

# Incipient speciation in the Chihuahuan Desert shrub *Berberis trifoliolata* under divergent climate scenarios

D.F. Angulo, L.D. Amarilla, and V. Sosa

**Abstract:** The discovery of biotic and ecological factors that influence genetic isolation in populations contributes to our understanding of speciation, one of the most important issues in evolutionary biology. In this paper, we ask whether differences in climate preferences are influencing genetic isolation in two assemblages of populations of *Berberis trifoliolata* Moric., the Northeastern and the Mexican Plateau groups. Agarito, as this species is known, is a remarkable shrub in the arid regions of North America and found mostly in the Chihuahuan Desert. Ecological niche modeling and two tests of niche evolution were carried out. The Ecological Niche Modeling suggests that the potential distribution of the Northeastern group does not predict that of the Mexican Plateau group, and nor does the latter predict the former. Tests of niche evolution indicate a divergent niche between these two groups. Among the most important climate factors detected in the multivariate niche evolution test are mean annual temperature and annual precipitation. We concluded that the two lineages exemplify an incipient speciation process.

**Key words:** North America arid lands, Chihuahuan Desert, divergent niche, ecological niche modeling, Mexican Plateau.

**Résumé :** La découverte de facteurs biotiques et écologiques qui influencent l'isolement génétique dans les populations contribue à mieux faire comprendre le phénomène de spéciation, une des questions les plus importantes de la biologie évolutive. Dans cet article, nous demandons si les différences dans les préférences climatiques influencent l'isolement génétique de deux assemblages de populations de *Berberis trifoliolata* Moric., les groupes du Nord-est et du Plateau mexicain. Agarito, tel qu'on le connaît, est un remarquable arbuste des régions arides de l'Amérique du Nord, que l'on trouve principalement dans le désert de Chihuahua. La modélisation des niches écologiques et deux tests d'évolution des niches ont été réalisés. La modélisation des niches écologiques suggère que la distribution potentielle du groupe du Nord-est ne prédit pas celle du groupe du Plateau mexicain, et que ce dernier ne prédit pas non plus celle du premier. Les tests d'évolution des niches indiquent une divergence de niche entre ces deux groupes. Parmi les facteurs climatiques les plus importants détectés par le test multivariable d'évolution des niches, se trouvent la température annuelle moyenne et les précipitations annuelles. Nous avons conclu que ces deux lignages illustrent un processus initial de spéciation. [Traduit par la Rédaction]

**Mots-clés :** terres arides d'Amérique du Nord, désert du Chihuahua, niche divergente, modélisation des niches écologiques, Plateau mexicain.

## Introduction

A number of phylogeographical studies carried out in recent years have revealed that widespread species are often subdivided into genetically distinct forms that lack morphological differences (e.g., Forsdyke 2013; Johnson et al. 2013; Govindarajulu et al. 2011; Pillon et al. 2009; Carstens and Satler 2013; Paupério et al. 2012). The discovery and characterization of these forms contribute to the understanding of speciation (Sites and Marshall 2003). In plants, as in most organisms, speciation is distinguished by the evolution of barriers to genetic interchange in populations that previously interbred. It is correlated with a number of biological and geohistorical influences that extend ecological opportunities (Rieseberg and Willis 2007).

Among the drivers of speciation, response to variation in climate in space and time is one of the most widely reported (e.g., Mittelbach et al. 2007; Jansson and Dynesius 2002; Barnosky 2005; Lovette 2005; Hoskin et al. 2011) and has recently received much attention (Hua and Wiens 2013). Two hypotheses have been proposed regarding climate and speciation: a scenario of niche conservatism and one of niche divergence. In the niche conservatism

hypothesis, differences in climate act as a barrier to the dispersal of allopatric populations (Wiens 2004b; Kozak and Wiens 2006). Niche conservatism occurs, in part, because of the inability of species to adapt to novel abiotic conditions, limiting their adaptation to the new ecological conditions and preventing gene flow between the two sets of populations (Wiens 2004a; Wiens and Graham 2005; Hua and Wiens 2010). Alternatively, populations experiencing different climatic conditions might undergo divergent selection that promotes reproductive isolation, as suggested by the hypothesis of ecological speciation (Schluter 2009), which corresponds to a divergent niche scenario.

