. Conserv Genet 11:1265-1271. https://doi.org/10.1007/s10592-009-9956-3

Vanselow K, Samimi C (2014) Predictive mapping of dwarf shrub vegetation in an arid high mountain ecosystem using remote sensing and random forests. Remote Sens 6:6709-6726. https://doi.org/10.3390/rs6076709

Wang YH, Yang KC, Bridgman CL, Lin LK (2008) Habitat suitability modelling to correlate gene flow with landscape connectivity. Landsc Ecol 23:989-1000. https://doi.org/10.1007/s10980-008-9262-3

Waske B, Braun M (2009) Classifier ensembles for land cover mapping using multitemporal SAR imagery. ISPRS J Photogramm Remote Sens 64:450-457. https://doi.org/10.1016/j.isprsjprs.2009.01.003

Webster NB, Van Dooren TJ, Schilthuizen M (2012) Phylogenetic reconstruction and shell evolution of the Diplommatinidae (Gastropoda: Caenogastropoda). Mol Phylogenet Evol 63:625-638. https://doi.org/10.1016/j.ympev.2012.02.004

Table 1(on next page)

Pairwise comparison of the resistance values obtained with Circuitscape using the vegetation cover as resistance surface

The numbers in the first column correspond to the geographic centroid of each one of the seven genetic groups recovered by López et al. 2017: 1) Guanaceví, 2) Los Herreras, 3), 4) Topia, 5) Progreso, 6) El Salto and 7) Las Peñas.

Table 1. Pairwise comparison of the resistance values obtained with Circuitscape using the vegetation cover as resistance surface

Pair	LGM	mid Holocene	Current
1,2	209.87	308.20	305.42
1,3	196.76	318.14	294.33
1,4	169.83	227.74	226.22
1,5	309.86	359.06	356.54
1,6	428.33	507.67	518.36
1,7	371.76	424.61	420.29
2,3	235.52	357.59	334.44
2,4	182.15	243.87	247.67
2,5	284.65	341.65	344.16
2,6	402.27	489.54	505.29
2,7	345.54	406.29	407.04
3,4	164.42	236.34	213.96
3,5	308.73	377.72	352.35
3,6	427.46	526.63	514.47
3,7	370.94	443.65	416.48
4,5	224.20	238.48	240.16
4,6	343.37	387.74	402.65
4,7	286.93	304.84	304.74
5,6	209.67	251.50	264.47
5,7	159.03	175.71	173.63
6,7	162.45	227.27	239.57

The numbers in the first column correspond to the geographic centroid of each one of the seven genetic groups recovered by López et al. 2017: 1) Guanaceví, 2) Los Herreras, 3), 4) Topia, 5) Progreso, 6) El Salto and 7) Las Peñas.

Table 2(on next page)

Mantel partial test of the effect of isolation by distance (IBD) and isolation by resistance (IBR) from climate and vegetation surfaces on the genetic differentiation of *Humboldtiana durangoensis* populations for the three temporal frames used in thi

R= Spearman correlation coefficent between pairwise genetic distances ($F_{ST}/(1-F_{ST})$) and the Euclidean distance from the geography and pairwise resistance of CIRCUITSCAPE. p= Statistical significance obtained from 1000 replicates.

Table 2. Mantel partial test of the effect of isolation by distance (IBD) and isolation by resistance (IBR) from climate and vegetation surfaces on the genetic differentiation of *Humboldtiana durangoensis* populations for the three temporal frames used in this study.

Resistance model	Comparison	LGM		mid Holocene		Current	
		R	p	R	P	R	P
Climate	**fst vs resistance**	0.474	0.094	0.368	0.122	0.418	0.111
	fst vs geogra|resistance	0.182	0.321	0.39	0.081	0.295	0.191
	fst vs resistance|geogra	0.105	0.398	-0.181	0.362	-0.074	0.476
Vegetation	**fst vs resistance**	0.057	0.382	-0.144	0.29	-0.111	0.344
	fst vs geogra|resistance	0.782	0.005	0.796	0.003	0.784	0.009
	fst vs resistance|geogra	-0.699	0.012	-0.725	0.013	-0.706	0.021

R= Spearman correlation coefficent between pairwise genetic distances ($F_{ST}/(1-F_{ST})$) and the Euclidean distance from the geography and pairwise resistance of CIRCUITSCAPE.

p= Statistical significance obtained from 1000 replicates.

