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Evidence of incipient speciation in the Neotropical mangrove *Pelliciera rhizophorae* (Tetrameristaceae) as revealed by molecular, morphological, physiological and climatic characteristics

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Two variants of the Neotropical mangrove species *Pelliciera rhizophorae* distributed along both sides of the Isthmus of Panama were detected by different colouration of the floral bracts and the size of the floral and vegetative structures. These findings raised questions concerning a possible speciation event in *P. rhizophorae*, for which a series of macro- and microscopic morphological traits (reproductive and vegetative structures), molecular markers from plastid DNA and climatic profiles were analyzed. Samples of *P. rhizophorae* were collected in three localities from the Panamanian Caribbean and Pacific coasts. The data obtained from molecular markers and morphological traits showed significant differences between the variants. The climatic profiles showed contrasting characteristics of rainfall and temperature in their habitats: variant A is found in wetter zones and variant B occupies drier zones. Evidence suggesting that a process of incipient speciation has occurred in *P. rhizophorae* in response to ecogeographical isolation due to climatic factors is presented. The presence of two geographically separate genetic-morphological groups, adapted to contrasting climatic conditions, will be the basis for suggesting the existence of incipient lineages in *Pelliciera*. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, ••, ••–••.

ADDITIONAL KEYWORDS: climate – molecular markers – morphology – speciation.

INTRODUCTION

The genus *Pelliciera* Planch. & Triana (Tetrameristaceae) comprises just one species, *P. rhizophorae* Planch. & Triana, which forms part of the Neotropical mangrove ecosystem that encompasses the Pacific Coast almost continuously from Costa Rica to Ecuador and some patches in the Caribbean, located in Nicaragua, Panama and Colombia (Jiménez, 1984). Given that its presence dates from the Eocene epoch (Graham, 1977) with a wide distribution in the Caribbean, *P. rhizophorae* is considered to be the oldest mangrove species in the Neotropical mangrove ecosystem; however, its populations have gradually reduced, possibly being displaced by interspecific competition with other groups such as *Rhizophora* L. and *Avicennia* L. (Jiménez, 1984). The most recent fossil records, found in Venezuela, suggest that its disappearance in the Caribbean occurred ca. 2 Mya (Lorente, 1986). Currently, the geographical distribution of *P. rhizophorae* populations in the Caribbean mangrove ecosystem is limited to isolated patches (Rull, 1998); whereas on the Pacific Coast, the species is distributed almost continuously from Costa Rica to Ecuador (Jiménez, 1984). Within the species, phenotypic differences have been detected according to descriptions of Colombian populations (Calderón-Saenz, 1984; von Prahl, 1987). Contrasting

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traits can be noted in the colour of the floral bracts (pink or red in Cartagena Bay-Colombian Caribbean vs. white in forests from the Colombian Pacific) and in height of mature trees (maximum 4 m in the Colombian Caribbean-Cartagena Bay vs. an average height of 15 m in the Colombian Pacific, reaching up to 27 m in Utria Cove-Chocó). On the Panamanian coast, small trees (maximum 3 m) with pink or red floral bracts are found in the Pacific-Panama Canal Zone, Chame and Dry Arc Region; whereas taller trees (up to 20 m) with white floral bracts are found in the Caribbean (Bocas del Toro). A recent study reported that nutrient limitation might be involved in the presence of dwarf forms of *P. rhizophorae* in populations located on the Panamanian Caribbean and Pacific coasts (Dangremond & Feller, 2014). Given that other environmental factors could be responsible for the existence of two morphological variants in *P. rhizophorae* species on different sides of the Isthmus of Panama, this study characterizes these variants and the climatic factors influencing the patterns of their geographical distribution.

Environmental factors have played an important role in the diversification processes in nature (MacColl, 2011). West-Eberhard argued that the most important initiator of evolutionary novelties is environmental induction given that populations of a species can be isolated due to an adaptive process under the influence of the environment (West-Eberhard, 2003). Given that climate is an important environmental factor determining the eco-geographical isolation of populations and based on ecological speciation when different populations of a species occupy different climate niches, it was hypothesized that contrasting conditions of their habitat may impose divergent selection that drives the evolution of reproductive isolation between them (Schluter, 2009; Hua & Wiens, 2013). Consequently, the formation of incipient species is the result of reproductive isolation, as one species cannot survive in the habitat of the other. Recent studies have reported the influence of climate in diversification processes (Kozak & Wiens, 2006; Nooryazdan *et al*., 2010; Pringle *et al*., 2012; Fregonezi *et al*., 2013; Pyakurel & Wang, 2013). Accordingly, ecology and speciation have given rise to a new hypothesis in the evolution of species that are separated by differences in climatic niche characteristics, known as niche conservatism (Wiens & Graham, 2005), which has an important role in allopatric speciation because it limits adaptation to ecological conditions.

Our most recent work on genetic structure and phylogeography of *P. rhizophorae* revealed the presence of two genetic clusters differentiated significantly by nuclear microsatellites and plastid haplotypes (Castillo-Cárdenas *et al*., 2014). Both genetic clusters were found on the Caribbean and Pacific coasts, but they were not mixed locally. The geographical distribution pattern of these genetic clusters permitted us to formulate new questions about the factors involved in the local establishment of populations. Around this scenario, we proposed the influence of climate as a factor affecting the survival of *P. rhizophorae* colonizers and preventing the random distribution of gene pools. Rainfall and temperature have a direct influence on the concentration of salt in the soil and, given that *P. rhizophorae* is the mangrove species least tolerant to high salt concentrations (Jiménez, 1984), the climate would be a determining factor in the spatial distribution of the genetic clusters. Taking into account these facts and our previous findings (Castillo-Cárdenas & Toro-Perea, 2012; Castillo-Cárdenas *et al*., 2014), our first objective was to compile new evidence regarding a diversification process that occurred in *P. rhizophorae*, revealed initially by DNA markers. We evaluated morphological traits of floral and vegetative structures and plastid DNA markers in order to characterize the two intraspecific variants recognized by contrasting phenotypic traits. Our second objective was to evaluate whether climate was the key factor in the diversification process. Based on the ecological speciation hypothesis, in which the variants of *P. rhizophorae* are products of incipient speciation due to reproductive isolation between populations adapted to different climatic conditions, we expected that the patterns of morphological and physiological differentiation between variants would be strongly correlated with local bioclimatic patterns.