To test whether speciation occurs in these two scenarios, ecological niche modeling has been employed to detect differences in climate in the habitats of lineages or closely related species (e.g., Fontanella et al. 2012; Zhao et al. 2013; Sánchez-Fernández et al. 2011; Theodoridis et al. 2013). Ecological niche models are created based on species occurrence data combined with environmental data, usually climate variables, and are generated with geographic information systems (see Elith and Leathwick 2009). These models identify locations with suitable abiotic conditions for species and a number of algorithms can be used to fit the models (Soberón and

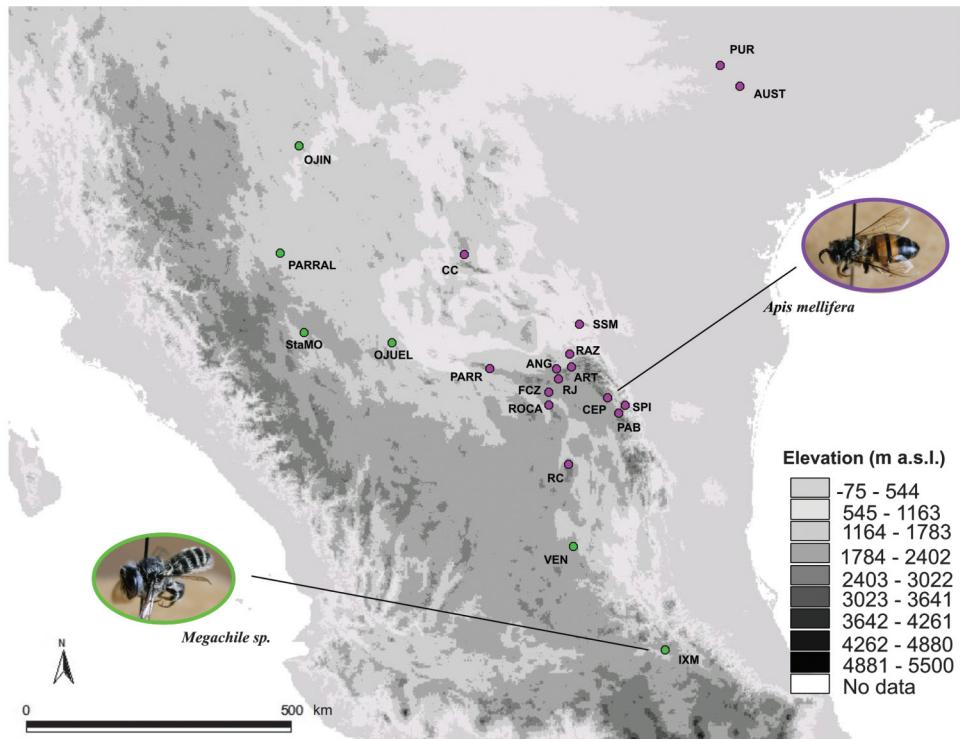
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**D.F. Angulo and V. Sosa.** Biología Evolutiva, Instituto de Ecología AC, Carretera antigua a Coatepec 351, 91070 Xalapa, Veracruz, Mexico.

**L.D. Amarilla.** Instituto Multidisciplinario de Biología Vegetal, CONICET-Universidad Nacional de Córdoba, Av. Vélez Sársfield 299, 5000 Córdoba, Argentina.

**Corresponding author:** Victoria Sosa (e-mail: [victoria.sosa@inecol.mx](mailto:victoria.sosa@inecol.mx)).

**Fig. 1.** Populations of *Berberis trifoliolata* sampled in the arid lands of North America. Light grey circles (green on the Web site only) indicate the Mexican Plateau populations and the dark grey circles (purple on the Web site only) indicate the Northeastern populations. Pollinators observed in IXM and CEP are shown. (For definitions of population abbreviations, see Table 1.)