Table 3(on next page)

Effect of the geographic distance (IBD), vegetation and climate on the genetic differentiation among the seven genetic populations of *Humboldtiana durangoensis* obtained from the distance based redundance analysis (dbRDA) for the three temporal frame

In the marginal test the effect of each one of the variables was evaluated separately, meanwhile, in the conditional test, the effect of the geographic distance was included as a covariate.F represent the proportion of variance, P the statistical significance and % var the percentage of variance explained from each variable

Table 3. Effect of the geographic distance (IBD), vegetation and climate on the genetic differentiation among the seven genetic populations of *Humboldtiana durangoensis* obtained from the distance based redundance analysis (dbRDA) for the three temporal frames used in this study

	Marginal tests			**Conditional tests**		
Variable	**F**	**P**	**% var**	**F**	**P**	**% var**
LGM						
Geographic	6.086	0.02	24.26			0
Vegetation	0.605	0.447	3.087	14.521	0.002	33.819
PCA1	1	0.327	4.999	0.244	0.623	1.013
PCA2	2.979	0.1	13.552	1.308	0.266	5.131
PCA3	0.098	0.748	0.515	1.536	0.238	5.956
mid Holocene						
Geographic	6.086	0.026	24.26			0
Vegetation	0.404	0.537	2.083	19.986	0	39.849
PCA1	2.106	0.166	9.976	1.916	0.177	7.286
PCA2	0.419	0.516	2.158	0.074	0.785	0.312
PCA3	1.211	0.284	5.992	2.888	0.105	10.473
Current						
Geographic	6.086	0.025	24.26			
Vegetation	0.235	0.636	1.224	17.844	6.00E-04	37.704
PCA1	0.408	0.525	2.101	0.138	0.704	0.578
PCA2	2.095	0.161	9.933	1.723	0.213	6.617
PCA3	1.48	0.231	7.228	2.82	0.108	10.258

In the marginal test the effect of each one of the variables was evaluated separately, meanwhile, in the conditional test, the effect of the geographic distance was included as a covariate.F represent the proportion of variance, P the statistical significance and % var the percentage of variance explained from each variable

Table 4(on next page)

Results of the Bayesian inference and model selection obtained from SUNDER to evaluate the relative effect of geography and vegetation cover on the genetic differetiation of the seven genetic groups of *Humboldiana durangoensis*.

G: Euclidean geographic distances; E: Resistance values obtained for the vegetation cover-; G+E: combined effect of both variables. The numbers inside brackets in the iteration column indicate the number fo times that each one of the three models has obtained the lower value of likelihood in ten independent runs. The parameter β represents the magnitude of the effect of the variable on the genetic covariance (-lower values indicate a more importanrt effect).

Tabla 4. Results of the Bayesian inference and model selection obtained from SUNDER to evaluate the relative effect of geography and vegetation cover on the genetic differetiation of the seven genetic groups of *Humboldiana durangoensis*.

Period	**Iteration**	**G**		**E**		**G+E**		
		Likelihood	**~~B~~g**	**Likelihood**	**~~B~~e**	**Likelihood**	**~~B~~g**	**~~B~~e**
LGM	(6, 3, 1)	-8975.22	4.13	-9053.74	521.65	-9044.04	4.15	1102.35
mid Holocene	(3, 3, 6)	-6712.19	3.29	-6672.19	524.79	-6638.51	3.52	2040.62
Current	(3, 2, 5)	-9942	3.63	-9964.55	530.97	-9890.39	3.29	1711.27

G: Euclidean geographic distances; E: Resistance values obtained for the vegetation cover; G+E: combined effect of both variables. The numbers inside brackets in the iteration column indicate the number fo times that each one of the three models has obtained the lower value of likelihood in ten independent runs. The parameter β represents the magnitude of the effect of the variable on the genetic covariance (lower values indicate a more importanrt effect).