MATERIALS AND METHODS

Sampling zones and plant material

Molecular analyses were carried out on 36 samples from nine *P. rhizophorae* populations distributed along both Neotropical coasts. Twelve individuals of *P. rhizophorae* from the Caribbean coast [Bocas del Toro (BTO), Panama; Cispata Bay (CIS) and Barbacoas Bay (BAR), Colombia] and 24 individuals from the Pacific Coast [Tempisque Gulf (TEM), Costa Rica; El Pedregal (PED), Chame Point (CHA) and Panama Canal Zone (PCZ), Panama; Utria Cove (UTR), Colombia; La Tola (TOL), Ecuador] were evaluated (Fig. 1). For the morphological analysis, three of the Panamanian populations included in the molecular analysis were chosen for their contrasting phenotypes revealed by floral bract colour (Table 1, Appendix). Each variant was characterized as follows: variant A with white to cream floral bracts and variant B with pink to red bracts. All morphological traits were measured in one population of variant A (BTO) and

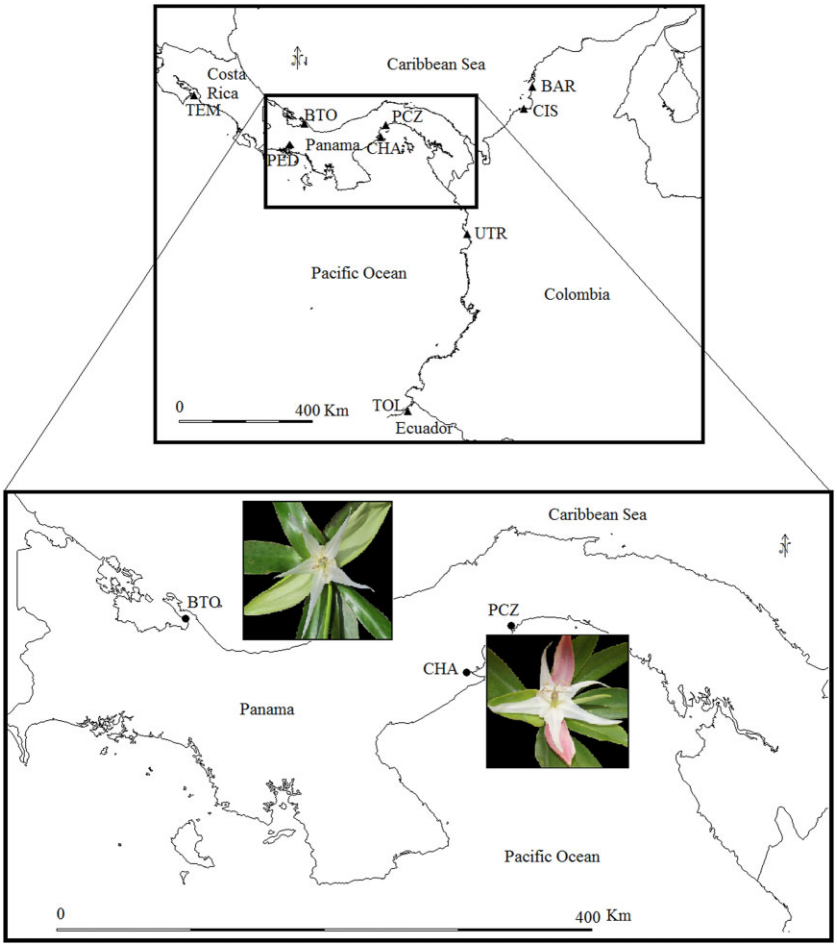

Figure 1. Sampling zones of *Pelliciera rhizophorae* and floral morphotypes. Triangles indicate the nine populations included in molecular analysis. Circles indicate the three populations *used in morphological analyses*. Population abbreviations: Tempisque (TEM), El Pedregal (PED), Chame Point (CHA), Panama Canal Zone (PCZ), Utria Cove (UTR), La Tola (TOL), Bocas del Toro (BTO), Cispata Bay (CIS), Barbacoas Bay (BAR).

Table 1. Collection localities for samples of *P. rhizophorae*

Variants	Variant A	Variant B	
Localities	Bocas del Toro (BTO)	Chame (CHA)	Panama Canal Zone (PCZ)
Coordinates	9°0′38.40″N, 81°47′27.66″O	8°38′37.74″N, 79°52′19.08″O	8°57′59.74″N, 79°34′14.38″O
Vegetative growth	10–15 m	50 cm–2 m	1 m–3 m
Floral bracts colour	White	Pink	Pink–Red
Clustering by microsatellite analyses[†]	Cluster II	Cluster I	Cluster I
Maximum temperature (°C)	29.5	31.2	30.9
Minimum temperature (°C)	22.4	23.3	23
Annual mean rainfall (mm)	3050	1643	1866
Flowering period	All the year, maximum at the end of year	February–June	February–June

[†]Castillo-Cárdenas *et al*. (2014).Description of genetic-morphological variants and climate profile of localities sampled.

two populations of variant B (CHA and PCZ). Two populations of variant B, both small patches, were analyzed in order to obtain a more representative group for this variant. Morphological traits were measured in five individual flowers and ten mature leaves collected from ten trees per variant, totalling 50 flower samples and 100 foliar samples per variant.