**Table 1.** Populations of *Berberis trifoliolata* studied, indicating population abbreviation, number of individuals ( $N_{ind}$ ), geographic region, latitude, longitude, and the elevation where they were collected.

Sample location	Abbreviation	$N_{ind}$	Region	N Latitude	W Longitude	Elevation (m a.s.l.)
US, Austin, Texas	AUST	5	Northeastern	30.14283	-97.96271	241
US, Purola, Texas	PUR	5	Northeastern	30.48536	-98.28256	300
Mexico, Real de Catorce, San Luis Potosi	RC	5	Northeastern	23.73769	-100.8455	1832
Mexico, at the Coahuila-Zacatecas border	FCZ	5	Northeastern	24.98063	-101.17952	1950
Mexico, Rancho Jaguey, Coahuila	RJ	5	Northeastern	25.23051	-101.01930	2075
Mexico, Parras, Coahuila	PARR	5	Northeastern	25.36014	-102.17508	1811
Mexico, Arteaga, Coahuila	ART	5	Northeastern	25.39958	-100.79894	1815
Mexico, La Angostura, Coahuila	ANG	6	Northeastern	25.33953	-101.04506	1804
Mexico, Ramos Arizpe, Coahuila	RAZ	4	Northeastern	25.61408	-100.83078	1297
Mexico, Cuatro Cienegas, Coahuila	CC	5	Northeastern	27.30289	-102.61372	1525
Mexico, Sierra de San Miguel, Nuevo Leon	SSM	5	Northeastern	26.11144	-100.65544	1103
Mexico, Pabellon, Nuevo Leon	PAB	6	Northeastern	24.60978	-100.00183	2063
Mexico, Cerro El Potosi, Nuevo Leon	CEP	10	Northeastern	24.88603	-100.18894	2130
Mexico, San Pedro Iturbide, Nuevo Leon	SPI	6	Northeastern	24.72400	-99.90897	1571
Mexico, Parral, Chihuahua	PARRAL	5	Mexican Plateau	27.32045	-105.71926	1816
Mexico, Ojinaga, Chihuahua	OJIN	5	Mexican Plateau	29.14910	-105.39052	1470
Mexico, Ojuelas, Durango	OJU	4	Mexican Plateau	25.79861	-103.78402	1473
Mexico, Santa Maria del Oro, Durango	StaMO	4	Mexican Plateau	25.98615	-105.32809	1400
Mexico, Rocamontes, Zacatecas	ROCA	5	Mexican Plateau	24.74164	-101.17501	1968
Mexico, Ixmiquilpan, Hidalgo	IXM	6	Mexican Plateau	20.61348	-99.23509	1846
Mexico, Ventura, San Luis Potosi	VEN	5	Mexican Plateau	22.38197	-100.77363	1777

Nakamura 2009). To detect comparable niches between two closely related species, several niche similarity metrics and randomization tests have been proposed. Warren et al. (2008) proposed a method that considers overlap between models of habitat suitability in the space of environmental variables and McCormack et al. (2010) proposed a multivariate approach that generates null expectations for niche divergence, which is applicable in lineages in the early stages of speciation.

We selected the shrubby species *Berberis trifoliolata* Moric. (Berberidaceae) to learn which factors lead to lineage divergence. *Berberis trifoliolata* is a shrub in the arid lands of North America; in the Sonoran Desert, it is found in Arizona and New Mexico and in the Chihuahuan Desert, populations spread from Texas to the southern part of the Mexican Plateau (see Fig. 1). Differences in annual mean temperature and annual precipitation in the areas that *B. trifoliolata* inhabits are remarkable (elevated temperatures

in the north and elevated precipitation in the south). Northernmost and southernmost populations are found in lower altitudes, whereas some populations in Chihuahua and Nuevo León reach higher altitudes along the Sierra Madre Oriental as well as in the elevated Mexican Plateau (see Fig. 1).