Table 5(on next page)

Average size (in mm) for the four measurements used in this study to evaluate the shell shape of seven genetic groups of *Humboldtiana durangoensis*. Shell height (SH), Shell width (SH) Aperture height (AH), Maximum Aperture width (AW). Additionally,

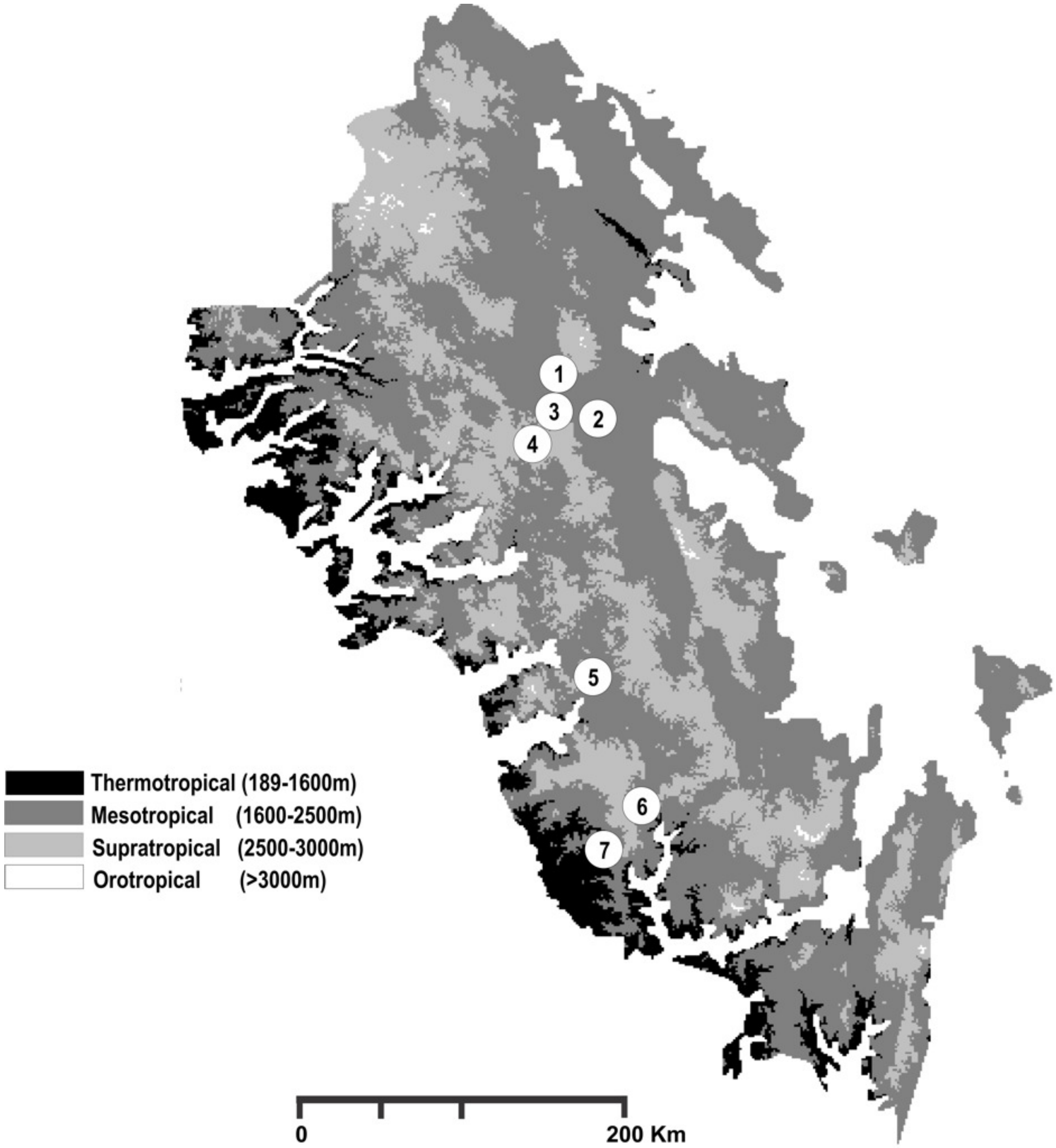
Table 5. Average size (in mm) for the four measurements used in this study to evaluate the shell shape of seven genetic groups of *Humboldtiana durangoensis*. Shell height (SH), Shell width (SH) Aperture height (AH), Maximum Aperture width (AW). Additionally, Globosity index (G), Spire Height (SP) and Shell Volume are showed

Group	SH	SW	ALH	AW	G	SP	V
Guanacevi	32.15	34.45	21.66	19.52	0.93	10.49	3.42
Los herreras	31.94	33	22.06	19.09	0.97	9.88	3.29
Potrero	32.86	35.4	22.32	20.34	0.93	10.54	3.47
Topia	31.13	32.56	23.18	18.98	0.96	7.95	3.22
Progreso	24.11	26.16	19.11	15.81	0.92	5	2.62
El Salto	28.94	31.3	21.28	17.97	0.93	7.66	3.16
Las Peñas	25.51	28.14	19.32	16.29	0.91	6.18	2.87

Figure 1

Geographic map of the Region Madrense Centro in the Mexican state of Durango.