Analyses of molecular data

DNA was obtained from dried leaves of *P. rhizophorae*, following the protocols described by Castillo-Cárdenas *et al*. (2014). The molecular analyses included three noncoding regions of plastid DNA (*psbJ–petA*, *psbD–trnT*, *atpI–atpH*). The PCR products were obtained using universal primers and standard protocols (Shaw *et al*., 2007) as follows: the PCR reaction was carried out in a total volume of 10 μL containing 1× PCR buffer, 3 mM $MgCl_2$, 200 μM each dNTP, 0.1 μM each primer, 1.25 U *Taq* DNA polymerase and 10 ng total DNA. A conventional protocol for PCR amplification was used: the initial denaturation step (94 °C for 2 min) was followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s. The final extension step used 72 °C for 2 min. Bidirectional sequencing for the three regions in the plastid DNA was run on a 3100 genetic analyzer (Applied Biosystems Inc.). Sequences were edited and assembled using Sequencher 4.6 software (Gene Code Corporation, 2007) and manually aligned using MacClade 4.1 (Maddison & Maddison, 2005). The best evolutionary model was obtained with jModelTest (Posada, 2008), which selected the JC model for the combined dataset. An unrooted tree using the neighbour-joining (NJ) method was created in MEGA 6 (Tamura *et al*., 2013). Branch support was provided by a bootstrap analysis of 1000 replicates. A network of haplotypes was built with NETWORK 4.6.1.3 (Fluxus-Technology-Ltd, 2004–2010), using the median-joining method.

Morphological traits and data analyses

The macroscopic traits measured in the two variants under study were length and width of the petals, sepals and bracts; length of the filaments, anthers and pistils; and length and width of the leaves. The measurements of these traits in flowers and mature leaves were made using a digital caliper. The microscopic traits included stomatal density in the adaxial and abaxial axes of the leaf, dividing the leaf blade into nine quadrants (I, apex, II, middle, III, base; a, narrow side, b, midrib, c, broad side). The stomatal counts were done for a 1 mm^2 area under a laser scanning confocal microscope (Olympus FV1200) with ×20 magnification.

The data resulting from the morphometric traits were analyzed using the STATISTICA program version 10 (StatSoft I, 2011). Average measurements of each foliar and floral trait were obtained. Data normality was tested using the estimator of kurtosis and symmetry. In all cases, it was necessary to apply non-parametric statistics to compare the two variants in independent groups, using the Mann–Whitney *U*-test. A data matrix with 11 foliar and floral morphometric variables was standardized and analyzed using UPGMA cluster analysis based on a Euclidean distance matrix of the software NTSYS 2.02g (Rohlf, 2000).

Analyses of climatic profiles

To assess whether the climate is a determining factor in the distribution of variants of *P. rhizophorae*, we analyzed 19 bioclimatic variables obtained from the WorldClim database (data recorded from 1950–2000, interpolated with a 1-km resolution): BIO1, annual mean temperature; BIO2, mean diurnal range (mean of monthly maximum–minimum temperature); BIO3, isothermality (BIO2/BIO7) (× 100); BIO4, temperature seasonality (SD × 100); BIO5, maximum temperature of warmest month; BIO6, minimum temperature of coldest month; BIO7, annual temperature range (BIO5-BIO6); BIO8, mean temperature of wettest quarter; BIO9, mean temperature of driest quarter; BIO10, mean temperature of warmest quarter; BIO11, mean temperature of coldest quarter; BIO12, annual rainfall; BIO13, rainfall of wettest month; BIO14, rainfall of driest month; BIO15, rainfall seasonality (coefficient of variation); BIO16, rainfall of wettest quarter; BIO17, rainfall of driest quarter; BIO18, rainfall of warmest quarter; and BIO19, rainfall of coldest quarter (Hijmans *et al*., 2005). The bioclimatic variables were extracted for 20 populations in which the presence of *P. rhizophorae* was recorded, including the locations of our study. A PCA was run to obtain a climatic profile of the niche that each variant occupies and morphological and climatic characterizations of the variants. A distance-based biplot was constructed based on climatic and morphological variables and individuals per variant. Finally, we analyzed the correlations between morphological traits and climatic variables using Pearson's correlation coefficient. All statistical tests were done using the software XLSTAT 2014 (Addinsof, 2007).

RESULTS

Molecular analysis

The combined plastid DNA dataset contained 3217 bp, with eight polymorphic sites (seven of which

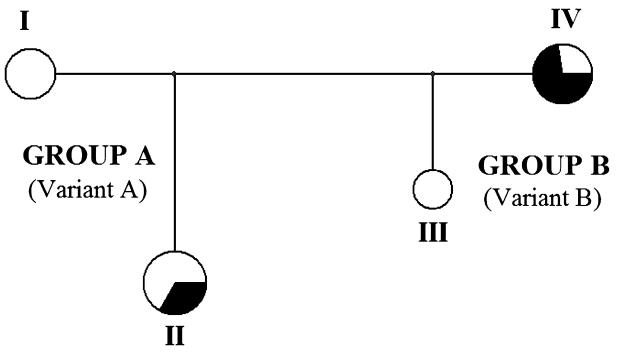

Figure 2. Network of haplotypes in *Pelliciera rhizophorae*. In black: haplotypes on the Caribbean coast; in white: haplotypes on the Pacific Coast.

were transversions) and no indels. Four haplotypes were found, two of which are geographically distributed on the Caribbean and Pacific coasts. The haplotype network revealed two groups with two haplotypes each (Fig. 2). Group A with haplotypes I and II comprises individuals with white to cream floral bracts (variant A) and group B with haplotypes III and IV comprises individuals with pink to red bracts (variant B). No shared haplotypes between variants were detected. The unrooted NJ tree revealed two strongly supported main branches (bootstrap > 80%) with a mix of populations belonging to both Neotropical coasts (Fig. 3A). Low variation was revealed in plastid DNA, but two clearly separated clades were found in *P. rhizophorae*. Clade A is composed of individuals from the Pacific Coast (TOL, Ecuador; UTR, Colombia; PED, Panama; TEM, Costa Rica) and Caribbean coast (BTO, Panama); Clade B is composed of individuals from the Panamanian Pacific populations (CHA, PCZ) and the Colombian Caribbean (CIS, BAR). The pattern of colouration in floral bracts was consistent within clades; thus, all individuals in Clade A have white to cream bracts (Fig. 3B) and all individuals in Clade B have pink to red bracts (Fig. 3C).