Agarito or algerita, as *B. trifoliolata* is commonly known, produces showy yellow flowers and red fruit. Populations from the northern part of its distribution are pollinated by alien bees, *Apis mellifera* Linnaeus, whereas southern populations are pollinated by native bees, among them *Megachile Latreille* sp. (Angulo et al. 2014; see Fig. 1). Our current phylogeographic analyses based on two plastid DNA regions and amplified fragment length polymorphism (AFLP) markers identified two groups of populations, one in the northeastern area of distribution and a second in the Mexican Plateau. Furthermore, there is no correlation between the genetic and geographical distances of these groups of populations. Both assemblages have different evolutionary histories and inhabit areas with different environmental conditions (Angulo et al. 2014). Agarito offers a good test case for understanding whether differences in climate have influenced the divergence of lineages with genetic isolation. *Berberis trifoliolata* is also a species for which a phylogeographic study has identified subdivided groups of populations that exhibit no morphological differences. Our hypothesis is that due to the complex topography and climate differences in its area of distribution, genetic differentiation in agarito occurred in a divergent niche scenario.

The objectives of this paper are: (i) to determine whether geographic or climatic factors have influenced the genetic divergence in *B. trifoliolata* populations and (ii) to determine whether divergence has occurred under a divergent or conserved niche scenario.

## Materials and methods

### Sampling

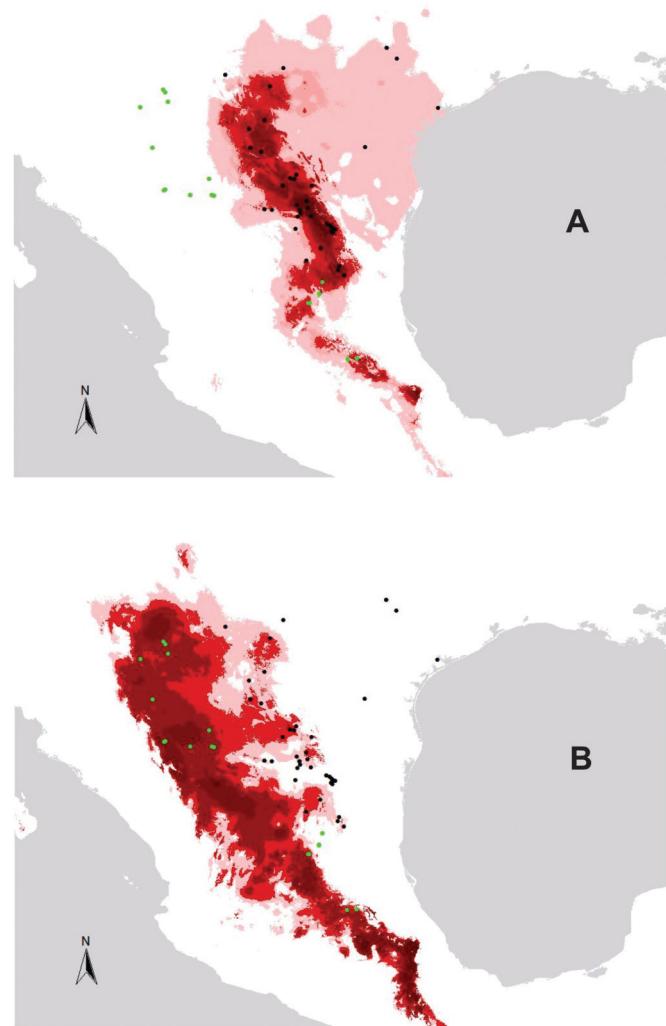
Twenty-one populations of *B. trifoliolata* were sampled including areas throughout its entire range of distribution for our current phylogeographic study (Fig. 1 and Table 1) (Angulo et al. 2014). They were used to build ecological niche models in this study. The geographical coordinates of these plants collected in the field were used and complemented with information available for specimens from herbaria such as ARIZ, ENCB, MEXU, TEX, and XAL to generate the Ecological Niche Modeling (ENM). Records separated by less than a kilometer were not included and a total of 66 records were used (Table 1).