In the upper section a digital elevation model (DEM) was used to highlight the different ombrothermal horizons defined by Macías-Rodriguez et al. (2017). The circles represent the geographic centroid for each one of the seven genetic groups of *Humboldtiana durangoensis* defined from microsatellite markers in López et al. (2017): 1 Guanaceví, 2 Los Herreras, 3 Potrero, 4,Topia, 5 Progreso 6, El Salto and 7 Las Peñas.In the lower section a suitability distribution map from Maxent is showed for the three temporal frames used in this study assuming a minimum training presence from the model A) Last Glacial Maximum (LGM, 0.172) (~~,~~ B) Mid Holocene (0.369)and C) Current time (0.347)


1
2
3
4
5
6
7
Thermotropical (189-1600m)
Mesotropical (1600-2500m)
Supratropical (2500-3000m)
Orotropical (>3000m)
0
200 Km

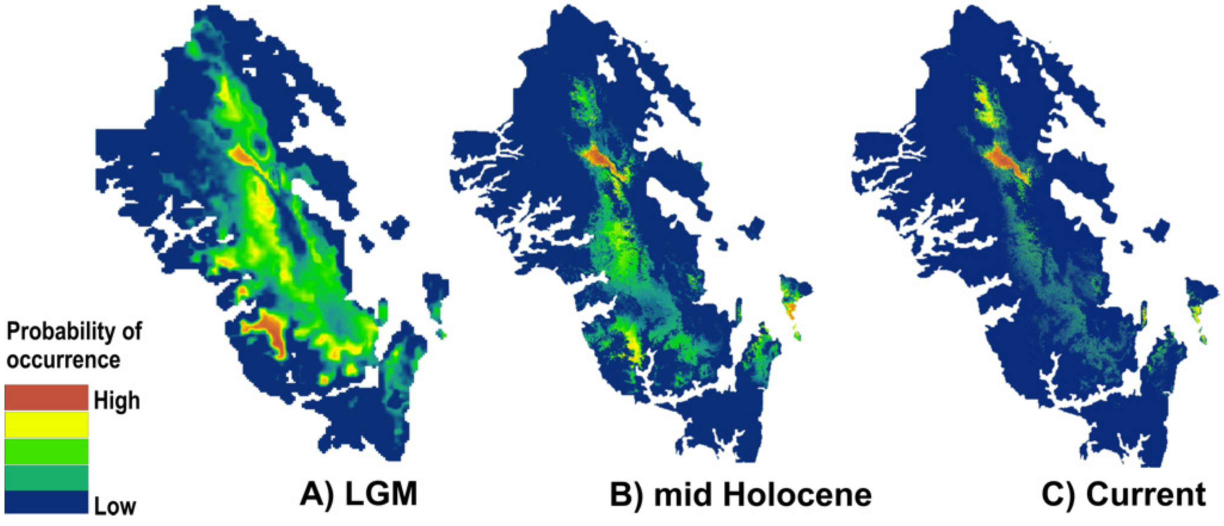
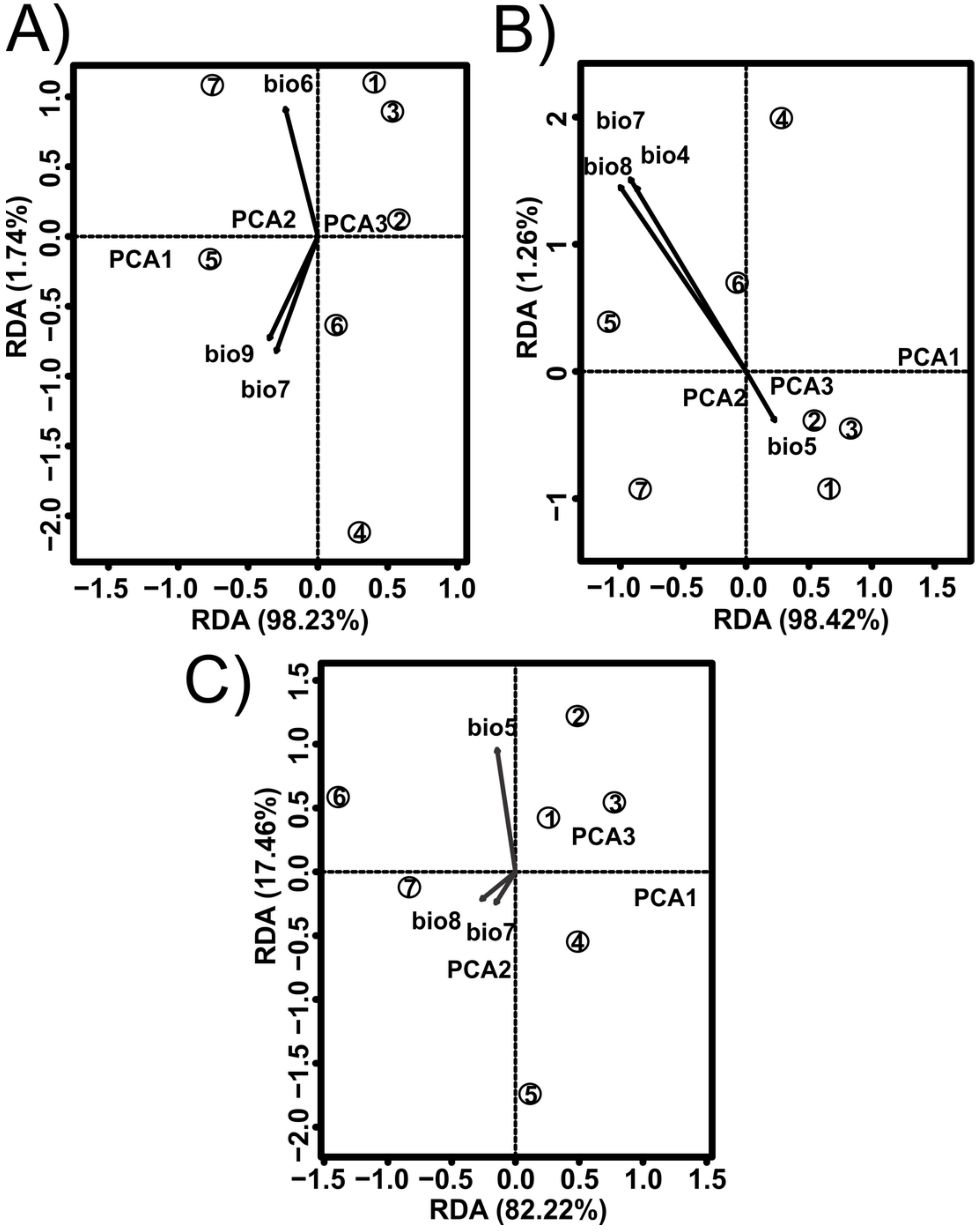