Floral morphometry

The *P. rhizophorae* flowers analyzed in this study typically had five white petals (in a few cases flowers with six or even seven petals were found), five white to cream sepals and two white bracts on all the variant A specimens collected in the Caribbean population of BTO (in both locations of the Pacific where variant B was collected, the bracts were pink or red) and five stamens with elongated anthers adhering to the lower part of the pistil. With respect to morphometry, the Mann–Whitney *U*-test showed highly significant differences between the two variants; i.e. measurements were larger for specimens of variant A than those of variant B (Table 2). Figure 4 compares each part of the flower for the two variants.

Leaf traits

The leaf blade, characterized by measurements of length, width and length/width ratio, of both variants of the species was oblanceolate and it was four times longer than wide; however, the size varied significantly between them, with the leaf area being greater in variant A (Table 2). Stomatal density, calculated in a 1 mm^2 area, averaged 128/89 (abaxial/adaxial axes) in variant A and 117/21 in variant B. These data indicate that stomatal density in the abaxial axis was similar for both variants; whereas highly significant differences in the adaxial axis were found between the two variants according to the Mann–Whitney *U*-test, which revealed a tendency for variant B to have hypostomatic leaves, with greater stomatal density toward the midrib (Table 3).

UPGMA cluster analysis

The morphometric data of the two variants were organized in a single matrix, standardized and submitted to Euclidean distance analysis. A cluster analysis using UPGMA revealed the presence of two groups with the samples corresponding to each variants (Fig. 5). Within both variants there was high morphometric variability, with variant B being subdivided into two populations: CHA and PCZ.

Climatic profile of the niche occupied by the two variants of *P. rhizophorae*

The characterization of the climatic profile based on the 19 bioclimatic variables of temperature and rainfall revealed differential patterns between zones. In the PCA analysis, the first two components of the 19 variables associated with each of the 20 locations where *P. rhizophorae* was found, described 73.54% of the data. The first component (52.39%) was formed by temperature and rainfall variables and the second component (21.15%) was defined primarily by temperature variables, especially the minimum temperature of the coldest month and the annual temperature range. Distribution of zones in the plot showed two clusters, each containing contrasting climatic characteristics. The cluster with high rainfall/low temperature zones comprised the BTO population, whereas the PCZ and CHA populations were in the low rainfall/high temperature cluster (Fig. 6). This analysis indicated that the two *P. rhizophorae* variants occupy separate climatic niches. The PCA of the morphological and climatic variables accounted for 92.23% of the total variance. The first factor

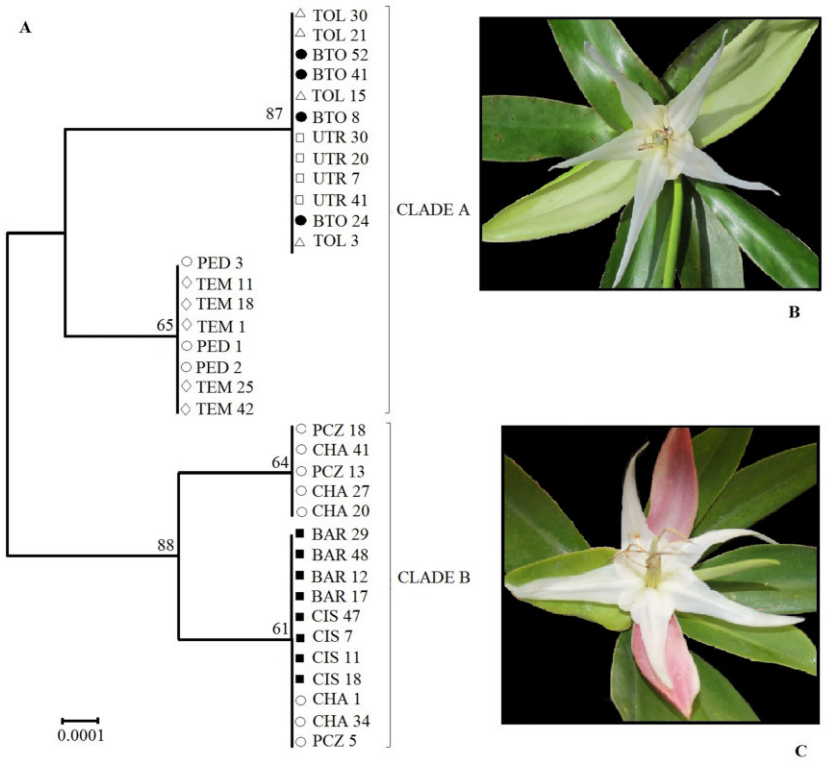

Figure 3. A, Unrooted neighbour-joining tree with 1000 bootstraps based on plastid DNA. Populations included in tree: Colombian Pacific Coast: UTR (white squares), Colombian Caribbean coast: CIS, BAR (black squares), Panamanian Pacific Coast: CHA, PCZ, PED (white circle), Panamanian Caribbean coast: BTO (black circle), Costa Rican Pacific Coast: TEM (white diamonds), Ecuadorian coast: TOL (white triangles). The scale bar indicates genetic distance. B, Flower variant A. C, Flower variant B.