### Ecological Niche Modeling

ENM was performed for each group of *Berberis* populations retrieved by our previous phylogeographic study. Nineteen environmental variables derived from temperature and precipitation data were obtained from WorldClim 1.4 (Hijmans et al. 2005) at a resolution of 1 km<sup>2</sup>. A correlation analysis was performed to eliminate correlated environmental variables using the program PAST version 2.12 (Hammer 2011) and only the least correlated variables (Pearson  $\leq 0.7$  based on all sample locations) were used.

All models were run in MaxEnt using the following values: convergence threshold =  $10^{-5}$ , maximum iterations = 1000, regularization multiplier = 1, and the default values for the other modeling parameters. MaxEnt uses only species presence data and performs well with low sample sizes (Phillips et al. 2006; Hernandez et al. 2006). Models were made by partitioning the records of the population between the two main groups found and analyses for each data set were performed using 46 records for the Northeastern group and 21 records for the Mexican Plateau group. Models were then evaluated by the area under the receiver-operating characteristic curve (AUC) (Peterson 2007; Lobo et al. 2008). AUC scores were calculated first using all records, and then using 75% training vs. 25% testing data sets (Fielding and Bell 1997).

**Fig. 2.** Ecological niche modeling for the two groups of populations of *Berberis trifoliolata*. Current predicted distributions for (A) the Northeastern group and (B) the Mexican Plateau group. Grey circles (green on the Web site only) indicate the Mexican Plateau populations and black circles indicate the Northeastern populations.



In addition, the models were evaluated using a null model procedure (Raes and ter Steege 2007). One thousand random presence data sets (equal to the number in our data set) were produced with ENMTools version 1.3 (Warren et al. 2010). The AUC for each data set was estimated to determine if the AUC of the *B. trifoliolata* data set exceeded the 95th percentile of the null-distribution dataset. A species model performs better than random (e.g., null model) and it is considered significant if the calculated AUC is greater than the corresponding 95th percentile AUC of the null-distribution (Raes and ter Steege 2007).

### Ecological niche evolution

Niche evolution analysis was based on raw environmental data and multivariate analysis following McCormack et al. (2010). The raw data of the uncorrelated environmental variables were extracted for the Northeastern and Mexican Plateau groups' occurrence points and the 1000 points taken randomly from the background area (polygons drawn around the occurrence points). Then, a principal components analysis (PCA) was run using the correlation matrix with Stata version 11.0 (StataCorp LP (StataCorp 2003)) to reduce the raw environmental data to a smaller set of axes, which explained most of the variation. The first four axes, which ex-

**Table 2.** Test of niche divergence in the two groups of populations of *Berberis trifoliolata* in the Chihuahuan Desert.

Pairwise comparison	Niche axes			
	PC 1	PC 2	PC 3	PC 4
Northeastern vs. Mexican Plateau (observed)	<b>1.68 C</b>	<b>0.94 D</b>	<b>0.62 D</b>	<b>0.95 D</b>
95% CI	3.3162 3.3189	0.7821 0.7893	0.1825 0.1855	0.6110 0.6125
% variance explained	40	34	14	6
Variable loadings	Bio2 Bio19	Bio11 Bio6	Bio1 Bio7	Bio12 Bio9
Biological interpretation	Temp & Prec	—	Temp	Prec & Temp

Note: Divergence on independent niche axes was analyzed with a principal components analysis. Bold values indicate significant niche divergence (D) or conservatism (C) compared with the null distribution based on background divergence between their respective geographic ranges. CI, confidence interval; PC, pairwise comparison; Prec, precipitation; Temp, temperature.

plained 94% of the total variance, were used for comparison between the two groups. On each of the four axes, niche divergence and conservatism were tested against a null model of background divergence by comparing the observed difference in mean niche values on a given axis vs. the difference in mean background values. Significance was assessed with 1000 jackknife replicates (deleting 25% of the matrix) of the background values. Significance for rejecting the null distribution was evaluated at the 95% level. Mean differences and jackknife replication were calculated using routines developed by McCormack et al. (2010).