Probability of occurrence
High
Low
A) LGM
B) mid Holocene
C) Current

Figure 2

Redundancy analysis (RDA) for the shell shape of *Humboldtiana durangoensis.*

A) Average size from traditional morphometrics B) Centroid from traditional morphometrics C) Consensus shape from geometric morphometrics and climate variables from Worldclim. The direction and size of the arrows indicate the correlation between climate variables and RDA axes. The circles represent the geographic centroid for each one of the seven genetic groups: 1 Guanaceví, 2 Los Herreras, 3 Potrero, 4,Topia, 5 Progreso 6, El Salto and 7 Las Peñas


A)
⑦
bio6
①
③
PCA2
PCA3
②
PCA1
⑤
⑥
bio9
bio7
④
RDA (1.74%)
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2.0
−1.5
−1.0
−0.5
0.0
0.5
1.0
RDA (98.23%)
B)
bio7
④
bio8
bio4
⑥
⑤
PCA1
PCA3
PCA2
②
③
bio5
⑦
①
RDA (1.26%)
2
1
0
−1
−1.0
−0.5
0.0
0.5
1.0
1.5
RDA (98.42%)
C)
②
bio5
⑥
③
①
PCA3
⑦
PCA1
bio8
bio7
④
PCA2
⑤
RDA (17.46%)
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2.0
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
RDA (82.22%)