Table 2. Morphometric description of floral and foliar structures in two variants of *Pelliciera rhizophorae*

Traits	*N*	Variant A mean (mm)	Variant B mean (mm)	Symmetry	Kurtosis
LPEL	100	68.17 ± 4.83	42.35 ± 8.00	−0.20	−1.36
APEL	100	13.57 ± 1.04	12.31 ± 1.95	−0.07	−0.57
LSEP	100	22.71 ± 1.89	13.79 ± 3.46	0.07	−1.18
ASEP	100	16.94 ± 1.50	10.45 ± 1.44	0.01	−1.57
LBRA	100	95.02 ± 7.30	54.17 ± 9.31	−0.07	−1.64
ABRA	100	44.65 ± 3.60	16.30 ± 2.57	0.04	−1.88
LFIL	100	39.58 ± 6.66	20.37 ± 5.07	0.19	−1.13
LANT	100	25.81 ± 2.82	18.98 ± 4.45	−0.40	−0.11
LPIS	100	65.10 ± 7.44	40.32 ± 8.19	−0.07	−1.31
LLEF	210	139.27 ± 18.61	108.73 ± 24.91	−0.15	−0.75
ALEF	210	37.31 ± 7.05	26.91 ± 4.59	0.16	−0.07
LLEF/ALEF	210	4.02 ± 2.95	4.02 ± 0.46	13.50	191.08

Mean values, standard deviations and symmetry and kurtosis tests.Trait abbreviations: ABRA, bract width; ALEF, leaf width; APEL, petal width; ASEL, sepal width; LANT, anther length; LBRA, bract length; LFIL, stamen filament length; LLEF, leaf length; LLEF/ALEF, ratio leaf length/leaf width; LPEL, petal length; LPIS, pistil length; LSEL, sepal length.

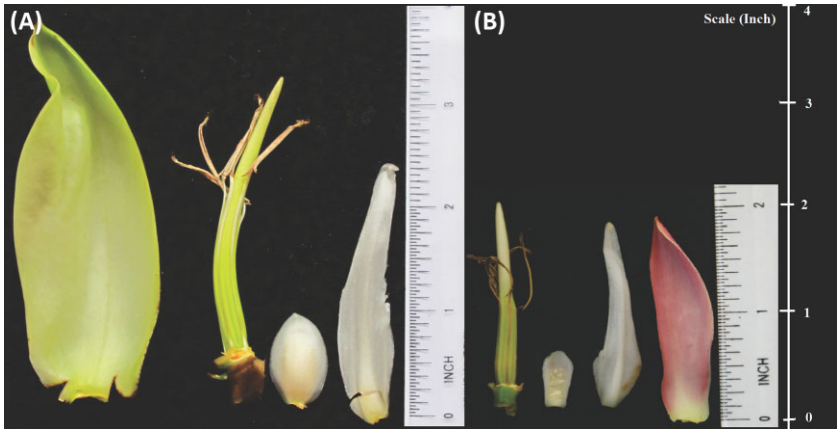

Figure 4. Floral structures in two variants of *Pelliciera rhizophorae*. *A*, Variant A (from left to right: bract, pistil and stamens, sepal, petal). B, Variant B (from left to right: pistil and stamens, sepal, petal, bract).

Table 3. Mean number of stomata in nine quadrants of adaxial axis in two variants of *Pelliciera rhizophorae*

Trait	Quadrant	*N*	Variant A	Variant B
Mean number of stomata	Ia	50	74.16 ± 16.08	1.84 ± 3.79
	Ib	50	106.84 ± 17.67	44.16 ± 18.51
	Ic	50	72.84 ± 16.76	2.48 ± 5.51
	IIa	50	93.12 ± 13.96	4.16 ± 7.93
	IIb	50	126.60 ± 16.37	73.92 ± 22.50
	IIc	50	96.04 ± 13.70	5.04 ± 9.71
	IIIa	50	72.40 ± 15.08	0.92 ± 2.27
	IIIb	50	84.00 ± 21.32	57.56 ± 14.87
	IIIc	50	76.72 ± 19.56	1.64 ± 5.94

Quadrant description: I, apex, II, middle, III, base; a, narrow side, b, midrib, c, broad side.

accounted for 81.50% of the total variance and had 28 variables with high contributions, whereas only two variables (APEL and BIO3) contributed to the variance accumulated in factor 2. The distance-based biplot graph (Fig. 7) revealed the separation between variants in terms of climatic variables and morphological traits. The majority of cases had significant correlation values ($P < 0.05$) between morphological and climatic variables. Negative correlations were found between morphological traits and ten of the 11 temperature variables; whereas six of the eight rainfall variables revealed a positive correlation with morphological traits (Table 4).

DISCUSSION

Intraspecific morphological variability and genetic differentiation of *P. rhizophorae*

Our earlier study with nuclear microsatellite markers showed the presence of geographically isolated genetic variants (Castillo-Cárdenas & Toro-Perea, 2012; Castillo-Cárdenas *et al.*, 2014). From these studies, two highly differentiated clusters were detected. No alleles shared between variants were found. The same differentiation pattern was supported by the plastid markers included in this study. These molecularly differentiated groups were also discriminated congruently by a different colouration of the floral bracts and a pattern of vegetative development that allowed us to characterize the two variants subject to study. In general, the morphological traits showed highly significant differences, except for the leaf length/width ratio, indicating that the shape of the leaf is conserved in both variants, but the dimensions were larger in specimens of variant A. Regarding this intraspecific variability, evidence at both the morphological and molecular levels allows us to support a diversification process occurred in *P. rhizophorae*.