An additional analysis of niche evolution was performed using a set of comparative similarity measures and statistical tests estimated with a graphical interface called ENMTools (Warren et al. 2010) that interacts with MaxEnt (Phillips et al. 2006; Phillips and Dudik 2008). This approach measures Schoener's (1968) similarity *D* and a measure derived from the Hellinger distance called *I* (Warren et al. 2008). Values for these measures ranged from 0 to 1, with values close to 1 indicating high similarity between niches, and values close to 0 indicating niche dissimilarity. Having obtained these values, we then carried out the background test of niche evolution. The background test asks whether the Ecological Niche Models of two or more entities are more or less similar than expected by chance considering the environment available to them (as opposed to the exact locations in which the species occur). Based on our errors of omission and commission for the background test, we assumed the area with a prediction threshold  $\geq 0.3$  of the ENMs generated with MaxEnt (Phillips et al. 2006; Phillips and Dudik 2008) as the background area. For both tests, we used 100 pseudoreplicates and significance for both analyses was tested with confidence levels of 0.05 and 0.01.

## Results

### Ecological Niche Modeling

The ENM results indicate that the models performed well (using all records and training-testing data with  $AUC > 0.95$ ). The  $AUC$  for each group was better than random (i.e., model  $AUC$  values exceeded the 95th percentile of the null  $AUC$  distributions). ENM for the Northeastern group indicates a potential distribution over the Sierra Madre Oriental mountain range, in the western part of the Tamaulipan Scrub and some areas in southeastern Texas (Fig. 2A), whereas in the Mexican Plateau group, the predicted occupancy area was adjacent to the western side of the Chihuahuan Desert (Fig. 2B). ENM in both groups predicted occupancy in the Mezquital Valley. The climate variables that contributed most to the model are different in the two groups. The mean temperature of the coldest quarter (Bio11) and mean temperature of the driest quarter (Bio9) made the greatest contributions for the Northeastern group, whereas for the Mexican Plateau group, precipitation in the coldest quarter (Bio19) and precipitation seasonality (Bio15) made the greatest contributions.

In the multivariate method to test scenarios of a conserved or divergent niche between the Northeastern and Mexican Plateau

**Table 3.** Contributions of uncorrelated environmental variables to the Ecological Niche Models in *Berberis trifoliolata*.