Adaptive evolution of *P. rhizophorae* in response to climate

One of the determining factors in the distribution and evolution of mangrove forests is the climate (Duke,

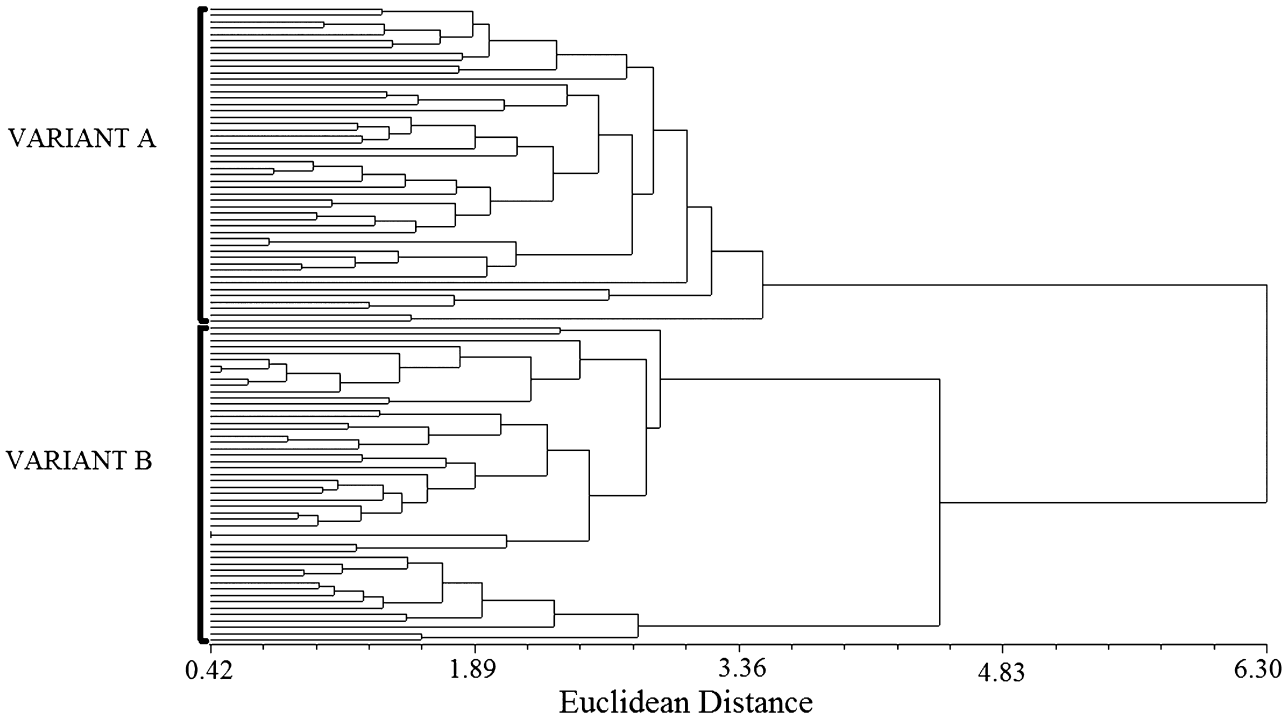

Figure 5. Clustering tree of floral and foliar morphometric traits in *Pelliciera rhizophorae* based on Euclidean distance.

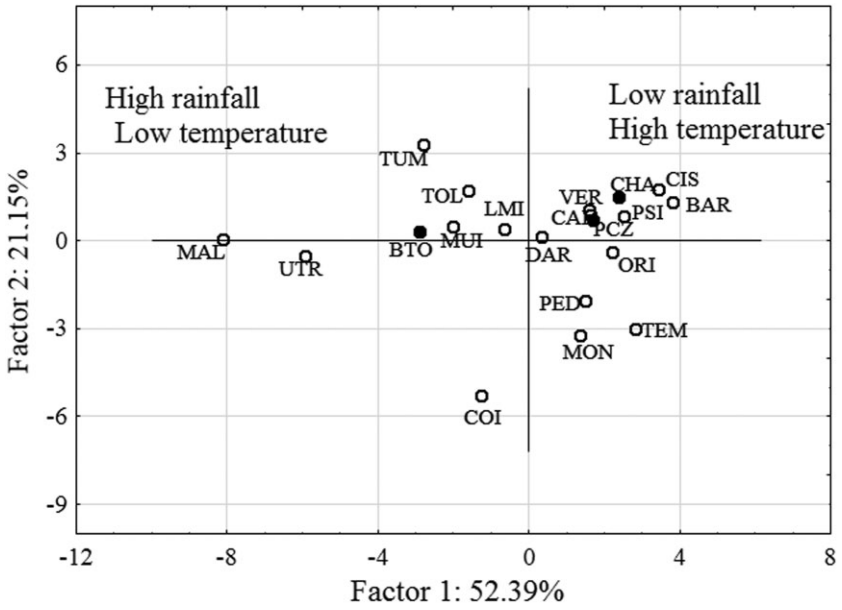

Figure 6. PCA of 19 bioclimatic variables at 20 localities with reports of current *Pelliciera rhizophorae* populations. Black circles show localities included in this study. Pacific Coast: Tempisque (TEM), Montijo Gulf (MON), Coiba Island (COI), Pedregal (PED), Oria (ORI), Pedasi (PED), Caimito Port (CAI), Veracruz (VER), Chame (CHA), Panama Canal Zone (PCZ), Darien (DAR), Malaga Bay (MAL), Utria Cove (UTR), Tumaco (TUM), Muisne (MUI), La Tola (TOL). Caribbean Coast: Bocas del Toro (BTO), Las Minas Bay (LMI), Cispata Bay (CIS), Barbacoas Bay (BAR).

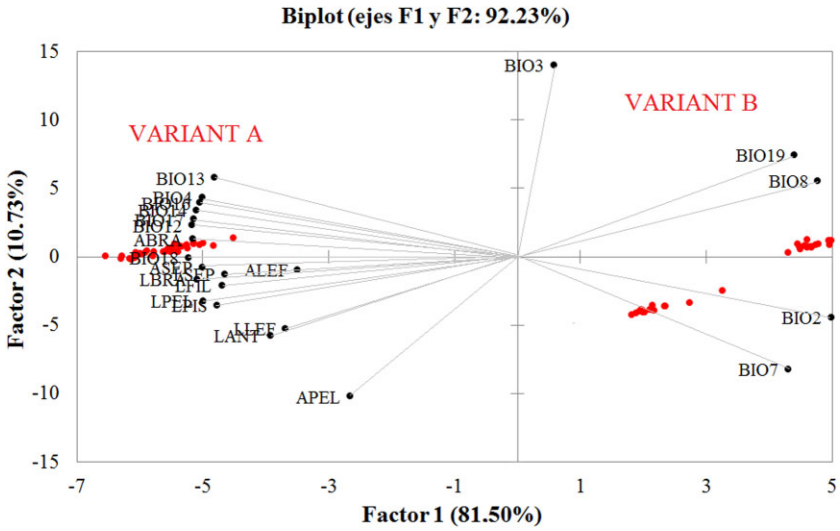

Figure 7. Principal components analysis of morphological and climatic variables for *Pelliciera rhizophorae*. Biplot vectors indicate strength and direction of factor loading for factor 1 and factor 2.

Table 4. Pearson's correlation between climatic and morphological variables

Variables	LPEL	APEL	LSEP	ASEP	LBRA	ABRA	LFIL	LANT	LPIS	LLEF	ALEF
BIO1	−0.938	−0.471	−0.865	−0.934	−0.961	−0.976	−0.874	−0.737	−0.888	−0.697	−0.662
BIO2	−0.837	−0.268	−0.809	−0.887	−0.885	−0.960	−0.800	−0.594	−0.783	−0.558	−0.609
BIO3	−0.299	−0.660	**−0.151**	**−0.119**	−0.215	**−0.013**	−0.212	−0.447	−0.312	−0.437	**−0.148**
BIO4	0.839	0.272	0.811	0.888	0.886	0.961	0.801	0.596	0.786	0.560	0.610
BIO5	−0.891	−0.361	−0.843	−0.917	−0.928	−0.978	−0.841	−0.663	−0.838	−0.625	−0.639
BIO6	−0.953	−0.531	−0.866	−0.931	−0.967	−0.962	−0.881	−0.770	−0.905	−0.730	−0.666
BIO7	−0.659	**−0.040**	−0.680	−0.759	−0.728	−0.857	−0.653	−0.397	−0.608	−0.367	−0.501
BIO8	−0.937	−0.681	−0.812	−0.859	−0.922	−0.852	−0.845	−0.823	−0.899	−0.785	−0.635
BIO9	−0.934	−0.462	−0.864	−0.933	−0.959	−0.978	−0.872	−0.731	−0.884	−0.691	−0.660
BIO10	−0.928	−0.444	−0.861	−0.932	−0.955	−0.980	−0.868	−0.719	−0.877	−0.680	−0.657
BIO11	−0.929	−0.447	−0.862	−0.932	−0.956	−0.979	−0.869	−0.722	−0.879	−0.682	−0.658
BIO12	0.899	0.377	0.847	0.921	0.934	0.980	0.848	0.675	0.847	0.637	0.643
BIO13	0.785	**0.194**	0.774	0.853	0.840	0.935	0.758	0.533	0.732	0.499	0.579
BIO14	0.870	0.322	0.830	0.906	0.912	0.973	0.826	0.635	0.817	0.598	0.628
BIO15	−0.926	−0.440	−0.861	−0.932	−0.954	−0.980	−0.867	−0.717	−0.876	−0.678	−0.657
BIO16	0.853	0.294	0.820	0.897	0.898	0.967	0.813	0.614	0.800	0.577	0.618
BIO17	0.888	0.356	0.841	0.916	0.926	0.978	0.839	0.660	0.835	0.622	0.638
BIO18	0.942	0.485	0.866	0.934	0.963	0.974	0.876	0.745	0.892	0.705	0.663
BIO19	−0.896	−0.727	−0.758	−0.794	−0.868	−0.770	−0.799	−0.819	−0.864	−0.783	−0.598

Morphological variables (abbreviations): ABRA, bract width; ALEF, leaf width; APEL, petal width; ASEL, sepal width; LANT, anther length; LBRA, bract length; LFIL, stamen filament length; LLEF, leaf length; LLEF/ALEF, ratio leaf length/leaf width; LPEL, petal length; LPIS, pistil length; LSEL, sepal length.Bioclimatic variables: BIO1, annual mean temperature, BIO2, mean diurnal range (mean of monthly maximum–minimum temperature), BIO3, isothermality (BIO2/BIO7) (× 100), BIO4, temperature seasonality (SD × 100), BIO5, maximum temperature of warmest month, BIO6, minimum temperature of coldest month, BIO7, annual temperature range (BIO5 and BIO6), BIO8, mean temperature of wettest quarter, BIO9, mean temperature of driest quarter, BIO10, mean temperature of warmest quarter, BIO11, mean temperature of coldest quarter, BIO12, annual rainfall, BIO13, rainfall of wettest month, BIO14, rainfall of driest month, BIO15, rainfall seasonality (coefficient of variation), BIO16, rainfall of wettest quarter, BIO17, rainfall of driest quarter, BIO18, rainfall of warmest quarter, BIO19, rainfall of coldest quarter.In bold letter: non-significant correlations ($P > 0.05$).