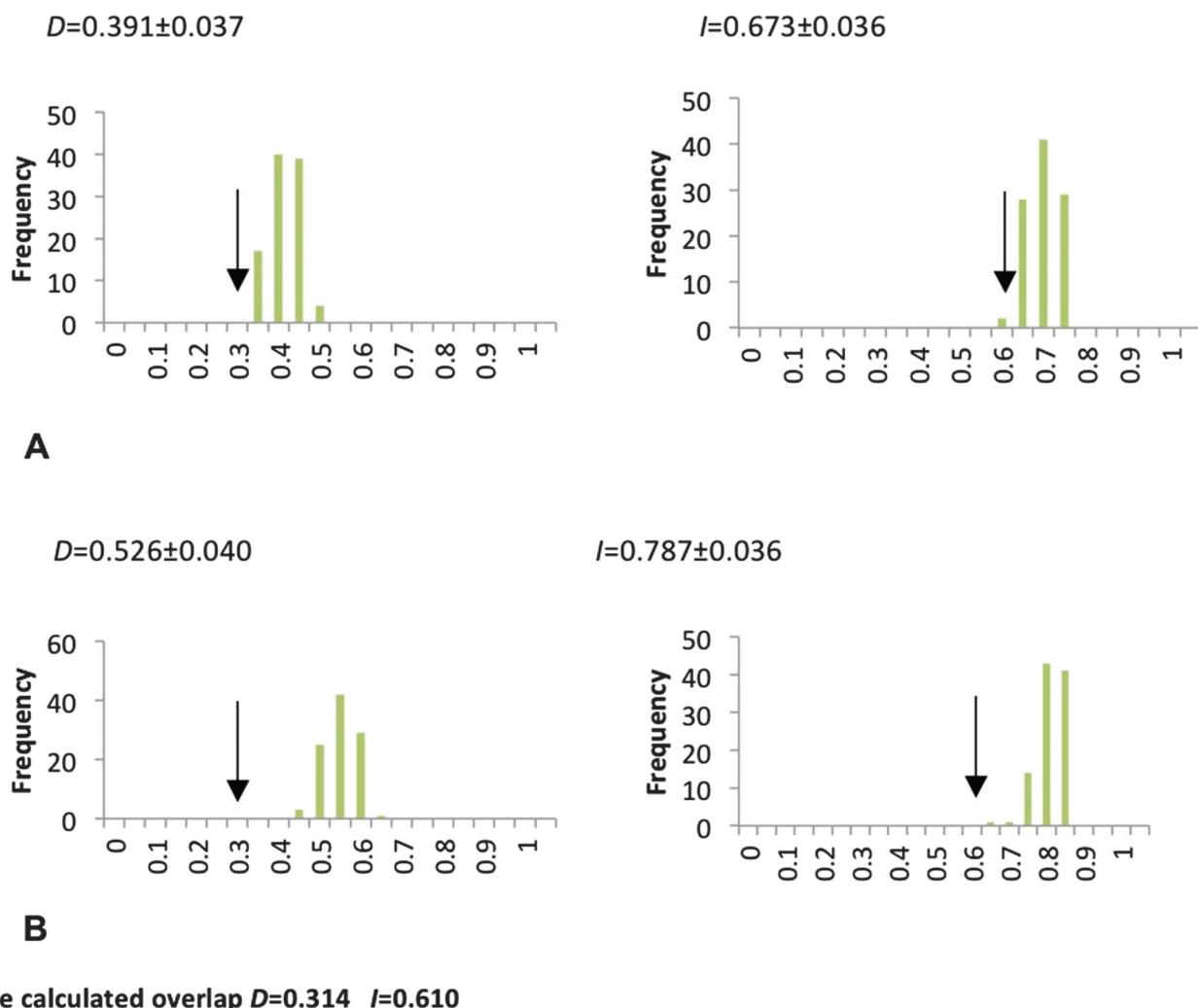
Variable	Northeastern	Mexican Plateau
Bio 1	2	0
Bio 2	3.9	0
Bio 3	1.2	9
Bio 6	2.7	24.5
Bio 7	32.2	1.9
Bio 9	17.4	1.5
Bio 11	0.7	1.2
Bio 12	13.3	6.9
Bio 15	14.8	28.3
Bio 19	11.8	26.7

groups, we detected divergence on niche axes between the two groups associated mainly with temperature. The first four PCA components explain 94% of the variation (Table 2). Only the first component — associated with diurnal temperature and cold precipitation — shows evidence of niche conservatism between the two groups. While the remaining components associated with annual temperature, the coldest and driest temperatures and annual precipitation indicate that both groups diverge in their niches (see Tables 2 and 3). In the background test of niche evolution between the Northeastern and the Mexican Plateau lineages, the *D* and *I* values were lower than expected from the null model, indicating niche divergence between the two groups (Fig. 3).