1995). In the case of *P. rhizophorae*, we propose that the climatic conditions are playing an important role in the species population dynamics. In the Neotropics the periods of climatic change occurring during and after the Quaternary defined the characteristics of the current climate, with two seasonal periods that alternate between wet and dry, affected by the Zone of Intertropical Convergence. In Panama the Caribbean lowlands have a pattern of higher yearly rainfall than the Pacific (Bartlett & Barghoorn, 1973), whereas in Colombia the inverse pattern occurs, with the coastal strips of the Caribbean being predominantly dry for the greater part of the year (Sanchez-Paez *et al*., 1997) and the Pacific has high rainfall (Chaves & Arango, 1997). With the purpose of supporting our hypothesis concerning the influence of the climate on the distribution of the genetic variants of the species, we performed a climatic characterization of the zones where the *P. rhizophorae* samples were collected. The bioclimatic data analyzed by PCA revealed a tendency toward two climatic zones with contrasting characteristics: (1) the dry zone, characterized by low rainfall (< 1850 mm yearly average), recorded in the Colombian Caribbean and the western zone of the Province of Panama up to the eastern side of the Azuero Peninsula, including the zone known as the Dry Arc Region of Panama; and (2) the wet zone, characterized by high rainfall (> 2000 mm yearly average), which corresponds to the Colombian Pacific, the Panamanian Caribbean and the western region of the Panamanian Pacific. Based on this climatic characterization and the distribution patterns for the *P. rhizophorae* variants, we can conclude that variant A tends to occupy the wetter zones and variant B occupies the drier zones with higher temperatures. Based on these tendencies, we suggest that the two variants evaluated in this study correspond to intraspecific lineages that occupy different climatic niches. Given these results, we can infer that the strongest barrier that impedes genetic exchange between *P. rhizophorae* populations is more environmental in nature than geographical. Recent studies have revealed that the influence of climatic factors can lead to the isolation of the population and later to incipient speciation as a consequence of the reproductive isolation of lineages that occupy different niches (Hua & Wiens, 2013).

The vegetative traits included in this study support our conclusions that climate is a key factor in a process of speciation in *P. rhizophorae*. This process, which would have occurred in response to the pressure exercised by the climatic factor, was evident in our study, noting that a phenotype associated with zones with a dry climate had a reduced stomatal density on the adaxial axis and a smaller leaf area than variant A, found in wet climate zones. The phenotype characteristic of variant B, which occupies dry zones, has an adaptive advantage, preventing the loss of water under these extreme climatic conditions. Stomatal density is a characteristic determined by the expression of the gene 'STOMAGEN' (Kondo *et al*., 2010), although it is also determined by environmental factors; however, the genetic and environmental influences on this character have been shown to be species-specific (Zhang *et al*., 2012). In the case of *P. rhizophorae*, we suggest that stomata development is a character strongly influenced by gene expression, which would explain the ecogeographical isolation of the variants.

Evidence of incipient speciation in *P. rhizophorae*

The description of the two variants found in *P. rhizophorae* takes us to just one scenario: the ecogeographical isolation to which populations have been submitted, due to the pressure of climatic factors, leads to a result that is consistently revealing patterns of micro- and macroevolutionary differentiation. Based on this study, it was possible to discriminate between the two groups that we initially named intraspecific variants of *P. rhizophorae*. In light of our current data, we suggest they could be divergent lineages of *Pelliciera*, one of which is adapted to dry climates and the other to wet climates. Despite not having information about the interbreeding or viability of possible crosses between these two lineages, it is probable that reproductive isolation is occurring at pollination given that the differential colouration of the floral bracts could be an important factor for attracting animal vectors as has been reported in other species (Sun *et al*., 2008). Regarding the physiology of the two lineages, we have evidence that suggests greater adaptability to a dry climate for the lineage that has fewer stomata in the adaxial axis, decreasing dehydration under the drought conditions to which it is subjected, the same as occurs in the grass *Imperata cylindrica* (L.) P.Beauv. (Hameed, Ashraf & Naz, 2009). Moreover, our data reveal a decreased level of vegetative growth in the dry climate lineage, which could indicate its potential to adapt to climatic pressures. Comparison of vegetative traits between closely related species has demonstrated a greater tolerance to stress in plants that are shorter and have smaller leaf blades than their taller associated congeners (Medrano, Castellanos & Herrera, 2006). Although the involvement of phenotypic plasticity in the morphological traits has not been discarded, it is possible that it may be contributing another piece of evidence for the adaptive process that has been occurring in *Pelliciera*. Recent studies have highlighted the key role of phenotypic

plasticity in promoting diversification at different levels of biological organization since the divergence within populations until the formation of new species through the action of genetic assimilation and phenotypic accommodation (West-Eberhard, 2005; Pigliucci, Murren & Schlichting, 2006; Pfennig *et al*., 2010).

Lastly, having compiled the biological evidence (molecular, morphological and physiological) and taking into account that the climatic niches of the lineages do not have characteristics in common, we can infer that the absence of genetic exchange suggests the presence of two incipient lineages in *Pelliciera*. However, we propose an incipient stage of speciation and new knowledge must be compiled in order to solve the taxonomic status of the lineages. With this purpose, cytological and palynological analyses are being developed.

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APPENDIX

Vouchers were deposited in Herbarium of Universidad de Panama.

Voucher information for this study. The order corresponds to: Variant, floral bracts colour, collectors, voucher number, voucher barcode number, location. in cat. (voucher in cataloging).

Variant A, white, J.A. Ramirez &D.E. Buitrago, in cat. Mouth of Cricamola river (Bocas del Toro).

Variant B, pink, J.A. Ramirez & M.F. Castillo, PMA 100446, 99753, Chame Point (Chame).

Variant B, red, D.E Buitrago & M.F. Castillo, PMA 109428, 99839, Diablo Heights (Panama Canal Zone).