## Discussion

Researchers have long identified the importance of both geographic and ecological barriers in the isolation of species (Stebbins 1950). Ecological barriers can influence adaptation to different environmental conditions and potentially affect the connection between populations which, in turn, promotes divergence (Rundell and Price 2009; Nosil et al. 2009). Our results from using two different niche approaches that incorporated null models of environmental background lead to a similar conclusion that niche divergence exists between the two genetic groups of agarito populations. The basic idea of using a null model is to avoid concluding that the divergence observed between species or groups is due to spatial autocorrelation in environmental variables between the regions over which the species or groups are distributed (Warren et al. 2008; McCormack et al. 2010). The two niche approaches have different formulations. Warren et al.'s (2008) background test is based not only on the current data, but also on the available habitat of the species to use as background for the niche model prediction. Whereas by using McCormack et al.'s (2010) approach, the inference does not rely on the use of Ecological Niche Models but rather on the raw environmental data. This multivariate method provides more detailed information on niche divergence, as it is in better keeping with the Hutchinsonian idea of the niche

**Fig. 3.** Background test null distributions for the Northeastern and Mexican Plateau lineages of *Berberis trifoliolata*. Arrows indicate results of niche overlap. Columns indicate the null distribution. (A) Background test comparing the Northeastern and Mexican Plateau groups. (B) Background test comparing the Mexican Plateau and Northeastern groups.



as a multidimensional hypervolume (Hutchinson 1957), in which some axes will remain conserved while others diverge.

Our results indicate that the genetic discontinuity between the Northeastern and Mexican Plateau populations of *B. trifoliolata* is strongly influenced by environmental factors (most notably temperature) and to a greater extent than by geographic distance or geological barriers, suggesting there are different climate selection pressures in each habitat. Some variables are similar between the two areas of *B. trifoliolata* distribution, such as mean diurnal temperature range (Bio2) and precipitation during the coldest quarter (Bio19). However, variables such as cold temperatures (Bio11 and Bio6), annual temperature (Bio1 and Bio7), and annual precipitation ranges (Bio12) are different between the two geographical distribution areas. It remains to be seen whether shifts in pollinators have also contributed to maintaining genetic isolation, as observed in other populations of agarito (Angulo et al., in press).

The trend toward a specialized niche also is observed in the ENM results, for which the potential distribution area of the Northeastern group predicts only some populations of the Mexican Plateau group and that of the Mexican Plateau group slightly predicts the Northeastern group. The multivariate niche evolution method suggests that although in general the niche is divergent, one of the four PC axes indicates strong evidence of a

conserved niche between both groups. *D* and *I* values from the background niche test indicated that there was no overlap of ecological niches between the two groups. Studies have shown that different climate habitats could act as a barrier to gene flow influencing reproductive isolation, and minimizing connectivity among populations (e.g., Sobel et al. 2010; Kozak and Wiens 2007). However, the evidence of a conserved niche from the multivariate niche method might suggest that although individuals of *B. trifoliolata* are able to colonize new areas, they inhabit zones where only certain climate variables change. As mentioned above, our results indicate that the climate variables which are the same for both groups are mean diurnal temperature range and precipitation during the coldest quarter.

The Northeastern and Mexican Plateau populations of *Berberis* have evolved in a divergent niche scenario. A divergent niche among close relatives has been detected in other plants as a response to different factors such as drought stress (*Mimulus* L.; Peterson et al. 2013), salt tolerance (*Mimulus guttatus* DC.; Lowry et al. 2008), and host plant adaptation with the beetle *Neochlamisus Karren* (Funk et al. 2011).

Our morphological observations have not detected diagnostic characters for the two groups of populations. Species concepts are being challenged by molecular data and there is some debate about whether cryptic taxa should be named (e.g., Kadereit et al.

2012; Slenzka et al. 2013). Furthermore, a DNA taxonomy system has been proposed as a platform for taxonomic knowledge and as a convenient tool for species identification (Tautz et al. 2003). Therefore, we will not name these two sets of populations as new species.

## Conclusions

Our results suggest that the divergence of the groups occurred under a divergent niche scenario. The main differences in climate variables between the two groups are cold temperatures, annual temperature, and annual precipitation ranges. We conclude that the two lineages exemplify an incipient speciation process.

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